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PRESENTAZIONE

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Presentazione

Carissime colleghe, carissimi colleghi,

dopo due anni vissuti in uno stato di sospensione dovuto alla paura di una pandemia che sembrava non doversi arrestare, siamo finalmente giunti ad un momento in cui, pur dovendo mantenere le necessarie precauzioni, possiamo ritornare a condurre le nostre esperienze professionali e di vita in modo diretto. Come disse il Sommo Poeta, di cui abbiamo ricordato i 700 anni dalla morte nell'anno appena passato, "E quindi uscimmo a riveder le stelle".

Con questa prospettiva, tenendo anche conto dei festeggiamenti degli 800 anni dalla Fondazione del nostro Ateneo, siamo quindi lieti di accogliere a Padova i Colleghi in presenza e di condividere i risultati del loro lavoro scientifico, anche con i Colleghi collegati online, nei giorni del 14, 15 e 16 settembre, in occasione del 75° congresso SIAI.

Raffaele De Caro, Michelangelo Cordenonsi, Veronica Macchi, Maria Teresa Conconi

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LETTURE MAGISTRALI

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Vol. 126, n. 1 (Supplement): 9, 2022
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Prima Lettura
Clinical Anatomy Between Research And Medical Practice?

Jose Ramon Sañudo Tejero[1]

[1]Università Complutense (Madrid, Spagna)

At the beginning of the twentieth century, many scholars considered Anatomy a "death science". The belief was that all the morphology was done. Biedermann W. (20th century) said, "*You, morphologist, make the luggage and go away!*". Santiago Ramon and Cajal wrote to his friend, the anatomist Federico Oloriz "*Descriptive anatomy is already an established science, and to discover new details, extremely demanding long-term research is needed. However, normal histology, embryology, and general physiology provide us with many questions still to be answered*". But Mr Ramon and Cajal also wrote, "*There are no expired scientific subjects, only men that are expired in their subject*".

Following the thesis of Abraham Flexner (1939) in his essay "*The Usefulness of Useless Knowledge*", my presentation will show how clinical anatomy, the modern view of anatomy, is a fundamental bridge between good clinical practice and research.

Anatomy and embryology are not two dead sciences. They are fundamental for four main reasons.

1 **MEDICAL BACKGROUND** permits us the knowledge of the medical past.
2 **MEDICAL VOCABULARY** Give a common terminology representing 75% of the medical language.
3 **GOOD MEDICAL PRACTICE**, avoid medical errors.
4 **MEDICAL RESEARCH**, basic for improving the medical practice.

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Seconda Lettura
From Single Cell Analyses to Molecular Histology of Tumor Ecosystem

Massimiliano Pagani[1,2]

[1]Istituto Fondazione Oncologia Molecolare (IFOM)
[2]Università di Milano

Single-cell RNA sequencing has become the technique of choice for defining the molecular blueprints, differentiation, and developmental trajectories of the different cells present in the tumor ecosystem. Single-cell technologies, though, rely on the physical dissociation of individual cells from the tissue. Thus, valuable topological information of expressed genes/proteins, as well as spatial reconstruction of molecular signals arising from cell-to-cell/matrix interfaces, are lost through the manipulation of the sample. To address these limitations, we are employing spatially resolved omics technologies that offer new insights into the molecular heterogeneity within tumor tissue, while preserving morphological clues. Indeed, spatial "ensembles" of cell types are crucial determinants of several functions, including immunity. The integration of these datasets will potentially lead to new drug and therapy concepts based on understanding of disease mechanisms, new clinical trial designs, and a new level of digital molecular pathology relying on multi-dimensional data.

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COMUNICAZIONI ORALI

Anatomia clinica e forense, anatomia per immagini, ingegneria tessutale e medicina rigenerativa

Prima sessione

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Malar bone anatomical measurements surgically oriented: a study on 36 fresh-frozen cadavers related to the placement of zygomatic implants

Carlo Barausse[1,2,3], Pietro Felice[2], Lucia Manzoli[1], Roberto Pistilli[4], Alessandra Ruggeri[1], Lucio Cocco[1], Stefano Ratti[1]

[1] Center of Experimental Clinical Surgery and Molecular Anatomy, Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy
[2] Unit of Oral Surgery, Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy
[3] Postgraduate School of Oral Surgery, University of Modena and Reggio Emilia, Italy
[4] Unit of Oral and Maxillofacial Surgery, San Camillo-Forlanini Hospital, Rome, Italy

Background: Malar bone could be a source of anchorage for zygomatic implants avoiding invasive reconstructive surgeries in the fixed rehabilitation of the fully edentulous and severely atrophic maxilla.[1] However, their placement in a limited bone volume required the carefully respect of the closed noble anatomical structures.

Aims: To measure malar and zygomatic arch bone and distances with the relevant anatomical structures, to guide the surgeon in a correct and safety zygomatic implant insertion.

Materials and Methods: Human fresh-frozen cadaver heads were bilaterally dissected. The main anatomical landmarks taken into consideration were: infraorbital foramen (IF), pyriform nasal aperture (PNA), external border of the orbital floor (OF), the anterior end of the zygomatic arch (A) and the most protruding point of the zygomatic arch (B)[2]. The following linear measurements were taken with a digital caliper (resolution 0.1 mm) from each zygoma: distance IF-PNA, IF-OF and A-B. Malar and zygomatic arch height and thickness were taken with A and B as reference points. Sex, age and ethnicity were also considered.

Results: A total of 72 malar bones from 36 fresh-frozen Caucasian cadavers were evaluated: 11 (30.6%) were female and 25 (69.4%) male. The mean age was 72.7 years. IF-PNA mean distance was 19.3 mm, IF-OF was 7.8 mm. Mean "A" height was found as 13.3 mm, while "B" 6.4 mm. A-B mean distance was 19.4 mm, while their thickness was 3.7 mm and 3.0 mm, respectively. *Discussion*: The cadaver anatomic study and dissection of the malar region is of paramount importance in order to guide and train the clinician in this type of surgery reducing the risk of intraoperative complications.

Conclusions: malar bone can offer adequate spaces to arbour two zygomatic implants for a fixed prosthetic rehabilitation. However, the surgeon should be aware of the anatomical limits to be respected and the knowledge of a mean distance value coming from a fresh-frozen cadaver can be helpful in daily clinical practice.

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Crime and Punishment – Part 1. Ischemic necrosis of the colon after Tauber's procedures. Unpredictable or avoidable?

Rafael Boscolo-Berto[1], Andrea Porzionato[1], Veronica Macchi[1], Raffaele De Caro[1]

[1] Institute of Human Anatomy, Department of Neurosciences, University of Padova, Padova, Italy

Tauber's procedure (antegrade sclerotherapy) is a minimally invasive approach used in treating male varicocele. Among the possible clinical consequences is the so-called ischemic colitis, i.e., ischemic necrosis of a part of the left colon of variable length. Professor Tauber himself described the occurrence of ischemic colitis since the introduction of the antegrade sclerotherapy as a clinical standard available for treating varicocele.

The pathogenetic explanation relies on the presence of an infrequently reported vascular communication between the internal spermatic vein and the colic veins. The sclerosing agent injected to obliterate the dilated veins of the pampiniform venous plexus (varicocele) can thus follow an abnormal route and cause a compromise of the colonic vascular homeostasis, with consequent ischemic necrosis.

Here we report a case of judicial interest that caused a heated juridical and medical-legal debate that dragged on in civil proceedings up to the court of appeal.

Immediately after performing the antegrade sclerotherapy of the left internal spermatic vein, the patient complained of severe testicular and abdominal pain but was discharged the day after without further investigation. After a few days, he went to the emergency department for worsening abdominal pain with intestinal obstruction. A septic shock due to the ischemic necrosis of the left colon from the splenic flexure to the sigma-rectum was detected. He underwent colon resection surgery with a temporary left colostomy, subsequently closed with a colorectal anastomosis.

The challenge involved several experts resistant to opposed positions, also based on the results of a scientific article published by the authors on the topic. However, the paper was misinterpreted, drawing inaccurate conclusions from correct assumptions and distorting the analysis of the clinical evolution and consequences to the patient.

After a struggling challenge between medicolegal and urology experts, the judge's consultants reached an unexpected conclusion, heavily relying on the report based on the principles and evidence of forensic clinical anatomy. The court, as a result, delivered a rigorous and exemplary judgment.

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Crime and Punishment – Part 2. Personal injury and damage due to medical malpractice: the anatomo-functional approach (boomerang effect)

Rafael Boscolo-Berto[1], Andrea Porzionato[1], Veronica Macchi[1], Raffaele De Caro[1]

[1] Institute of Human Anatomy, Department of Neurosciences, University of Padova, Padova, Italy

Antegrade sclerotherapy of the internal spermatic vein (Tauber's procedure) is a minimally invasive approach to treating male varicocele. The procedure's possible clinical consequences are ischemic colitis, testicular atrophy, and other minor events (e.g., infection, orchitis). Ischemic colitis is due to the presence of a vascular communication between the internal spermatic vein and the colic veins, through which the sclerosing agent injected to obliterate the dilated veins of the pampiniform venous plexus (varicocele) can wander, causing an impairment of the colonic vascular homeostasis, with consequent ischemic necrosis. Similarly, testicular atrophy is due to the spread of the sclerosing agent caudally toward the testis, resulting in ischemic orchitis of the gonad, potentially culminating in its atrophy.

The damage resulting from a civil offense, such as the ascertained medical malpractice, must be compensated with specific reference to valuation barèmes reported in devoted guidelines. The medicolegal expert expresses the quantum of permanent biological damage in percentages. However, percentages reported in such guidelines must be regarded as merely indicative, as they refer to ordinary cases and hence require adjustments in particular cases with especially harsh consequences. Moreover, it is necessary to distinguish between coexisting impairments, which affect different organs or functional systems, and concurrent impairments, which affect the same organ or system.

There is, however, no agreement on how to apply this principle. In cases where more than one damage item applies, there are no arithmetic formulas, but an appraisal should be made of the overall effect of the impairments on the injured party's functioning.

Here we present a case of judicial and clinical interest as reporting the consequences of antegrade sclerotherapy of the internal spermatic vein followed by ischemic necrosis of the left colon with testicular atrophy. In the damage ascertainment, particular importance was given to the medicolegal considerations underlining the anatomo-functional dimension of the damage in the loss of the testicle. Applying the principles of forensic clinical anatomy has made it possible to scientifically support a "concurrent" interpretation instead of the "differential" one of the damages suffered by the patient. This approach bypassed the one proposed by the court's consultants and led the judge to re-evaluate the estimated damage coherently to our interpretation.

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The oculomotor cistern: a morphometrical study for clinical considerations

Barbara Buffoli[1], Simona Serioli[2], Caterina Franco[1], Stefania Castrezzati[1], Francesco Doglietto[3,4]

[1] Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[2] Division of Neurosurgery, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, Spedali Civili of Brescia, University of Brescia, Brescia, Italy
[3] Division of Neurosurgery, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, Spedali Civili of Brescia, University of Brescia, Brescia, Italy
[4] Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy

The morphological features of the oculomotor cistern (OMC) have been focused by several radiological and surgical studies [1-4], although the modalities of tumor growth through this space are poorly understood. The aim of this study was to describe anatomical features of the OMC from a morphological point of view.

The morphometrical study was performed on four fixed adult cadaver heads. Seven hemisellae were used for the collection of the samples. One hemisella was analyzed by sagittal sections, respecting the course of the oculomotor nerve, whereas 6 hemisellae were included to obtain coronal sections. 30 serial sections/sample were evaluated using an optical light microscope and quantitative measurements were performed by a digital scanner (Aperio CS2®, Leica Biosystems, Wetzlar, Germany) and the software QuPath v0.2.3.

The OCM had a larger craniocaudal diameter (3218.76 +/- 634.68 μm) than the medio-lateral one (1214.55 +/- 438.03 μm), without point of weakness. The average area of OMC at the point of maximum extension was 3,1 +/- 0.9 mm^2). At the lever of the porus, the nerve was located near the superior edge of the cistern level, while in the more ventral sections the nerve was close to the lower border. At the level of the anterior clinoid process, the meningeal layers were no longer recognizable and replaced by the neural ones: the dura became the epineurium, whereas the pia-arachnoid continues as the perineurium.

The morphometrical data collected in this study could be significant to understand compression and invasion of the OMC by tumor growth for both better preoperative planning and adequate postoperative follow-up.

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The incidence and position of facial pigmented lesions as individualizing anatomical features for personal identification

Annalisa Cappella[1,2], Riccardo Solazzo[3], Barbara Bertoglio[4], Debora Mazzarelli[4], Marina Dobosz[5], Claudia Dolci[6], Cristina Cattaneo[4]

[1] Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Italy
[2] UO Laboratorio di Morfologia Umana Applicata, IRCCS Policlinico San Donato, San Donato Milanese, Italy
[3] Università degli Studi di Perugia, Italy
[4] LABANOF (Laboratorio di Antropologia e Odontologia Forense), Sezione di Medicina Legale, Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Italy
[5] Dipartimento di Ingegneria Civile e Ambientale, Università degli Studi di Perugia, Italy
[6] LAFAS (Laboratorio di Anatomia Funzionale dell'Apparato Stomatognatico), Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Italy

The issue of personal identification (PI) may be problematic in those frameworks where primary methods using genetics or fingerprints are not applicable. The chosen PI method mostly depends on the availability and typology of antemortem (AM) data: in some contexts, photographs may be the only available AM material to be analysed and compared with postmortem (PM) one. The use of photos in supporting the PI is not a novelty in literature [1] and it recently received great attention in "special" disasters because photos can be easily provided by the families or retrieved from social networks that are nowadays worldwide used [2-4]. Being exposed in photographs, the face may ensure to retrieve salient information about the so called "personal descriptors", as relatively time-stable pigmented lesions. Nonetheless there still is an urge to collect empiric data on identifier features visible on photos and research has to advance in case of secondary methods based on them.

This study aims at collecting empiric data on the frequency of specific facial identifier features as pigmented lesions and to verify their potential in the PI. A retrospective analysis on stereophotogrammetric facial 3D models of 1039 Italian subjects (aging from 4 to 84 years old) has been conducted in order to investigate the incidence and position of pigmented lesions (discriminated according to size (1-3 mm or 3>mm) and location in 12 different facial regions). A survey on the patterns expressed by the analysed subjects was performed to verify the possible uniqueness and differences among different individuals: for each individual two codes were generated based on the available collected information and a probabilistic likelihood ratio approach was provided in terms of uniqueness.

The results have revealed that 82% of our reference population exhibits a unique pattern if the lesions are discriminated according to position, and incidence and size, while a lower percentage (74%) expresses a unique pattern if size is not considered.

The data on incidence and position of facial pigmented lesions, their generated codes, and the probabilistic approach here presented, all constitute a starting point for the use of non-standard identifier features in the PI.

In a forensic era where all methods, regardless of the kind of provided data, must postulate probabilities when supporting results, facial pigmented lesions proved to be valuable and potential characteristics to use as auxiliary secondary identifying method, building the foundations for advancing the research on unique anatomical features to apply to this demanding topic.

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Condylar Hyperplasia: a morphostructural analysis

Antonio Centofanti[1], Michele Runci Anastasi[2,3], Ludovico Magaudda[1], Maria Righi[1], Angelo Favaloro[1], Piero Cascone[2], Giuseppina Cutroneo[1]

[1] Department of Biomedical and Dental Sciences and Morphofunctional Images Policlinico Universitario "G Martino", Messina, Italy
[2] Unicamillus Università Medica, Roma, Italy
[3] IRCCS Centro Neurolesi "Bonino-Pulejo," Messina, Italy

Condylar hyperplasia is a rare disease that modifies the anatomy of the condylus and the mandible, determining a progressive development of a typical facial asymmetry, as direct consequence of an excessive unilateral growth of the condylar cartilage. The pathological condition is not described in any other joint and it is generally observed in young patients between the ages of 11 and 30 years. Some patients present as first symptoms pain and joint noise as a result of functional pathology; the secondary symptom is the difficulty in opening the mouth. Many authors have studied this pathology, because it is a frequent clinical condition and of great interest for maxillofacial surgeons for therapeutic solution, but the etiology remains unknown. It was observed that after a successful orthognathic surgery, with a rediscovered facial symmetry, after several years the patient again showed a facial asymmetry with condylar hyperplasia, for this reason the surgeons choose condylectomy as the golden standard. The aim of the present work was to evaluate, with different methods, the morphological characteristics of the disease, to try to identify the cause that triggers the increase this pathology. For this study we used 5 patients with condylar hyperplasia (4 female and 1 male) who were treated with proportional condylectomy and a control patient. The samples were observed under the optical microscope, the SEM and the confocal laser microscope. Our results support the hypothesis that indicates an increase of cartilage tissue activity, with metabolic disorders of the matrix.

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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Anatomical position of the mental foramen: hints for a correct localization

Daniele Gibelli[1], Carlo Martinenghi[2], Sandro De Nardi[3], Michaela Cellina[4]

[1] Dipartimento di Scienze Biomediche per la Salute Università degli Studi di Milano
[2] Unità Clinica di Radiologia Diagnostica IRCCS Ospedale San Raffaele, Milano
[3] Radiology 3D sas Milano
[4] Reparto di Radiologia Ospedale Fatebenefratelli ASST Fatebenefratelli Sacco, Milano

Anatomical localization of the mental foramen (MF) is crucial in odontological surgery procedures[1]. With time several articles have focused on the correct anatomical assessment of MF: however, very few data are available about the correlation between different metrical parameters.

One hundred CBCT scans were randomly selected, equally divided between males (mean age: 53.7±16.0 years) and females (mean age: 50.7±18.7 years). On each CBCT, MF width, height, depth, distance from the inferior and superior mandibular edge, position according to teeth and dental apices were assessed.

Differences according to sex and side for each metrical measurement were assessed through two-way ANOVA test; differences according to position according to teeth and dental apices were analysed through Chi-square test. Pearson's test and one-way ANOVA test were used to test possible correlations among all the parameters ($p<0.01$).

Depth, distance from inferior and superior mandibular edges were significantly higher in males than in females ($p<0.01$). A positive correlation between height and width was found both in males and in females, and between depth and distance from the inferior mandibular edge only in females ($p>0.01$).

Results represent a novel contribution to literature about anatomical characteristics of MF, useful in surgical and anaesthetic procedures.

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Updated report on the study of the Neapolitan "eared skull"

Veronica Papa[1,2,3], Elena Varotto[3,4], Silvio del Pizzo[2,5], Salvatore Troisi[2,5], Fabiana Di Ciaccio[2,5], Silvia Sofia Staiano[3], Stefano Vanin[6], Mauro Vaccarezza[7,8] Laura Landini[3,9], Carlo Cantile[10] and Francesco Maria Galassi[3,4].

[1] Department of Sport Sciences and Wellness, University of Naples "Parthenope," Naples, Italy
[2] School of Science, Engineering, and Health, University of Naples "Parthenope," Naples, Italy
[3] Forensic Anthropology, Paleopathology, and Bioarchaeology (FAPAB) Research Center, Avola, Italy
[4] Flinders University, College of Humanities, Arts and Social Sciences, Archaeology, Adelaide 5001, SA, Australia
[5] Department of Sciences and Technologies, University of Naples "Parthenope" Naples, Italy
[6] Departmenf of Life Science, Environment and Earth (DiSTAV), University of Genoa, Genoa, Italy
[7] Curtin Medical School, Faculty of Health Sciences, Curtin University, Bentley, Perth, Western Australia, Australia
[8] Curtin Health Innovation Research Institute (CHIRI), Faculty of Health Sciences, Curtin University, Bentley, Perth, Western Australia, Australia
[9] Department of Civilizations and Forms of Knowledge, University of Pisa, Pisa, Italy
[10] Department of Veterinary Sciences, University of Pisa, Pisa, Italy

The purpose of this study is to report updates and further details on the study of the so-called eared-skull, which is currently exhibited in the hypogeum of the musealised Church of Santa Luciella ai Librai in Naples (Italy).

The study started in 2017 and used a multidisciplinary approach combining morphological, archaeo-anthropological, chronological, entomological, taphonomical, and photogrammetric analyses.

As the authors previously reported, human remains in the hypogeum were dated. The entomological analysis revealed insects associated with body decomposition and wooden coffins.

The morphological and anthropological study of the skull demonstrated it is that of an adult male. Since the skull showed the absence of the sagittal suture, a diffuse cranial porosity, and only the coronal suture was clearly visible, a more precise range for age at death of this individual could not be estimated.

The skull consists of the neurocranium and the nasal bone; more interestingly, the temporal bones were found to be outwardly rotated, which had been wrongly assumed to be mummified auricular structures in the past. Furthermore, a cerebral fragment was found inside the cranial cavity.

Histological analysis was performed on the brain fragment. The sections were stained with Hematoxylin and Eosin as well as with Luxol Fast Blue methods.

These staining revealed a tissue with a diffusely vacuolized extracellular matrix of granular aspect. Moreover, isolated corpuscles and aggregates of discoid corpuscles (10 to 20 microns of diameter) have been detected within the tissue. These aggregates had a burnished-bronze color, were unstained with the used methods, and can be interpreted as fungi or bacteria. Finally, Luxol Fast Blue staining highlighted the possible presence of myelin residues in the investigated tissue.

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ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Importance of the anatomical elements of the palatal vault for the treatment of complte edentulous patients

Elena Sapte[1], Jula Cazacu Corina[2], Jula Radu[2], Grammatikis Efstratios[2], Manea Mircea[4], Corina Stefanescu[3]

[1] Department of Anatomy, Faculty of Dental Medicine, "Ovidius" University of Constanța, Romania
[2] PhD student, Department of Anatomy, Faculty of Dental Medicine, "Ovidius" University of Constanța, Romania
[3] Department of Prosthetics, Faculty of Dental Medicine, "Ovidius" University of Constanța, Romania
[4] Student, Department of Anatomy, Faculty of Dental Medicine, "Ovidius" University of Constanța, Romania

The main purpose of complete edentulous maxillary patients therapy with complete dentures is based on patient acceptance of the consequences of tooth loss and its psychological modeling in order to be harmonized with this prosthetic construction. The mission taken by the dentist is a complex one, including the integration of the notions of anatomy and oral physiology that definitely influence the success of the treatment. The prosthetic fieldrepresents the total biological surface with which the prosthetic piece has a contingency, whether direct or indirect, and which cooperates with its support, maintenance and stability. Support is the manner in which occlusal pressures are transmitted to the prosthetic field, maintaining counterbalancing forces that tend to detach the denture, and stabilization constrains the horizontal movement tendencies of the prosthesis. The significance of these notions is related to the fact that, once assured, they will direct the equilibrium attributes necessary for the prosthesis both during the performance of dento-maxillary functions, but also in rest position.

The study was designed to evaluate the main aspects of the palatal vault in complete edentulous patients, features seen in the context of the regressive phenomena of the elderly adult dento-maxillary system. The analysis of the anatomical structures capable of influencing the success of conventional prosthetic treatment by means of the complete dentures is also concentrated on the median and paramedian components, which in the anterior-posterior sense are incisive papilla, palatine folds, intermaxillary suture and median palatine suture, and, not in the end, the vibratory line Ah. The main objective underlying the study is to highlight the morphological profile of the palatal bone partition structures, namely: the shape, location, dimensions and analysis of any particularities that could affect or modify the application of prosthetic treatment.

The study was conducted at the Faculty of Dental Medicine, in Mobile Denture Department, on 100 complete edentulous patients who presented themselves for oral rehabilitation of the dento-maxillary system. The evaluation was done clinically and paraclinically by photostatic examination of prosthetic fields and study casts made after impressions.

A thorough knowledge of palatal anatomical relief is essential to understand the direction of approach in establishing the treatment of complete edentulous patients.

The data show that there is not a similar prosthetic field among edentulous fields, that the anatomy of each patient is unique, and as a consequence, the treatment is necessary to be applied by analyzing thoroughly each anatomical structure capable of influencing the success of the therapy.

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Reference data on facial symmetry in healthy Italian male children: a three-dimensional stereophotogrammetric survey

Chiarella Sforza[1], Riccardo Solazzo[2], Claudia Brunetti[1], Josh Yang[3,4], Noha Hassan[3], Loris Marcolongo[3], Claudia Dolci[1]

[1] LAFAS (Laboratorio di Anatomia Funzionale dell'Apparato Stomatognatico), Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Italia
[2] Università degli Studi di Perugia, Italia
[3] UO Chirurgia Maxillo-Facciale e Odontostomatologia, Fondazione IRCCS Cà Grande Ospedale Maggiore Policlinico, Milano, Italia
[4] Harvard School of Dental Medicine (HSDM), Boston, MA, United States

In everyday clinical and diagnostic practices, the evaluation of facial symmetry is an important part of the examination. Our understanding of facial symmetry involves many objectives as the definition of normal morphology, the appreciation of malformations and dysmorphisms, the evaluation of growth and development, the comprehension of evolution and the assessment of treatments and related outcomes. Indeed, reference data on symmetry of the whole face and/or of limited regions is fundamental for advancing in all the above purposes.

Lately, the analysis of facial symmetry made much progress, passing from direct methods to indirect ones through three-dimensional (3D) imaging systems [1]. Stereophotogrammetry has recently proven to be reliable in assessing facial symmetry in adults [2]. This safe 3D system can be applied also to children: facial asymmetry, when early and promptly detected in young patients, can be a significant clue to consider for driving the diagnostic process and treatment choices. Unfortunately, reference data on facial symmetry in growing persons are still incomplete. Therefore, knowledge on facial symmetry must improve and assure comparable data to use in literature [3].

This study aims to quantify facial symmetry in a healthy subadult Italian male population considering different facial thirds. We combined surface- and landmarks-based approaches on 3D facial models of 74 male children acquired using the Vectra M3 stereophotogrammetric system (Canfield Scientific Inc., Fairfield, NJ). To explore the potential impact of permanent teeth development and eruption on facial symmetry, the sample was divided in two different groups: children with ages between 4 and 8 years old (n=35) and children with pre-puberal ages ranging from 9 to 12 (n=39). For each facial model, the hemi-face and facial thirds (upper (UT), middle (MT), and lower (LT)) of each side were separated using anatomical landmarks, thus obtaining regions of interest (ROI) based on the territory of distribution of trigeminal branches. The symmetry was quantified calculating the root mean square values (RMS), namely the average point-to-point distance between the original and the reflected ROI: the right ROIs were mirrored across the sagittal plane, registered onto the equivalent left region by corresponding landmarks, and then superimposed again by using the iterative closest point algorithm (automatic best-fit) according to a previous validated protocol [2]. The lower the RMS value, the higher the degree of symmetry of corresponding points belonging to mirrored ROIs.

The entire protocol was performed with VAM (Vectra 3D Analysis Module), the software provided with the Vectra system. A 2-way ANOVA test was used to verify significant differences between the RMS values of different thirds and between the values of thirds in the two age classes.

Although no significant interaction was found between age and facial thirds ($p = 0.901$), results revealed significant differences in symmetry between the two age groups (younger chil-

dren were more symmetrical, $p = 0.046$) and when facial thirds were considered ($p < 0.001$). RMS values ranged between 0.34 mm (for MT) to 0.40 mm (for UT and LT) in younger children while from 0.37 mm (for MT) to 0.42 mm (for UT and LT) in pre-puberal children; overall hemi-facial symmetry was 1.5-2 times lower than those measured in the facial thirds (RMS = 0.6 mm). Among individuals of a same age group, statistical analysis using Bonferroni correction indicated differences between MT and the other two thirds (MT vs UT, $p = 0.001$; MT vs LT, $p = 0.004$): MT resulted the more symmetrical facial region. On the contrary, no significant differences were found between symmetry of UT and LT ($p=1.000$).

With this study we have provided objective and quantitative reference data on facial symmetry of Italian boys aged between 4 and 12 years, which can be used as important comparative data for further clinical and research purposes. Healthy children are characterized by RMS values of symmetry ranging from 0.34 to 0.40 mm in case of facial regions and from 0.61 to 0.64 mm when the hemi-face is considered. Since no consensus on the amount of 3D symmetry to considered as clinically acceptable exists [4], further investigations on larger randomized samples aiding to address this question are needed. Indeed, determining the clinical relevance of the amount of facial symmetry in children might facilitate in future several clinical practices and diagnostic processes which currently are still problematic and limited.

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Fetal anomalies: a comparison between prenatal ultrasound and post-mortem findings

Maria Alessandra Sotgiu[1], Pasquale Bandiera[1], Alessio Pirino[1], Vittorio Mazzarello[1], Marco Ferrari[1], Giampiero Capobianco[2], Andrea Montella[1]

[1]Department of Biomedical Sciences, University of Sassari, Sassari, Italy
[2]Department of Medicine, Surgery and Pharmacy, University of Sassari, Sassari, Italy

Introduction: Most fetal anomalies can be identified by fetal ultrasound. However,

fetal autopsy remains the gold standard procedure to confirm the abnormalities. As a result of advancement in prenatal imaging techniques and time consumption, there is a general decline of rate of autopsy. Even if false positive diagnosis in prenatal ultrasonogram leading to termination of pregnancy (TOP) is extremely rare, associated malformations can be missed by ultrasound. The aim of the present study was to compare the prenatal diagnosis with the post-mortem findings, evaluating if the quality of prenatal diagnosis of fetal anomalies improved during the time of this study.

Material and Methods: Records of all fetal autopsies performed at the Institute of Human Anatomy, University of Sassari, from January 2003 to December 2021 were reviewed.

The following data were extracted and registered in a database: woman's age, obstetric history (number of pregnancies, births, and terminations due to fetal anomalies), maternal risk factors, gestational age at last ultrasound, results of ultrasound examination and the autopsy findings.

Terminations carried out for chromosomal aberrations were excluded.

All the fetuses were examined with photography (supine, prone, and lateral positions), external and internal examination. Organs were dissected, separated, and weighted individually. All autopsies were performed by two Anatomists with experience in fetal pathologies.

Results: During the study period there were 298 fetal autopsies. All fetuses underwent autopsy following TOP between 12 and 23+6 weeks' gestation. Mean maternal age was 29 years. Structural anomalies were the most common reason for autopsy (n = 208; 69.8%) followed by intrauterine fetal death (n = 90; 30.2%). The sex differentiation was 38.9% (116/298) of females and 60.7% (181/298) of males. It was not possible to determine the sex in one case. Cardiovascular, digestive, and nervous were the most common types of fetal anomalies diagnosed in our study.

There were no false-positive sonographic diagnoses influencing outcome in the autopsies performed. However, foetal autopsy provided further diagnostic information in 34% of cases.

Conclusion: Our study demonstrates that there is high agreement between prenatal and autopsy diagnosis after TOP for fetal anomalies. Autopsy is particularly useful in the case where fetal ultrasound cannot provide the anatomic details necessary for accurate diagnosis.

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NFkB and SIRT1 evaluation to discriminate antemortem lesions from postmortem damages in wounds. Potential markers of skin wound vitality in forensic practice

Gabriella Teti[1], Paolo Fais[2], Susi Pelotti[2],Valentina Gatta[1], Laura Ingrà[1], Simone Gavelli[2], Mirella Falconi[3]

[1] Department of Biomedical and Neuromotor Sciences, University di Bologna, Bologna, Italy
[2] Department of Medical and Surgical Sciences, University of Bologna
[3] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna

In forensic autopsy cases wound examination is one of the most important issues in order to clarify the causal relationship between the cause of death and wounds detected in cadavers. Forensic pathologists need to determine whether a mechanical wound has vital reactions or not, and to estimate the postinfliction interval. However, it is often difficult to discriminate antemortem wounds from postmortem damages when they are inflicted very close to the time of death. In order to explore novel markers for vitality of acute mechanical wounds, we investigated the expression of the inflammatory related marker nuclear factor kappa B (NFkB) and analyze its correlation with the silent information regulator sirtuin 1 (SIRT1) protein, in human skin wounds. Human skin tissue samples (1-1.5 cm^2) were taken at the amputation site (antemortem lesion) of different lower limbs undergoing surgery for traumatic or natural pathologies, from patients of both sexes aged between 54 and 75 years. Samples were immediately processed for western blot and immunohistochemistry analysis to evaluate the expression of NfKB and SIRT1 proteins. Results were compared to wounds skin samples inflicted on the same lower limb, at the level of sural triceps, the same days of sample collection (postmortem lesion).

Results showed high level of cytoplasmic and nuclear staining of NFkB in epidermal cells of antemorten lesions compared to post-mortem ones, where the NFkB signal was mainly localized in keratinocytes nuclei. Quantitative analysis of stained areas demonstrated 2-fold increase of NFkB protein in ante-mortem lesions. On the contrary, SIRT-1 levels were almost absent in antemortem lesions, while a high nuclear staining was detected in keratinocytes in postmortem lesions. Quantitative analysis of stained area demonstrated a 3 fold increase of SIRT1 protein in post-mortem lesions.

In conclusion, the analysis of NFkB and SIRT1 proteins showed a quantitative and qualitative different pattern of expression correlated with their time of infliction interval. They represent good candidates in forensic pathology as biomarkers in wound vitality estimation.

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Anatomia clinica e forense, anatomia per immagini, ingegneria tessutale e medicina rigenerativa

Seconda sessione

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Laser micro-anatomical dissection reveals the recovery of mtDNA depletion in the ileum of a Mitochondrial Neuro-Gastrointestinal Encephalomyopathy (MNGIE) patient receiving liver transplant

Elisa Boschetti[1], Irene Neri[1], Leonardo Caporali[2], Roberto D'Angelo[3], Rita Rinaldi[3], Valerio Carelli[1], Roberto De Giorgio[4], Stefano Ratti[1], Lucio I. Cocco[1], Lucia Manzoli[1]

[1] Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy
[2] IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
[3] IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy
[4] Department of Translational Medicine, University of Ferrara, Ferrara, Italy

Introduction: Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an extremely rare disease caused by TYMP mutations, a gene encoding for thymidine phosphorylase (TP). Defective TP fails to convert the nucleosides thymidine and deoxyuridine into respective nucleotides, thus causing accumulation and thereby imbalance of mitochondrial nucleotide pool, impaired mtDNA replication and depletion. Since mtDNA is required to ensure oxidative phosphorylation, metabolically active tissues in MNGIE patients may not achieve sufficient energy production. Permanent tissue replacement of TP, via bone marrow or liver transplant, is nowadays the only life-saving approach that stably reverts the nucleoside imbalance, improving some of the MNGIE clinical features and increasing patients' quality of life. However, the follow-up of patients who have undergone allogeneic hematopoietic stem cell or liver transplantation showed that fatal massive gastrointestinal bleeding can occur even in the transplanted patients, and gastrointestinal abnormalities such as angiopathy, fibrosis and hypoxia, never revert.

Aim: This study aims to elucidate whether the reintroduction of TP after transplant is effective in recovering mtDNA copy number in a normal range.

Methods: Ileal full thickness biopsies were collected from 5 MNGIE (1F; 20-38 years) patients and n=5 CTR (1F; 32-55 years). Formalin fixed-paraffin embedded tissue were sectioned at 10 μm and stained with hematoxylin and eosin. Based on morphologic criteria, each ileal layer (mucosa; submucosal with small vessels deprived of any blood cells; circular muscle layer, myenteric plexus and longitudinal muscle layer) were microdissected and total DNA extracted. mtDNA copy number was assessed using droplet digital PCR in each layer of full thickness ileal samples of a native MNGIE cohort vs. non-MNGIE controls and in a patient pre- and post-TP replacement.

Results: We detected mtDNA depletion in each layer of native-MNGIE ileum. Liver transplant allow recovery of mtDNA copy number in each layer except from mucosa, in which copies tended to increase, but did not reach control values.

Conclusion: This study suggests that a timely TP replacement is needed in order to maximize therapeutic success before irreversible degenerative tissue changes and also to prevent fatal gastrointestinal complications in MNGIE.

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Modulation of post-ischemic myocardial remodelling in a large animal model

Paolo Contessotto[1], Doriana Orbanić[2], Mark Da Costa[3], Chunsheng Jin[4], Peter Owens[5], Niclas Karlsson[4], José Carlos Rodríguez-Cabello[2], Peter Dockery[6], Michelle Kilcoyne[3,7,8], Abhay Pandit[3]

[1] Department of Molecular Medicine, University of Padua, Italy
[2] GIR Bioforge Lab, CIBER-BBN, Universidad de Valladolid, Valladolid, Spain
[3] CÚRAM SFI Research Centre for Medical Devices, NUI, Galway, Ireland
[4] Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden
[5] Centre for Microscopy and Imaging, NUI, Galway, Ireland
[6] Anatomy, School of Medicine, NUI, Galway, Ireland
[7] Advanced Glycoscience Research Cluster, NUI, Galway, Ireland
[8] Microbiology, School of Natural Sciences, NUI, Galway, Ireland

Myocardial infarction (MI) is a major cause of mortality due to ischemic damage to cardiac muscle cells. Given the characteristics of the cardiac extracellular matrix (ECM) post-ischemia, we designed an elastin-based injectable hydrogel by using catalyst-free click chemistry covalent bonding between two elastin-like recombinamers (ELRs) [1]. The proposed hydrogel included functional domains for cell adhesion (RGD) and matrix metalloproteinases (MMPs) cleavage sites which are sensitive to cleavage during the inflammatory phase post-MI. Stromal cell invasion and endothelial cell sprouting within the hydrogel was seen *in vitro*. In addition, we have developed a clinically relevant ovine model of non-transmural infarcts induced by multiple suture ligations to test the efficacy of the ELRs-made hydrogel *in vivo*. Importantly, complete functional recovery of ejection fraction was achieved by intramyocardially injecting the degradable ELRs-hydrogel. Moreover, we observed reduced fibrosis and increased angiogenesis in the ELRs-hydrogel-treated ischemic core region compared to the untreated animals. Thorough validation of the beneficial effect was performed by RNA-seq, proteomic, glycomic, and histological analyses. Finally, we observed that a crucial role was played by GATA4+ cardiomyocytes which survived in the border zone of the infarct. Thus, we propose that our functionalised ELRs-hydrogel favours cardiomyocyte preservation in the border zone by modulating the ischemic core. Remarkably, the functional benefits obtained by the timely injection of the ELRs-hydrogel in a clinically relevant MI model constitutes a leap forward in the field and could be adapted for further clinical translation.

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Skin ageing and inflammation: role of Hyaluronan derived anti-inflammatory molecules on inflammatory mediators' expression

A Donato[1], E Belluzzi[2], R Venerando[1], P Ruggieri[2], V Vindigni [3], P Brun[1]

[1] Department of Molecular Medicine, Histology Unit, University of Padova, Padova, Italy
[2] Department of Surgery, Oncology and Gastroenterology (DiSCOG), University of Padova, Padova, Italy
[3] Department of Neuroscience, University of Padova, Padova, Italy

Background: Inflammation plays an important role in the structural and functional modifications that lead to skin ageing. For this reason, reduction of inflammation, cellular oxidation and dermal extracellular matrix (ECM) alterations may prevent the ageing process.

Aim: to investigate the expression of pro-inflammatory markers and ECM molecules in human dermal fibroblasts derived from young and middle-aged women and the effects of a lactose-modified chitosan (Chitlac®, CTL), alone or in combination with mid-MW hyaluronan (HA), on inflammatory mediators

Materials and Methods: Skin biopsies taken from women of different ages were used to analyse the expression of pro-inflammatory cytokines (by immunohistochemistry) and in *in vitro* model of inflammation was used to assess changes in cell viability (MTT test), pro-inflammatory mediators, metalloproteinases (MMPs) and ECM molecules expression at gene and protein level (qPCR and Elisa assay) or intracellular ROS generation in dermal fibroblast cultures obtained from skin of women with different ages.

Results: The expression of pro-inflammatory molecules was age-related in both skin tissue and inflamed dermal fibroblast cultures. CTL, HA and their combinations counteracted the oxidative damage, stimulating the expression of ECM molecules and, when added to inflamed cells, restored the baseline levels of IL1-β, TNFα, GAL-1, GAL-3 and MMP-3.

Conclusions: HA and CTL mixture attenuated macrophage-induced inflammation, inhibited MMP-3 expression, exhibited anti-oxidative effects and exerted a pro-regenerative effect on ECM.

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Expression studies on MAGI2 in the renal glomerulus and its regulation in different glomerulopathies

Eleonora Hay[1,2], Florian Siegerist[1], Maximilian Schindler[1], Sophia-Marie Bach[1], Antonio De Luca[2], Giovambattista Capasso[3,4], Christos Chatziantoniou[5], Christos Chadjichristos[5], Thorsten Wiech[6], Nicole Endlich[1]

[1] Department of Anatomy and Cell Biology, University Medicine Greifswald, Greifswald, Germany
[2] Department of Mental and Physical Health and Preventive Medicine, Section of Human Anatomy, University of Campania "Luigi Vanvitelli", Naples, Italy
[3] Biogem: Research Institute of Molecular Biology and Genetics Institute, Ariano Irpino, Italy
[4] Department of Translational Medical Sciences, University of Campania "L Vanvitelli", Naples, Italy
[5] UMRS 1155, INSERM, Sorbonne Université, Paris, France
[6] Institute of Pathology, Nephropathology Section, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Background: Interdigitating podocyte foot processes are an essential part of the kidney filtration barrier. They form highly specialized cell-cell contacts that are ultrastructurally described as a slit diaphragm (SD). The SD is composed of a multiprotein complex that contains features of adherens as well as tight junctions which link the large extracellular domain of nephrin to the cytoskeleton. One tight junction-associated protein that has been shown to be localized within the SD complex is MAGI2 (membrane-associated guanylate kinase inverted 2), which is specifically expressed in podocytes.

The aim of this work was to study the expression of MAGI2 in glomerular diseases and to evaluate whether a different regulation of this protein occurs in different glomerulopathies. Additionally, we wanted to investigate the influence of MAGI2 and nephrin on their relative localization.

Methods: We investigated the Magi2 regulation in three different rodent models of glomerulopathy as well as in the genetic focal segmental glomerulosclerosis (FSGS)-like zebrafish model. MAGI2 expression was evaluated in kidney biopsies of patients affected by minimal change disease (MCD), primary and secondary focal segmental glomerulosclerosis in comparison with healthy controls. Immunostaining was performed and slides were imaged using confocal laser scanning and super-resolution 3D-structured illumination microscopy (3D-SIM). Filtration slit density (FSD) was determined on human biopsies by PEMP (**p**odocyte **e**xact **m**orphology **m**easurement **p**rocedure) [1] and MAGI2/Nephrin ratio measurements were performed with a customized macro for the ImageJ-based platform FIJI. Using LC-MS/MS, RNA sequencing, and Taqman RT-qPCR, expression studies were performed in murine glomeruli freshly isolated or *in vitro* de-differentiated over time (Glom*Assay*) [2]. To evaluate the coregulation of Magi2 and nephrin in the zebrafish model, the respective orthologues (*nphs1* and *magi2a*) were knocked-out using CRISPR/Cas9. Transmission electron microscopy was used to investigate the effects of the knockout from an ultrastructural point of view. The expression of these two proteins in both strains was analyzed by immunofluorescence staining and proteinuria was assessed using an automated screening approach.

Results: MAGI2 was expressed in a linear pattern along the filtration slit and colocalized with nephrin in both healthy filtration slit and effaced areas regardless of the species (human, mouse, rat and zebrafish). Magi2 was uniformly down-regulated in animal models of glomerulopathy as well as in de-differentiated podocytes. A reduction of MAGI2 was found in human biopsies of patients suffering from primary FSGS, but not in MCD and secondary FSGS. Transmission electron microscopy revealed podocyte foot process effacement in both CRISPR/Cas9 zebrafish knockout models, while the morphology of the glomerular basement membrane and glomerular endothelial cells was well preserved. Additionally, Nphs1 expression was signifi-

cantly reduced in *magi2a*-KO larvae, whereas interestingly Magi2a expression was unchanged in *nphs1*-KO larvae.

Conclusions: Here, we have demonstrated that the expression of MAGI2 in the glomerulus is associated with the expression of nephrin in all the species investigated. By using knockout larvae, we showed that Nphs1 expression is dependent on Magi2a and not vice versa. MAGI2 is downregulated in glomerular diseases, presumably as a consequence of podocyte dedifferentiation. Finally, the expression of MAGI2 seems to be specifically regulated depending on the podocytopathy investigated, suggesting a role for this protein to distinguish between different glomerulopathies.

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Piezo1/2 mechanosensors in the infrapatellar fat pad-synovial membrane anatomo-functional unit: microscopic distribution of osteoarthritis pain-implied receptors

Elena Stocco[1], Aron Emmi[1], Rafael Boscolo-Berto[1], Martina Contran[1], Elisa Belluzzi[2,3], Marta Favero[4,5], Roberta Ramonda[2], Andrea Porzionato, Pietro Ruggieri[3], Raffaele De Caro[1], Veronica Macchi[1]

[1] Department of Neuroscience, Section of Human Anatomy, University of Padova, Padova, Italy
[2] Musculoskeletal Pathology and Oncology Laboratory, Department of Surgery, Oncology and Gastroenterology (DiSCOG), University of Padova, Padova, Italy
[3] Orthopedics and Orthopedic Oncology, Department of Surgery, Oncology and Gastroenterology (DiSCOG), University of Padova, Padova, Italy
[4] Rheumatology Unit, Department of Medicine-DIMED, University—Hospital of Padova, Via Giustiniani, 2, Padova Italy
[5] Internal Medicine I, Cà Foncello Hospital, Treviso, Italy

The Infrapatellar Fat Pad (IFP) is a fibro-adipose tissue of the knee recently reconsidered as part of a single anatomo-functional unit (AFU) together with the synovial membrane (SM). Interestingly, several evidences highlight the AFU role in those mechanisms triggering and perpetuating the onset/progression of osteoarthritis (OA) disease; however, its contribution to OA-associated pain has been only supposed and strong experimental evidences are lacking. Within this scenario, the recent discovery of the mechanoceptive Piezo ion channels (i.e., Piezo1 and Piezo2) in mammals and consciousness on their role in mediating both mechanoceptive/inflammatory stimuli could shed some light on knee OA pain. For this purpose, the IFP-SM AFUs of both healthy donors (non-OA IFP-SM AFUs, n=10) and OA patients (OA IFP-SM AFUs, n=10) were processed by histology and immunohistochemistry. After the attribution of a histopathological score to IFP-SM AFUs to confirm intrinsic differences between the two groups, the mechanosensors Piezo1 and Piezo2 were investigated for their specific presence and localization/distribution pattern. In addition, immunostaining for detection of monocytes/macrophages (CD68), peripheral nerve endings (PGP9.5) as well as neoangiogenesis signs (YAP1) also occurred for a broad tissue characterization. According to the study gathered data, here it was possible to provide for a better description of the IFP-SM AFU microscopic features in both healthy and pathological conditions, highlighting peculiar differences in the study cohort. Specifically, immunopositivity towards Piezo1/2, CD68 and YAP1 markers was detected at vessels level in the OA- IFP-SM AFUs compartments, differently from the non-OA-group. A correlation with pain was also inferred, thus suggesting for new and effective targets in OA management.

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Correlation between intestinal dendritic cells and neuroendocrine system in Inflammatory Bowel Diseases

Simona Pergolizzi[1], Alessio Alesci[1], Eugenia Rita Lauriano[1], Giacomo Picciolo[2], Debora Di Mauro[2], Alba Migliorato[2], Angelo Favaloro[2]

[1] Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Viale Ferdinando Stagno d'Alcontres, 31 - 98166, Messina
[2] Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali, Policlinico Universitario "G Martino", Viale Gazzi – 98125, Messina

Crohn's disease (CD) and Ulcerative colitis (UC) are the most common forms of inflammatory bowel diseases (IBDs) (Yang et al., 2020), affecting people around the world. The pathogenesis of both CD and UC involves genetic factors, changes in the gut microbiome, and activated immune cells including macrophages, dendritic cells (DCs), and T helper (Th) cells which play an important role in the development and in the maintenance of the diseases. Intestinal DCs are the major source of proinflammatory mediators, including cytokines, reactive oxygen, and acts as key cells in the induction and maintenance of chronic inflammation in IBDs. It has been described an increment of number in DCs colonic mucosa of patients with CD and UC. Serotonin (5-hydroxytryptamine, 5-HT) is both a classical neurotransmitter regulating sleep, appetite, mood, and a vasoactive amine participating in many other organ systems such as in intestinal motor and secretory function and has significant effects on inflammation and immunity (Wu et al., 2019). In recent studies, it has been claimed that antigen-presenting cells (APCs), mast cells, and lymphocytes are able of synthesizing and/or releasing neurotransmitters such as dopamine, 5-HT, glutamate, and ACh (Pergolizzi et al, 2021). Paraformaldehyde-fixed intestinal tissues, obtained from the stricture sites of patients with CD and UC were analyzed by immunostaining for Langerin/CD207, serotonin and vesicular acetylcholine transporter. As controls, unaffected (normal) intestinal biopsies were also investigated. The aim of this study was to characterize the human gut dendritic cells of CD and UC patients, with Langerin/CD207 that is a c-type lectin expressed by different types of DCs, and to verify the co-expression of serotonin and vesicular acetylcholine transporter, demonstrating the link between cells, gut enterochromaffin cells or autonomic nerves in immune activation and generation of intestinal inflammation. This study confirms the significant role of dendritic cells in human intestinal mucosa supporting the hypothesis that the abnormal immunological reaction plays an important role in the pathogenesis of IBDs. The increased knowledge of the cholinergic immune cell system may be useful in the development of therapeutic strategies for the treatment of gastrointestinal disorders.

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Fascial alterations in the diabetic foot: an Ultrasound Imaging study

Carmelo Pirri[1], Carlo Biz[2], Veronica Macchi[1], Raffaele De Caro[1], Carla Stecco[1]

[1] Department of Neurosciences, Institute of Human Anatomy, University of Padova
[2] Orthopedics and Orthopedic Oncology, Department of Surgery, Oncology and Gastroenterology (DiSCOG), University of Padova

Background: The diabetic foot, in its various forms, represents a growing problem in the general population and a reason for great medical and social expenditure due to the disability it can entail. Charcot foot represent a severe form of diabetes' podiatry complications. Diabetic neuropathy, peripheral vasculopathy, susceptibility to infections and anatomical-histological alterations of soft tissues and bones contribute most of all to the development of progressive deformities that alter the biomechanics and functionality of the foot [1]. Increasing attention to this complication of diabetes has already expanded to the study of soft tissues and of the plantar fascia.

Objective: To evaluate by ultrasonography the thickness of the crural fascia and the plantar fascia in different predetermined locations in patients with Charcot neuro-arthropathy. To correlate the results with clinical parameters of the diabetic patient: diabetes type and years of diabetes diagnosis, BMI, ABI, NSS, NDS, SF-12, retinopathy, nephropathy. To compare the results with a control group of healthy subjects.

Materials and Methods: We collected anamnestic data of enrolled diabetic patients and healthy volunteers. We assessed the Ankle-Brachial Index (ABI) and the Neuropathy Disability Score (NDS). Then the patients filled in the surveys SF-12 and Neuropathy Symptoms Score (NSS). We performed a series of ultrasound scans of the foot and leg of healthy subjects and of diabetic patients with Charcot's foot. We measured; the thickness of the plantar fascia near its calcaneal insertion and in near its distal insertion; the thickness of the crural fascia in the anterior and posterior portions, each in two different points.

Results: The results of the ultrasound imaging measurements of Charcot patients showed a statistically significant positive linear correlation ($p<0.05$) between the thickness of the posterior compartment of the crural fascia and the years of diabetes. At the level of the lateral gastrocnemius, the thickness of the crural fascia was also positively correlated in a relevant way to the value of the Neuropathy Disability Score ($r=0.5779$, $p=0.0008$). In patients with Charcot's foot, compared to healthy controls, it was found a statistically significant thickening of: crural fascia, at the level of the medial gastrocnemius ($p=0.03$); crural fascia, at the level of the lateral gastrocnemius ($p=0.03$); plantar fascia, at the level of the calcaneal insertion ($p <0.0001$); plantar fascia, at the level of the middle third ($p <0.0001$).

Conclusions: the study carried out shows that the plantar fascia is not the only fascial structure to be altered in Charcot's foot. The incision of the crural fascia, thickened and rigid, during the release of the muscle-tendon junction of the gastrocnemius could give an additional explanation for the effectiveness of this surgery technique in reducing the equine attitude of the ankle induced by the shortening of the Achille's tendon. The fact that changes in fascial thickness appear to be early findings in the natural history of diabetic disease combined with the fact that such thickening appears to be directly related to the development of diabetic complications make ultrasonography to be considered as a potential diagnostic test in the follow-up of the Charcot foot patient. also consider its accuracy, reproducibility, availability, speed and non-invasiveness.

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Proinflammatory psoriatic cytokines early affect tight junction molecular composition in 3D organotypic cultures of normal human skin

Federica Riva[1], Jasmine Pusceddu[1], Silvia Orio[2], Franz Baruffaldi Preis[3], Francesca Prignano[4], Elena Donetti[2]

[1] Dipartimento di Sanità Pubblica, Medicina Sperimentale e Forense, Unità di Istologia ed Embriologia generale, Universita` degli Studi di Pavia
[2] Dipartimento di Scienze Biomediche per la Salute, Universita` degli Studi di Milano
[3] Centro Ustioni e Chirurgia Plastica Ricostruttiva, ASST Grande Ospedale Metropolitano Niguarda, Milano
[4] Dipartimento di Scienze della Salute, Sezione di Dermatologia, Universita` degli Studi di Firenze

Thanks to its unique anatomical position and the peculiar 3D architecture, the human epidermis represents not only an efficient physical/chemical barrier, but also an immunological shield against potential incoming pathogens in the ever-changing environment. A pivotal role in the physical barrier is played by tight junctions (TJs), i.e. claudin -mediated intercellular attachment structures, localized in the upper granular layer. A barrier impairment associated to an alteration in TJ proteins is described in psoriasis, but the specific contribution due to proinflammatory cytokines is not elucidated yet. In the present study we investigated by immunofluorescence analysis the modulation of the expression of claudin 1 (CLDN-1), a transmembrane integral membrane TJ protein, and of the scaffold plaque protein Zonula Occludens 1 (ZO-1) after the incubation with interleukin (IL)-17 (50 ng/ml), IL-22 (100ng/ml), IL-23 (50ng/ml), tumornecrosisfactor (TNF)-alpha (100ng/ml) aloneorincombination (MIX) for 24 (T24) and 48 (T48) hours. We considered as experimental model the standardized 3D organotypic cultures of normal human skin (n=7)[1-3]. In controls, CLDN-1 immunopositivity increased from the basal layer upwards, but its expression was early reduced in the basal and suprabasal layers in all experimental groups at T24. Throughout the epidermal layer, TNF- alpha induced the strongest inhibition of CLDN-1 distribution, while only IL-23 up-regulated its expression in the granular layer. At T48, only skin samples incubated with MIX showed a homogeneous decrease of CLDN-1 in all the epidermal compartment and IL-23 and TNF-alpha did not affect CLDN-1 expression.

At T24, ZO-1 expression in control samples increased gradually, starting from the basal layers towardstheepidermalsurfaceandwasheterogeneouslyaffectedinalltheexperimentalgroups. Interestingly, at T48, the incubation with all cytokines, alone or in combination, promoted an increase of ZO-1 immunopositivity in the basal and suprabasal layers.

The present results strongly suggest that the i) broadening of ZO-1 expression and ii) the downregulation of CLDN-1, i.e. a typical feature of psoriasis, can be induced as early as 24 hours, in a time- and cytokine-dependent manner. These observations give further insights into the early processes leading to the formation/progression of psoriatic plaques.

To complete this study, parallel experiments are ongoing on calcium-differentiated human primary keratinocytes.

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Testing the angiogenic potential of 3D printed scaffolds *in ovo*: an ethical, economical and eco-friendly solution for critical-size bone defects

Virginia Stanzani[1], Alberto Portone[2], Marta Checchi[1], Elena Veronesi[2,3], Serena Truocchio[4], Marzia Ferretti[1], Alberto Ferrari[2,5], Carla Palumbo[1]

[1] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Via del Pozzo 71, 41124 Modena, Italy
[2] Scientific and Technological Park of Medicine "Mario Veronesi", Via 29 maggio 6, 41037 Mirandola, Italy
[3] Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, Via del Pozzo 71, 41124 Modena, Italy
[4] Biomedical and Neuromotor Sciences - DIBINEM - Alma Mater Studiorum - Università of Bologna, Italy
[5] Department of Engineering "Enzo Ferrari", University of Modena and Reggio Emilia, Via P Vivarelli 10, 41125 Modena, Italy

In the last years, Additive Manufacturing (AM) technologies has paved the way to one of the greatest scientific progresses in medicine: the possibility of printing patient-customized tissues with peculiar geometries and highly personalized shapes. Conditions such as critical-size bone defects still represent an unmet need and one of the major challenges in the clinical scenario in the field of Bone Tissue Engineering (BTE). In order to repair successfully a non-self-healing damage, the implanted scaffolds must promote both angiogenesis and osteogenesis, but the present approaches have mainly focused by mostly of researchers on the osteogenic potential of the materials, relegating angiogenesis to a secondary role. Instead, vascularization is essential not only for bone formation, but also for a successful bone healing, and the development of biomaterial should consider preliminarily the angiogenic response other than an adequate pore structure, a high biocompatibility and good mechanical properties. For this reason, we exploited the chick chorioallantoic membrane (CAM) assay to evaluate the angiogenic response of 3D printed scaffolds through an extrusion-based technique. This simple, economic and well-known model, revisited in an ethical key (since sustains the 3Rs rule), allowed us to select the chemical composition, the internal architecture and the external geometry of the scaffolds with the higher angiogenic potential, overcoming the limitations of 2D cell cultures and animal experimentation[1]. In fact, the first objective of the study was to select the 3D scaffold composition/geometry that could have the best impact onto the CAM in terms of vascular response and the consequent regenerative ability to recover critical-size bone defects. The second goal was to promote an ethical, economical and eco-friendly solution to address BTE issues, by means of the scleral ossicles (SOs) and their derivatives (Pal-OS powder), subjects of patent application and trademark filing, respectively. Specifically, SOs are naturally decellularized bone segments that are located in the eye-bulge of lower vertebrates, such as birds, and could be extracted at zero cost from poultry waste. Since they have displayed outstanding angiogenic and osteogenic and properties both *in vitro* and *in vivo*[2], the final aim was to 3D print through the same extrusion-based technique the SOs in the form of powder, mixed with tunable materials such as hydrogels. In conclusion, the thus produced scaffolds were tested *in ovo* to evaluate if their angiogenic ability was maintained or enhanced by means of different geometries and the inclusion in other biomaterials.

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Anatomia clinica e forense, anatomia per immagini, ingegneria tessutale e medicina rigenerativa

Terza sessione

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Cancer-nerve Crosstalk in Human Cholangiocarcinoma

Valerio de Franchis[1], Simonetta Petrungaro[1], Enrico Mandolini[1], Francesca Somma[1], Elena Pompili[1], Antonio Filippini[1], Eugenio Gaudio[1], Cinzia Fabrizi[1], Claudia Giampietri[1]

[1] Department of Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza University of Rome, Rome, Italy

Cholangiocarcinoma (CCA) is a tumor with high tendency to infiltrate nerves. Recent studies highlighted a key role of Schwann cells (SC) in cancer progression. This aspect is still uninvestigated in CCA

We observed through a 3D model of perineural invasion the neurotropism of a CCA cell line (HuCC-T1), towards sciatic nerve explants, while no migration was observed using the cholangiocyte controls (H69).

Migration and invasion of HuCC-T1 is fostered by the SC conditioned media. Neither the HuCC-T1 nor the H69 control produce factors capable of modulating neuritogenesis in PC12.

Western blots performed on HuCC-T1 cells incubated for 48h with conditioned media from SC show a downregulation of E-Caderin indicative of epithelial-mesenchimal transition (EMT) and an upregulation of the proliferating nuclear antigen (PCNA) as compared to the controls.

Our data indicate that SC may regulate EMT, migration, invasion and proliferation in Cholangiocarcinoma.

we will further investigate this phenomenon by looking for potential mechanisms and molecular pathways involved

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Histopathology of human obese adipose organ

Angelica Di Vincenzo[1], Jessica Perugini[1], Eleonora Di Mercurio[1], Tonia Luca[2], Mario Tomasello[2], Giovanni Lezoche[1], Mario Guerrieri[1], Fabio Malavasi[3], Armando Gabrielli[4], Patrizia Sabatelli[5], Luciano Merlini[6], Antonio Giordano[1], Sergio Castorina[2] and Saverio Cinti[1]

[1]Dpt Medicina Sperimentale e Clinica, UNIVPM, AN
[2] Dpt Scienze Mediche, Chirurgiche e Tecnologie Avanzate UNICT, CT and "GB Morgagni" Mediterranean Foundation, CT
[3] Dpt Scienze Mediche UNITO e Centro Ricerca Medicina, Sperimentale (CeRMS) and Fondazione Ricerca Molinette Onlus, TO
[4] Dpt di Scienze Cliniche e Molecolari UNIVPM, AN
[5] CNR, Istituto di Genetica Molecolare, BO Laboratorio di Biologia Cellulare Muscolo-Scheletrica, IOR-IRCCS, BO
[6] Clinica del Muscolo, Ospedale Villa Erbosa, Gruppo San Donato BO

Since 2003 there is a large consensus on the fact that obese adipose tissues are inflamed. This low-grade chronic inflammation is sustained mainly by macrophages and is linked to insulin resistance (1, 2). Data suggest: insulin resistance induces pancreatic beta cells hyperactivity, but a progressive increase of noradrenergic nerve fibers in endocrine pancreas induces an insulin secretory decline and type2 diabetes (T2D) (3,4). In 2005 we showed that inflammation is due to death of obese adipocytes that are reabsorbed by macrophages forming characteristic histopathologic figures called crown-like structures (CLS) (5).

Visceral adipocytes are smaller than subcutaneous adipocytes and a positive correlation exists between size of adipocytes and number of infiltrating macrophages, thus a higher population of inflammatory cells was expected in subcutaneous fat, but data showed the reverse (6). These data raised the idea that visceral adipocytes are more fragile and die at a lower size than subcutaneous adipocytes, thus inducing a higher level of inflammation in visceral fat than in subcutaneous fat (7). This theory offered an explanation to the well-known clinical data claiming visceral obesity as the condition more frequently linked to the usual obesity-related disorders and mainly to the T2D (8). In 2018 we showed data confirming a lower critical death size for visceral adipocytes (9). In 2013 we showed that obese adipocytes die with a specific mechanism called pyroptosis (10).

Another well-known aspect of obese adipose organ is fibrosis. Several aspects of this pathology need to be clarified. In this work we studied by morphology (including electron microscopy), morphometry, immunohistochemistry and molecular biology, subcutaneous (SC) and omental (OM) biopsies from obese subjects (n 50) undergoing bariatric surgery. Lean patients (n 12) treated by cholecystectomy were used as controls.

A wide range of fibrosis was detected: from a maximum of about 11% of tissue (OM) occupied by Sirius Red-stained fibrous strands to value similar to controls (1-2%). Average value: about 5% (similar, but lower data in SC fat). We than checked the presence on adipocytes of the vital protein perilipin1 and found that a large proportion (about 10-15%) of adipocytes lacked the protein (with positive correlation between OM and SC fat, $p=0.007$) suggesting their death (5, 11). Electron microscopy showed aspects suggesting collagen production by adipocytes, thus we checked collagen production by hMADS (a well-accepted model of human adipocytes in vitro). Data suggest that mature adipocytes produce more collagen (I, III and VI) than normal human dermal fibroblasts (HDFn) especially when they are hypertrophic due to fatty acids addition to the culture system. Interestingly palmitate induced hypertrophic hMADS showed a ColVI reduction compared with mature adipocytes ($p=0.05$). Of note, CD68 immunoreactive macrophages significantly increased in obese fat, but only a minority were involved in CLS. The large majority of macrophages were in fact detected in fibrotic areas with a positive correlation both in OM and SC ($p=0.002$ and $p=0.0001$respectively).

While the increased fibrous collagen (I and III) is widely accepted (12) the amount of the non-fibrillar collagen VI in obese fat is debated (13). Our data showed a reduction in gene expression and the analysis of adipose tissue from two lean patients with a rare mutation inducing a reduction of active collagen VI production (14) showed high levels of fibrosis (2-3 times that observed in obese patients) and adipocytes death comparable with that observed in obese fat suggesting a causative role for this gene.

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Morphological and molecular analysis of the effect of hyaluronic acid on gingival collagen turnover: a split-mouth design study

Sara Meroni[1], Nicoletta Gagliano[2], Mariana A. Rojas[3], Gaia Pellegrini[1], Andrea Pilloni[3], Aurora Cristofalo[1], Claudia Fortunati[1], Claudia Dellavia[1]

[1] Department of Biomedical, Surgical and Dental Sciences, University of Milan, 20133 Milan, Italy
[2] Department of Biomedical Sciences for Health, University of Milan, 20133 Milan, Italy
[3] Department of Oral and Maxillofacial Sciences, Sapienza University of Rome, 00161 Rome, Italy

Background and aim: Wound healing is a complex pathophysiological process orchestrated by a variety of factors. It has been recognized the importance of the first 24 hours in the oral repair process [1]. Hyaluronic acid, a major component of the extracellular matrix (ECM), plays a fundamental role in different biological processes related to tissue repair and regeneration [2]. The aim of the present study was to assess the effect of HA on collagen turnover pathways in the early healing response of human gingiva.

Material and Methods: In this split-mouth design study, 8 patients underwent two surgical periodontal procedures in which sutured margins of vertical releasing incisions (VRIs) were either treated with Hyaluronic acid (HA, intra-surgical and topical application) or left to heal spontaneously (CT) were included. After 24 hours, gingival biopsies were collected at the level of VRIs, and half of the sample was processed for histological analysis with Haematoxylin and Eosin, Sirius red and Masson trichrome. The second half underwent molecular analysis for the evaluation of lysyl oxidase (LOX), matrix metalloproteinase 1 (MMP-1), and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) by Real-Time PCR.

Results: The histological analysis showed, in both experimental conditions, that collagen fibres were arranged in dense bundles extending in all directions, maintaining the physiological organization typical of the irregular dense connective tissue of the gingiva. At polarized light, no signs of newly deposited collagen were detected and the pattern was similar in CT and after HA treatment. LOX and TIMP-1 gene expression resulted significantly up-regulated in HA compared to CT ($p<0.05$). MMP-1 gene expression was not significantly affected.

Conclusions: This study assessed for the first time the in vivo effects of exogenous HA on collagen expression in early wound healing of human gingival tissue. The morphological analysis did not reveal significant differences in CT and HA. Interestingly, the molecular analysis suggests that HA is able to trigger the up-regulation of LOX and TIMP-1 that are responsible, respectively, for collagen cross-linking and collagen breakdown inhibition. Overall, these findings are consistent with the hypothesis that Hyaluronic acid could favour tissue repair in early gingival wound healing.

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The non-neuronal cholinergic system modulates adipocyte inflammation associated with obesity

Jessica Perugini[1], Ilenia Severi[1], Eleonora Di Mercurio[1], Martina Senzacqua[1], Giovanni Lai[1], Saverio Cinti[1], Antonio Giordano[1]

[1] Section of Neuroscience and Cell Biology, Department of Experimental and Clinical Medicine, Marche Polytechnic University, Ancona, Italy

Non-neuronal cholinergic signaling plays an important role in physiological processes, including inflammation and immunity (1). Interestingly, recent evidence demonstrates the presence of a non-neuronal cholinergic pathway in adipose tissue, where immune cells secrete acetylcholine (Ach) to activate beige adipocytes during thermogenesis (2). Obesity is a state of chronic low-grade systemic inflammation that is characterized by increased proinflammatory cytokine secretion from adipose tissue and the infiltration of immune cells, including macrophages, into the white adipose tissue (WAT), forming distinctive crown-like structures (3).

Here we evaluated whether the non-neuronal cholinergic system occurs in obese and inflamed fat and whether macrophages are the cellular players responsible for secreting Ach. By RT-qPCR, we found that all the components of the non-neuronal cholinergic system molecular machinery significantly increased in subcutaneous and visceral WAT from high-fat diet obese mice compared with mice fed a normal diet. By immunohistochemistry and confocal microscopy, we found that white adipocytes expressed butyrylcholinesterase (BChE) and about 40–50% of macrophages infiltrating obese WAT expressed choline acetyltransferase (ChAT), the enzyme for Ach biosynthesis. Interestingly, ChAT specific staining was also found in macrophages infiltrating human obese fat. Thus, to assess the role of Ach in obese WAT we tested its ability to counteract inflammation and insulin resistance in TNFα-treated human white adipocytes. Ach stimulation blunted TNFα-induced inflammation and insulin resistance by reducing the expression of MCP1, IL6 and IL1β and increasing the expression of Glut4 and IRS1. Importantly, Ach was also able to ameliorate the insulin-dependent glucose uptake.

Collectively, these data suggest that a consistent proportion of macrophages infiltrating obese WAT produce and secrete Ach, which in turn exerts anti-inflammatory and insulin-sensitizing effects on human adipocytes. Promoting cholinergic transmission could represent a novel approach to treat obesity and associated diseases

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D_2-H_3 receptor-receptor interactions in the carotid body: a descriptive multispecies study

Elena Stocco[1], Maria Martina Sfriso[1], Silvia Barbon[1], Aron Emmi[1], Diego Guidolin[1], Camillo Di Giulio C[2], Veronica Macchi[1], Raffaele De Caro R[1], Andrea Porzionato[1]

[1] Department of Neuroscience, Section of Human Anatomy, University of Padova, Padova, Italy
[2] Department of Neuroscience, Imaging, and Clinical Sciences, University "G d'Annunzio" of Chieti-Pescara, Chieti, Italy

The carotid body (CB), located at the carotid bifurcation, is the main peripheral arterial chemoreceptor showing a fundamental role in maintenance of O_2, CO_2/H^+, and glucose homeostasis in response to hypoxia, hypercapnia or reduced blood pH. Typically, physiopathological/environmental stimuli trigger the CB's release of neurotransmitters/modulators acting on different receptors, including metabotropic receptors located in CB type I/II cells and afferent nerve fibers. Most metabotropic receptors are G protein-coupled receptors (GPCRs) that function interacting with G-proteins inside the cell, amplifying extracellular signals to produce strong, varied, and cell-specific responses. It is well established that GPGRs can undergo physical receptor-receptor interactions (RRIs), leading to homo/hetero-complexes in a dynamic equilibrium; however, physical proximity (≤10 nm) and colocalization are the fundamental requirements for RRI occurrence. Among the several GPCRs identified in the CB[1], the Dopamine and Histamine receptors D_2R and H_3R are included; however, differently from striatal membrane preparations, their possible heterodimerization has never been demonstrated here. The aim of this work was to verify D_2R and H_3R colocalization in the CB, thus suggesting a possible interplay that in turn may be responsible of specific D_2R-H_3R antagonistic functional implications. The CBs of both Sprague Dawley rats (n=3) and human donors (n=3) were dissected and immunohistochemistry (tubulin βIII and S100 staining for type I and II, respectively; D_2R and H_3R) and *in situ* proximity ligation assay (PLA) occurred. According to our analyses, all the samples showed the presence of positive D_2R/H_3R elements; hence, PLA assay followed by confocal microscopy analysis revealed red clusters surrounding DAPI-stained nuclei, corroborating possible D_2-H_3 RRIs. Additionally, D_2R-H_3R heterodimers were mainly detected in type I cells; however, type II cells involvement cannot be excluded as previously highlighted also for $A_{2A}R$-D_2R heterodimers [2]. RRIs may have a role in CB cells function modulation; clarifying RRIs establishment in the CB may guide towards comprehension of its finely regulated plastic behaviour.

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Reconstruction of mandibular defects: an anatomy and clinical study

Stefano Ratti[1], Alice Marzi Manfroni[1], Lucia Manzoli[1], Achille Tarsitano[1] and Alessandra Ruggeri[1]

[1] DIBINEM (Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy)

Objectives: Mandibular defect is one of the common defects of facial region, which can seriously affect the appearance, chew and speech. Functional reconstruction of mandibular defect is due to restore denture, occlusal function and mandibular bone. Vascularized bone grafting on mandibular defect is an effective method for reconstruction especially in larger defects treatment.

To promote the reconstruction of mandibular defect treatment and improve the quality of reconstruction, in this study was evaluated the possibility of the use of a galeo-pericranial flap, pedicled and free, evaluating the vascular caliber and course of superficial temporal vessels comparing with facial and superior thyroid vessels and its osteogenic potential.

Materials and methods: Ten cadaveric anatomical bilaterally head and neck specimens, obtained by donors, were used to simulate the surgical procedure evaluating the vascular calibers of the temporal and cervical arteries. For the clinical study was used Augmented Reality (AR) navigation in order to obtain three-dimensional image of the vascular pedicle supplying the flap.

Mandibular defects were realized and then replaced using the pedicled flaps. The Ethics Committee for application of the galeo-pericranial free flap to mandibular reconstruction was obtained.

Results and conclusion: The facial arteries seem to have a slightly larger diameter, while there is no clear anatomical evaluation concerning the caliber of cadaveric superior thyroid vessels in literature. Clinical results not demonstrated to have a functional issue at either the recipient or donor site. All patients showed good healing during recovery and follow-up visits with an average follow-up of nine months.

Microsurgical free flap is shown to be a valuable and reliable method in head and neck surgery.

In particular, galeo-pericranial flaps for mandibular reconstruction demonstrated improved functional and morphological outcomes, with higher success rates.

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Normal gut morphology is affected by SARS-CoV-2 infection superimposed on an ileal diverticulum

Mariaconcetta Sicurella[1], Roberta Gafà[2,3], Paola Boldrini[4], Angelina Passaro[2,5], Luca Maria Neri[2,4]

[1] Department of Environmental and Prevention Sciences, University of Ferrara, Ferrara, Italy
[2] Department of Translational Medicine, University of Ferrara, Ferrara, Italy
[3] Oncological and Medical Department, University Hospital of Ferrara Arcispedale Sant'Anna, Ferrara, Italy
[4] LTTA Electron Microscopy Center, University of Ferrara, Italy
[5] Research and Innovation Section, University Hospital of Ferrara Arcispedale Sant'Anna, Ferrara, Italy

The enterocyte gut monolayer is a functional structure characterized by a regular colonnade of cylindric cells that enlarge their adsorbing surface with a huge number of microvilli at the endoluminal side. This functionality is reinforced by ordered junctions between neighbouring cells, to avoid diffusion of nutrients without an ordered absorption.

The COVID-19 disease mainly affects the respiratory tract, with severe fatal consequences attributed to a surge of inflammatory events described as the "cytokine storm" in a significant number of patients [1-4]. However, this disease may affect different apparatus as witnessed by the wide spectrum of clinical manifestations and has been associated with extrapulmonary tropism and organ dysfunction.

Gastrointestinal (GI) symptoms have been recorded in more than 10% of affected patients [5] and the digestive tract may be also affected by SARS-CoV-2.

Human intestinal biopsy tissues were obtained from a COVID-19 patient with a large ileal diverticulum that underwent surgical resection due to massive endoluminal bleeding. A pre COVID-19 patient with a similar ileal diverticulum was used as control.

In COVID-19 samples, transmission electron microscopy disclosed several morphological and functional alterations: microvilli appeared shorter, disorganized, and not homogeneous when compared to the control; the number of mitochondria increased significantly; cell junctions were disorganized or absent and cell borders became tortuous. Alterations in the nuclear structure was also observed, with pyknotic, shrunken nucleus, condensed chromatin mostly localized at the nuclear periphery, typically referred to apoptotic nuclei.

The presence of SARS-CoV-2 in the digestive tract is responsible of relevant changes of the normal ultrastructural morphology of the ileal mucosa.

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Human placental tissue morphology during and after SARS-CoV-2 infection: focus on vascular and inflammatory aspects

Simioni C.[1,2], Gafà R.[3,4], Sanz J. M.[5], Bortolotti D.[5], Rizzo R.[5], Passaro A.[3,6], Neri L. M.[2,3]

[1] Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy
[2] Laboratory for Technologies of Advanced Therapies (LTTA)-Electron Microscopy Center, University of Ferrara, Ferrara, Italy
[3] Department of Translational Medicine, University of Ferrara, Ferrara, Italy
[4] Oncological and Medical Department, University Hospital of Ferrara Arcispedale Sant'Anna, Ferrara, Italy
[5] Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Ferrara, Italy
[6] Research and Innovation Section, University Hospital of Ferrara Arcispedale Sant'Anna, Ferrara, Italy

It is already widely known that SARS-CoV-2 infection is interpreted as a multi-organ disease, and several studies highlighted the relevance of SARS-CoV-2 in inducing vascular injury, also in the placenta [1,2].

SARS-CoV-2 enters human cells via angiotensin-converting enzyme 2 (ACE-2) and, in the placenta, abundance of this receptor on the syncytiotrophoblast and on the decidua section, could potentially contribute to vertical transplacental transmission to the fetus.

The placenta morphological analysis was carried out on the following patient cohort: women with running COVID-19 infection at delivery or women healed from a COVID-19 infection (COVID-19 negative at delivery). Samples were studied by Immunohistochemistry (IHC) and Transmission Electron Microscopy (TEM). Spike protein, ACE-2, the glycoprotein Basigin (CD147), the Vascular Endothelial Growth Factor (VEGF) and the CD68 marker were evaluated.

Virus-induced inflammation was documented by increased macrophage infiltration into tissue, as observed by expression of the CD68 marker. Interestingly, the infiltration was more pronounced in post-infection condition, thus suggesting the hypothesis of long Covid disease involving also placenta. Moreover, by IHC it was observed an increased tissue vascular damage, suggesting also an altered maternal vascular system.

Additionally, the expression and localization of the autophagic marker LC3B with viral presence has been detected and shown by Multiplexed Immunohistochemical Consecutive Staining on Single Slide (MICSSS).

This evidence may provide important information, underpinning the scientific literature, on the impact that SARS-CoV-2 may have on the placenta and on possible mother-to-fetus transmission, involving both the vascular and inflammatory aspects.

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Cryopreserved versus decellularized trachea: from anatomy to translational medicine

Elena Stocco[1,2], Silvia Barbon[1,2], Marco Mammana[2,3], Alex Pontini[4], Giovanni Zambello[3], Giulia Zivelonghi[1], Anna Rambaldo[1], Diletta Trojan[5], Alice Bianchin[5], Francesca Favaretto[5], Alberto Elmi[6], Domenico Ventrella[6], Fiorella Calabrese[7], Maria Laura Bacci[6], Veronica Macchi[1,2], Maria Teresa Conconi[8], Federico Rea[2,3], Raffaele De Caro[1,2], Andrea Porzionato[1,2]

[1] Department of Neuroscience, Section of Human Anatomy, University of Padova, Padova, Italy
[2] Life Lab Program, Consorzio per la Ricerca Sanitaria, Padova, Italy
[3] Thoracic Surgery and Lung Transplant Unit, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Padua, Italy
[4] Burn Unit, Plastic Surgery, Padua University Hospital, Padua, Italy
[5] Fondazione Banca dei Tessuti di Treviso Onlus, Treviso, Italy
[6] Department of Veterinary Medical Sciences, University of Bologna, Ozzano Dell'Emilia, Italy
[7] Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Italy
[8] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy

Long-term airway stenting is the only established treatment option in patients affected by significant tracheal lesions; however, this strategy is not free from drawbacks (life quality deterioration, infection, stent migration). Tracheal replacement, by the adoption of airway substitutes, has been proposed as an alternative approach: currently allogenic cryopreserved segments are promising but the effect of cryopreservation on antigen presenting cells remains controversial. In parallel, engineered substitutes based on decellularization are also intriguing but identification of an extracellular matrix (ECM)-respectful method is a challenge. Within this scenario, the aim of this study was to develop and characterize a newly pig-derived acellular trachea which was compared to the cryopreserved counterpart. The fresh tissue was also included as control group. Tracheal segments of 4 cm in length were processed by a *physical+enzymatic+chemical* method based on lyophilization, osmotic shock in deionized water (dH_2O), DNase, trypsin + EDTA and Tergitol™. In parallel, the cryopreserved samples were prepared according to a well-established protocol run by the "Fondazione Banca dei Tessuti Treviso (FBTV)". Once proved the decellularization method efficiency by histological analysis and DNA quantification assay (<50 ng/mg of tissue), each ECM (native/cryopreserved/decellularized) was broadly characterized for presence/maintenance of tissue specific proteins (histology, second harmonic generation (SHG) microscopy), and ultrastructure (Scanning Electron Microscopy (SEM)); hence, glycosaminoglycans (GAGs) content was also determined. To assess absence of cytotoxicity, the extract-test assay was performed in vitro using human bone marrow-derived mesenchymal stem cells (HM1SV40); in parallel, heterotopic implant in BALB/c mice occurred to prove in vivo cryopreserved/decellularized ECMs biocompatibility (end point 15 days; immunohistochemical analyses). Finally, orthotopic positioning of the grafts in swine animal model of disease was attempted (window-like defect, 3 tracheal rings). According to the study evidences, the decellularization protocol effectively removed the immunogenic material after 12 decellularization cycles (void lacunae, no respiratory epithelium cells) while preserving cartilage ECM architecture; cryopreservation led to the maintenance of a native-like cartilage ECM, with well identifiable chondrocytes' nuclei and an epithelium with a partially compromised integrity. Focusing on ECM, histological and SEM analyses showed a preserved structural/ultrastructural organization and collagen content in the cartilaginous compartment of the decellularized tracheas; conversely, the GAGs appeared significantly reduced, as also proved by the biochemical assay. Evaluation of eventual cytotoxicity confirmed no toxic effect on cells in vitro, as also corroborated by subcutaneous implants analysis, showing no significant inflam-

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matory reaction triggered by the tracheas (cryopreserved/decellularized). Finally, it was possible a successful heterotopic positioning of the cryopreserved/decellularized scaffolds; in particular, no fracture/rupture of the decellularized grafts occurred while suturing, thus suggesting their good manipulability despite a slightly lower stiffness versus the cryopreserved counterpart. Tracheal replacement by decellularized supports may represent a promising option in clinical practice; however, a fine research on the most adequate surgical technique to assure for graft re-vascularization is required to guarantee for grafts survival and optimal integration in the defect site.

Stili di vita e prevenzione: scienze del movimento, della nutrizione e del benessere

Prima sessione

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Vitamin D cytoprotection in human liver cell lipotoxicity and its transcriptomic fingerprint: a comparison of natural and synthetic formulations

Desirée Bartolini[1], Linda Zatini[2], Giada Marcantonini[2], Anna Migni[2], Anna Maria Stabile[1], Tiziana Frammartino[3,4], Angela Guerrini[3,4], Stefano Garetto[3,4], Jacopo Lucci[3,4], Alessandra Pistilli[1], Gabriele Di Sante[1], Francesco Galli[2] and Mario Rende[1]

[1] Department of Medicine and Surgery, Section of Human, Clinical and Forensic Anatomy, University of Perugia, Perugia, Italy desireebartolini@unipgit (DB)
[2] Department of Pharmaceutical Sciences, University of Perugia, 06126 Perugia, Italy
[3] Natural Bio-Medicine SpA, Loc Aboca 20, Sansepolcro, AR, Italy
[4]Innovation & Medical Science Division, Aboca SpA Societa` Agricola, Loc Aboca 20, Sansepolcro, AR, Italy

Vitamin D (VD) is considered to have a fundamental role in promoting bone health by increasing gut calcium absorption and in the maintenance of serum calcium and phosphate concentration [1]. In addition to calcium/phosphate metabolism and bone homeostasis regulation, VD has antiproliferative, anti-inflammatory and antifibrotic properties. These protective actions may have an impact in the progression of chronic liver disease including NAFLD (Non-alcoholic fatty liver disease) [2].

The aim of this study is to assess the cytoprotection function and the corresponding transcriptomics fingerprint of VD (10 nM) in human hepatocytes (HepaRG) exposed to steatosis and lipotoxicity by means of oleic and palmitic acids (OA-PA) supplementation. A natural source of vitamin D (a Shiitake Mushroom extract; SM-VD2) was compared with a synthetic form (VS-D3) and the active metabolite ($1,25(OH)_2D$) of the vitamin in protecting and the presence of lipotoxicity was assessed by the cellular production of the reactive oxygen species (ROS) and induction of specific transcriptomic changes.

Cell viability data demonstrated that the *in vitro* model of steatosis produced conditions of sub-maximal lipotoxicity and cellular damage. Transcriptional modifications indicated the modulation of genes associated with long-chain fatty acid β-oxidation, ROS production, cholesterol synthesis, AMPK activity, and hepatocyte apoptosis, liver fibrosis and damage. the different VD formulations showed similar efficacy in improving both the levels of steatosis and ROS. However, formulation-specific modifications of the cellular transcriptome were observed. Transcriptional fingerprints and biological functions identified by "Ingenuity Pathway Analysis" (IPA) showed higher similarities when the mushroom extract was compared with 1,25(OH)2-D metabolite than VS-D3. In conclusion, VD showed efficient protection against lipotoxicity in HepaRG cells. Comparable cytoprotection activity was observed for the natural and synthetic formulations, but transcriptomics data highlighted some specificities in the mechanism of action.

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Histological assessment of lipid deposition in tissues from genetically modified mice with deficiency or overexpression of apolipoprotein A-I

Elsa Franchi[1], Giulia Chiesa[1], Stefano Manzini[1], Alice Colombo[1], Marco Busnelli[1]

[1] Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milano, Italy

The reverse cholesterol transport is a multistep process whereby excess cholesterol is transported by HDL from the peripheral tissues to the liver for excretion. In this study, the impact of genetic manipulation of HDL/apoA-I levels on lipid deposition in liver and kidney was investigated.

Mice with extremely low plasma HDL levels, deficient for both murine apoA-I and apoE (DKO), were compared with mice characterized by elevated HDL, deficient for both apoA-I/apoE, but overexpressing human apoA-I (DKO/hA-I). Mice, both female and male, were fed a standard rodent diet until one year of age. Plasma lipids were quantified by enzymatic methods. Liver and kidney morphology was evaluated by light microscopy on frozen sections.

Plasma total cholesterol concentration in DKO mice was comparable with that of wild type mice and 3-fold lower than that observed in DKO/hA-I mice. Plasma HDL-C was almost absent in DKO mice and strongly elevated in DKO/hA-I mice. The H&E-stained sections did not reveal the presence of steatosis in liver parenchyma as well as of foam cells in renal glomeruli of both genotypes. The neutral lipid-specific staining with Oil Red O showed instead interesting differences. In the hepatic parenchyma, an increased accumulation of lipids around the centrilobular vein was observed only in DKO/hA-I mice. In addition, within the glomeruli of DKO/hA-I mice, lipid accumulation was significantly higher than in DKO, both in females and males.

Although DKO mice are almost completely devoid of HDL and prone to atherosclerosis development, they do not exhibit steatosis or other signs of abnormal lipid accumulation in the liver and do not develop glomerular lipidosis.

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L'esposizione *in utero* al cannabinoide JWH-133 compromete la durata della vita riproduttiva della prole femminile

Paola Grimaldi[1], Elisa Innocenzi[1], Alice Zucchi[1], Ambra Colopi[1], Susanna Dolci[1]

[1] Dipartimento di Biomedicina e Prevenzione, Sezione di Anatomia Umana, Universita` di Roma Tor Vergata, Italia

L'uso di cannabis durante la gravidanza è associato a esiti neonatali negativi, come nascita pretermine, ritardo di crescita intrauterino, basso peso alla nascita e alterazioni dello sviluppo neurologico (1-2). Tuttavia, è ancora poco noto l'impatto sulla linea germinale dei nascituri. La comprensione dell'effetto dell'esposizione alla cannabis durante la gravidanza sulle gonadi della prole ha un'importanza rilevante, poiche' qualsiasi alterazione delle cellule germinali potrebbe essere trasferita alle generazioni successive (3). La cannabis agisce attraverso due tipi di recettori: CB1 e CB2. Abbiamo precedentemente dimostrato che gli ovociti fetali esprimono il recettore CB2 a un livello superiore rispetto al CB1. Il trattamento *in vitro* di ovociti fetali con JWH-133, un agonista selettivo di CB2, induce un'accelerazione dell'ingresso e della progressione meiotica. L'accelerazione della meiosi è accompagnata da un forte aumento della percentuale di cellule γ-H2AX-positive, parallelamente a un aumento delle cellule TUNEL-positive, suggerendo un esteso processo apoptotico. Ora dimostriamo che l'esposizione *in vivo* alla droga JWH-133 durante la vita fetale, attraverso la somministrazione del farmaco a femmine gravide, causa una significativa riduzione del numero di follicoli primordiali e primari nelle ovaie delle neonate (4).

In eta` adulta, le femmine esposte *in utero* presentano una ridota riserva ovarica che si traduce in una riduzione prematura e precoce della vita riproduttiva.

Punti conclusivi rilevanti:

A) il recettore cannabinoide CB2 è un induttore pro-meiotico dello sviluppo degli ovociti;
B) l'esposizione *in utero* al cannabinoide JWH-133, agonista del recettore CB2, compromette lo sviluppo del pool di follicoli nell'ovaio della prole;
C) le femmine adulte esposte all'agonista del recettore CB2 nella vita fetale mostrano una ridota riserva ovarica e una durata della vita riproduttiva piu` breve.

Rilevanza traslazionale: il consumo di marijuana durante la gravidanza puo` potenzialmente compromettere la riserva ovarica e ridurre la durata della vita riproduttiva delle femmine esposte *in utero*.

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Sagittal whole body posture during walking assessed by 3D gait analysis in healthy population

Angela Lucariello[1], Allegra Fullin[2], Daniele Di Tella[3], Angelica Perna [3], Germano Guerra[3], Antonio De Luca[2], Paolo De Blasiis[2]

[1] University of Naples "Parthenope", Department of Sport Sciences and Wellness, Naples, Italy
[2] University of Campania "Luigi Vanvitelli", Department of Mental and Physical Health and Preventive Medicine, Section of Human Anatomy, Functional Anatomy Laboratory, Naples, Italy
[3] Department of Medicine and Health Sciences "Vincenzo Tiberio", University of Molise, Campobasso, Italy

Posture can be evaluated by clinical and instrumental methods. 3D motion analysis is the gold standard for the static and dynamic postural assessment. Conventional stereophotogrammetric protocols are used to assess the posture of pelvis, hip, knee, ankle, trunk (considered as a single segment) and rarely head and upper limbs during walking. A few studies analysed the multi-segmental trunk and whole-body kinematics. Aim of our study was to evaluate sagittal spine and whole-body posture during walking in healthy subjects by 3D gait analysis using a new marker set.

Fourteen healthy subjects were assessed by 3D-Stereophotogrammetry using DB-Total protocol. Excursion range, Absolute Excursion range, Average, intra-subject Coefficient of variation (CV) and inter-subjects Standard Deviation Average (SD-Average) of eighteen new kinematic parameters about sagittal spine and whole-body posture were calculated.

Analysis of DB-Total parameters showed a high intra-subject repeatability (CV < 50%) and a high inter-subject repeatability (SD-Average < 1) for the most of new kinematic parameters.

The present study introduced new postural values characterizing sagittal spine and whole-body posture of healthy subjects during walking. DB-Total parameters may be useful for understanding multi-segmental body biomechanics and as benchmark for pathological patterns.

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In vitro effects of electronic cigarette and heated tobacco product (IQOS) on oral cells

Lorella Marinucci[1], Maddalena Coniglio[2], Chiara Valenti[2], Alessandro Di Michele[3], Monia Billi[4], Stefano Bruscoli[5], Paolo Negri[2], Guido Lombardo[2], Stefano Cianetti[2], Stefano Pagano[2]

[1] Department of Medicine and Surgery, Section of Biosciences and Medical Embryology, University of Perugia, Perugia, Italy
[2] Department of Medicine and Surgery, Odontostomatological University Centre, University of Perugia, Perugia, Italy
[3] Department of Physics and Geology, University of Perugia, Perugia, Italy
[4] Department of Medicine and Surgery, Section of General Pathology, University of Perugia, Perugia, Italy
[5] Department of Medicine and Surgery, Section of Pharmacology, University of Perugia, Perugia, Italy

With approximately 1.3 billion smokers worldwide, 11.6 million in Italy and 8 million smoking-related deaths annually[1], tobacco smoking is one of the most serious public health problems. It has long been recognized as a major etiological factor in many malignancies, cardiovascular and respiratory diseases[2,3]. To help smokers stop and/or mitigate smoke-related health damage, electronic devices were introduced on to the world market in the early 2000s such as electronic cigarettes or Heated tobacco products (HTP), like IQOS device.

The aim of this in vitro study was to investigate the biological effects of electronic cigarette (e-cigarette) and heated tobacco product (IQOS) extracts respect to tobacco smoke extract on human gingival fibroblasts and human oral keratinocytes analysing cell viability, morphology, migration, apoptosis, cell cycle and related gene expression. The cells were treated with aerosols at different concentrations. This study is inspired by a preliminary evaluation performed by the same authors exclusively on IQOS device[4].

In particular cell viability was analysed by MTT assay, cell morphology using scanning electron microscope (SEM) and cell migration by Scratch assay, a method to mimic the migration of the cells during wound healing *in vivo*. Apoptosis and cell cycle were analysed with flow cytometry and the related-gene expression of p53, Bcl2, p16 and p21 was indagated using real-time PCR. All investigations were evaluated after 24h an *in vitro* exposure.

Undiluted tobacco smoke extract induced significantly inhibition of cell viability and cell migration, caused morphological alterations and induced an increase in cell death. No alterations or damage were observed after treatment with e-cigarette extracts. IQOS extract induced proliferation as highlighted by an increase of cell viability, cell migration and alterations of cycle analysis.

Comparing the different cigarette extracts, tobacco smoke turns out to be the most harmful, e-cigarette did not determine morphological and functional alterations and IQOS must be carefully investigated for its possible clinical effects on oral cell populations.

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Effects of Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) on HUVECs from women affected with gestational diabetes

Silvia Sancilio[1], Gianmarco Stati[1], Lucia Centurione[1], Roberta Di Pietro[1]

[1] Department of Medicine and Ageing Sciences, "G d'Annunzio" University of Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy

Pregnant women affected with gestational diabetes (GD) exhibit systemic low-grade inflammation and tend to develop cardiovascular and metabolic diseases. GD is associated with an increased oxidative stress and the overexpression of inflammatory cytokines, both of which can lead to endothelial dysfunction and vascular desease[1,2]. As such, GD could be viewed as a sort of 'short lived' metabolic syndrome that poses a risk for a variety of serious medical conditions.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a multifunctional cytokine that signals through a complex receptor system that triggers both cell survival and apoptosis pathways[3]. TRAIL is a regulator of endothelial cell physiology, and it sequentially affects functions, including immune response, inflammation, and vascular homeostasis. In human umbilical vein endothelial cells from healthy pregnant women (C-HUVECs), TRAIL increases endothelial nitric oxide synthase (eNOS) phosphorylation and upregulates nitric oxide (NO), nitrotyrosine (NT) and prostanoid production without triggering the NFκB/IκB pathway[4]. Moreover, TRAIL does not induce apoptosis but survival both in C-HUVECs and in HUVECs under normal physiological conditions, as in other tissue cell populations.

The aim of the present work was to study HUVECs chronically exposed to hyperglycaemia and to a pro-inflammatory environment during pregnancy to identify TRAIL effects on GD-HUVECs compared with C-HUVECs from healthy women. Tissue specimens, HUVECs and peripheral blood were obtained from umbilical cords of 14 GD women (age 35.4±4.93) and 12 control women (age 34.6±4.93). As compared to controls, GD-HUVECs exhibited an increased eNOS and NT immunoreactivity (immunohistochemistry); an up-regulation of TRAIL-R1 (death receptor), -R3 and -R4 (decoy receptors) in cultured cells (flow cytometry) and in tissue sections (immunohistochemistry) and an increased number of apoptotic cells after in vitro TRAIL treatment (TRAIL 100 ng/mL and 1000 ng/mL). Interestingly, we observed that the white blood cells of GD women showed higher expression levels of all TRAIL-Rs and sTRAIL (TNFSF10) in comparison with C-HUVECs (flow cytometry). These findings suggest the possible involvement of TRAIL in the inflammatory process of GD and shows that primary HUVECs are a valuable model to assess the biological activity of TRAIL. With mounting evidence indicating the importance of metabolic syndromes in human disease, this model provides an exceptional opportunity for research aimed at identifying the biochemical processes underlying their onset.

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Cardiovascular changes in dietary-induced obese rats: anti-inflammatory and antioxidant effect of tart cherry supplementation.

Daniele Tomassoni[1], Ilenia Martinelli[2], Vincenzo Bellitto[2], Proshanta Roy[2], Maria Vittoria Micioni Di Bonaventura[2], Seyed Khosrow Tayebati[2], Consuelo Amantini[1], Carlo Cifani[2] and Francesco Amenta[2]

[1] School of Biosciences and Veterinary Medicine, University of Camerino, 62032 Camerino, Italy
[2] School of Medicinal and Health Products Sciences, University of Camerino, 62032 Camerino, Italy

Obesity is a risk factor for cardiovascular diseases related to inflammation and oxidative stress [1]. In the last decade, considerable interest has been shown in diet supplementation with natural bioactive compounds and their ability to preserve or improve cardiovascular health [2]. Assumption of red fruits rich in bioactive phytochemicals attracts great attention since their benefits have been attributed mostly to their possible antioxidant properties [1]. In the present work, we have evaluated the oxidative status and inflammation in the heart of dietary-induced obese (DIO) rats exposed to a high-fat diet compared to a standard diet. Moreover, the possible protective effects of tart cherry seed powder and seed powder plus tart cherry juice were analysed.

The oxidative stress was assessed by investigating protein oxidation and 4-hydroxynonenal expression in heart samples. Western blot assays and immunohistochemistry, followed by the morphological analysis on the expression of proinflammatory cytokines and endothelial adhesion molecules were done. In DIO rats, cardiomyocyte hypertrophy was accompanied by an increase in oxidized proteins and lipid peroxidation. The intake of tart cherry seeds powder and juice significantly countered the above changes. Heart injury was associated with NF-κB activation and increased endothelial adhesion molecules and cytokines (TNF-α, IL-1β, and IL-6) expression levels. An anti-inflammatory effect, with downregulation of different parameters analysed, was induced by tart cherry consumption. These effects could be related to the reduction of systolic blood pressure and glycemic values induced by the supplementation. Our findings support the view of an antioxidant and anti-inflammatory activity of tart cherry juice and seeds. This dietary supplementation can contribute to prevent or counter obesity-linked cardiovascular disease.

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Stili di vita e prevenzione: scienze del movimento, della nutrizione e del benessere

Seconda sessione

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Visually impaired dancers vs other sound input-based sport athletes: survey on psychological well-being and quality of life

Giuditta Carretti[1], Daniela Mirandola[1], Eleonora Sgambati[2], Mirko Manetti[1] and Mirca Marini[1]

[1]Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, 50134 Florence, Italy
[2] Department of Biosciences and Territory, University of Molise, 86090 Pesche, Isernia, Italy

Sport practice has the widely demonstrated potential of promoting well-being and physical/mental health, especially in disabled individuals. Nowadays, visually impaired people can participate in several sports commonly adapted and played substituting visual input with auditory or tactile ones. By integrating movement and music, dance can simultaneously promote physical and emotional involvement and enhances vicarious sense recruitment. On these premises, we performed a survey to assess the psychological well-being (PWB) and quality of life (QoL) in visually impaired athletes, comparing dancesport vs other sound input-based sports. Twenty-one visually impaired dancers and twenty-seven visually impaired athletes practicing adapted baseball, showdown, blind futsal, or blind tennis completed a structured self-report survey including the Italian version of PWB-18 scale and the Short Form-12 (SF-12) questionnaire. Dancers reported significantly higher scores in PWB-18 autonomy, environmental mastery, and self-acceptance along with a higher PWB total score than the other athlete group. Similarly, the SF-12 questionnaire results demonstrated significantly higher scores in both physical and mental QoL of visually impaired dancers compared with other athletes. Those results were further strengthened by the statistically significant data of the applied multiple linear regression model revealing the role of sport-specific practice as independent predictor of both PWB and QoL. In conclusion, our findings suggest that, given its peculiarities, the practice of dancesport may have a stronger positive impact on PWB and QoL of visually impaired individuals than other sound input-based sports.

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Acute effects of fasted training on physical performance and training stress in highly-conditioned subjects

Morucci G[1,2], Ryskalin L[1,2], Levi Micheli M[3,4], Cannataro R[5], Scala A[3,4], Orsini A[2], Branca JJV[3,4], Gulisano M[3,4], Gesi M[1,2]

[1] Dept of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy
[2] Center for Rehabilitative Medicine "Sport and Anatomy", University of Pisa, Italy
[3] Human Anatomy Section, Dept of Experimental and Clinical Medicine, University of Florence, Italy
[4] Sports Science Laboratory "Mario Marella", University of Florence, Italy
[5] Galascreen Laboratories, University of Calabria, Rende (CS), Italy

Background: Over the years, the effects of fasted training on the performance of competitive and amateur athletes has gathered increasing attention. This is mostly due to the theoretical vantages regarding the greater reliance on fatty acids as the energetic substrate and the increased lipolysis in adipose tissue during exercise in the fasted state. At the same time, fasted training is especially preferred by amateur athletes, since it allows them to train early in the morning before work.

Questions/purposes: The present study aims to evaluate the effects of a single training session in fasting conditions on physical performance, body composition, and training stress in competitive athletes.

Methods: Twelve competitive male athletes in different sports were firstly scored for anthropometric measurements (weight, height, BMI), body composition (Bioelectrical Impedance Analysis, BIA, Akern srl, Italy), salivary cortisol levels (Immunoenzymatic Assay, Diametra srl, Italy), the velocity at maximal oxygen uptake (treadmill incremental vVO_2max test) and maximal dynamic strength by two different exercises, namely 1-RM Repetition Maximum by Brzycki formula in both bench press and lat pull-down with pronated grip. Each subject randomly performed two equal-volume single training sessions both in normal conditions (i.e. no fasting) and after 12-14 hours of fasting. During the training sessions, the Heart Rate and the Ratings of Perceived Exertion (Borg CR10 scale) were monitored both during the training session and at the end of each session.

Results: No differences were found between the training session performed either in normal or fasted conditions regarding all the measurements except for the Heart Rate in the first 5 minutes of aerobic exercise (-3,2% in fasted training, $p<0.05$) and the lat pull-down maximal strength test (-19% in fasted training, $p<0.05$); after the training session, salivary cortisol levels were 49% higher in fasted state compared with no fasted condition, though being not statistically significant ($p=0.07$); with reference to BIA variables, the phase angle showed a slight but significant decrease (-1.3%, $p<0.05$) at the end of the training session in the fasted conditions, while a minor reduction in the resistance occurred in fasted *vs.* no fasted training (-1.4%, $p<0.05$ *vs* -3.5%, $p<0.002$, respectively), suggesting a higher catabolic state.

Conclusion: In highly-conditioned subjects, the acute response to a single standard training session in fasting conditions does not show excessive athletic and metabolic body stress but rather induces a reduction of dynamic strength and higher catabolism at the end of the training.

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Role of enteric glial nlrp3 inflammasome in gut barrier alterations associated with high fat diet-induced obesity

Carolina Pellegrini[1], Vanessa D'Antongiovanni[2], Cristina Segnani[1], Chiara Ippolito[1], Laura Benvenuti[2], Rocchina Colucci[3], Clelia Di Salvo[2], Luca Antonioli[2], Matteo Fornai[2], Nunzia Bernardini[1]

[1]Unit of Histology and Medical Embryology, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
[2]Unit of Pharmacology and Pharmacovigilance, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
[3] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy

Background: Nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain containing protein 3 (NLRP3) inflammasome activation in immune/inflammatory cells contributes to enteric inflammation and bowel dysmotility in obesity (1). However, the involvement of NLRP3 inflammasome in enteric glial activation and gut barrier alterations associated with obesity has not yet been investigated.

Aim: This study examined the interplay among enteric glia NLRP3 inflammasome activation and intestinal barrier impairments in high-fat diet (HFD)-induced obesity

Methods: Wild-type C57BL/6J mice were fed with HFD or standard diet for 8 weeks. The remodelling of intestinal epithelial barrier in the colonic wall were assessed by standard histological staining, immunohistochemistry and immunofluorescence and western blot. Double-immunofluorescence of ASC and GFAP-positive glial cells was performed to characterize inflammasome activation in enteric glial cells. The molecular mechanisms underlying the interplay between altered intestinal barrier, enteric gliosis and NLRP3 activation in the setting of obesity were investigated with *in vitro* co-culture experiments enteric glial cells (EGCs) and intestinal epithelial cells (IECs).

Results: After 8 weeks of HFD, animals displayed a remodeling of mucus layer, decrease in expression of tight junction proteins along with an increase in proliferating epithelial cells in colonic crypts. HFD animals were also characterized by increased ASC immunopositivity in GFAP-positive glial cells. In co-culture experiments, exposure to palmitate and lipopolysaccharide, besides to induce intestinal epithelial barrier alterations, promotes enteric gliosis with consequent hyperactivation of enteric glial NLRP3/caspase-1/IL-1β signaling pathway. The glial-derived IL-1β release contributes to exacerbate the disruption of IEB. Such an effect was abrogated upon incubation with IL-1β receptor antagonist, anakinra.

Conclusions: Glial NLRP3 inflammasome acts as a regulatory hub linking EGCs and IECs, representing a promising therapeutic target for the treatment of gut barrier alterations related to obesity.

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A predictive model of falling risk and frailty condition in the elderly

Valentina Presta[1], Laura Galuppo[2], Giancarlo Condello[1], Luca Ambrosini[1], Prisco Mirandola[1], Marco Vitale[1,3], Giuliana Gobbi[1]

[1] Department of Medicine and Surgery, Human Anatomy, University of Parma, Parma, Italy
[2] AUSL Reggio Emilia, Reggio Emilia, Italy
[3] Movement Analysis Laboratory (LAM), Parma University Hospital, Parma, Italy

Fall events are challenging for the elderly, caregivers, and the community in which they live[1]. Public healthcare and stakeholders involved in the management of the elderly should constantly improve the prevention strategies of fall events, as they are known to be risk factors for disability, hospitalization, and mortality[2,3]. We aimed at creating a screening and predictive protocol of fall events in the elderly as a replicable model in clinical settings.

Fifty subjects (17 males and 33 females) were recruited (mean age 76.9 ± 3.69 yrs) for a clinical geriatric evaluation and posture/gait performance analysis. Participants underwent a complete anamnesis, fall history, medical equipment as well as tests and questionnaires battery. After a body composition analysis, posture was evaluated through stabilometry. Gait performance included a battery of standardized tests (10-meter walking test, six minutes walking test, timed up and go test).

Results showed that path length, sway area, and sway speed were predictive factors of fall events, whereas the six minutes walking test was found to be a predictor of frailty condition. Of note, timed up and go test was predictive of both frailty and fall events.

We conclude that stabilometric parameters together with the gait test performances (six minutes walking and timed up and go tests) should be included in a screening protocol model for the elderly to prevent fall events and to recognize frailty conditions at earlier stages.

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A non-invasive method for the evaluation of adolescent idiopathic scoliosis: morphological analysis of the spine with infrared thermography

Federico Roggio[1], Luca Petrigna[1], Bruno Trovato[1], Marta Zanghì[1], Martina Sortino[1] and Giuseppe Musumeci[1]

[1] Department of Biomedical and Biotechnological Sciences, Human, Histology and Movement Science Section, University of Catania, Via S Sofia n°87, 95123, Catania, Italy

Background: Adolescent idiopathic scoliosis (AIS) is defined as a three-dimensional spine deformity with a multifactorial etiology involving genetic, environmental, and lifestyle factors. The clinical evaluation of AIS is based on the anterior trunk flexion test, Adams test, and the measurement of the hump that occurs through the scoliometer. Currently, the gold standard for diagnosing scoliosis is radiography. It is configured as disease identification and monitoring method; however, the use of radiography is associated with awareness of the potential adverse effects of exposure to x-rays. In this context, several non-invasive methods have been introduced with high reliability in evaluating scoliosis. Infrared thermography (IRT) is a non-invasive method that provides information on the body's thermal, metabolic and vascular conditions in the area of interest. It is effective for assessing asymmetries in temperature distribution, making it versatile in monitoring scoliosis throughout its course. The muscles of the convex side of scoliosis are characterized by a more intense electromyographic activity than the concave side, the thermography analyzing the temperature radiated by the muscles can compute the present asymmetries.

Aim: This study aimed to analyze the thermal differences of the back surface between the convex and concave sides of scoliotic individuals compared with non-scoliotic individuals.

Methods: A sample of 48 individuals with and without adolescent idiopathic scoliosis diagnosed by radiography was collected. We used infrared thermography, FLIR E54, to assess the presence of asymmetrical muscle activity. For the statistical analysis, ANOVA and Tukey's test were employed to analyze the temperature of the trapezius, latissimus dorsi, and quadratus lumborum muscles, dividing the individuals by topography of scoliosis, i.e., thoracic scoliosis, thoracolumbar scoliosis, and individuals without scoliosis.

Results: The mean age of the sample is 12.2 ± 2.29 years, height 153 ± 13.4 cm, weight 48.0 ± 13.5 kg. In the group with thoracic scoliosis, the differential (Δ) of the mean temperature of the trapezius muscle is 0.47 ° C ± 0.16 ° C, Δ of the latissimus dorsi is 0.37 ± 0.30, Δ of the quadratus lumborum is 0.63 ± 0.35. In the group with thoracolumbar scoliosis, Δ of the trapezius muscle is 0.19 ° C ± 0.22 ° C, Δ of the latissimus dorsi is 0.42 ± 0.21, Δ of the quadratus lumborum is 0.49 ± 0.26. In the group without scoliosis, Δ of the trapezius muscle is 0.09 ° C ± 0.09 ° C, Δ of the latissimus dorsi muscle is 0.12 ° C ± 0.09 ° C, Δ of the quadratus lumborum is 0.21 ° C ± 0.27 ° C.

Conclusion: The hypothesis that the IRT analysis differs in individuals with thoracic, thoracolumbar scoliosis, and without scoliosis was confirmed. In individuals with scoliosis, the convex side always has a higher surface temperature than the convex side. Furthermore, the topographic classification can guide clinicians toward a precise IRT analysis of scoliosis.

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Tessuto muscolare e connettivo

Prima sessione

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Analysis of breast cancer-associated fibroblasts in 2D and 3D cell culture systems: development of an in vitro model to deactivate cancer-associated fibroblasts and study their influence on cancer growth

Angelica Avagliano[1], Vittoria Acampora[1], Pietro Carotenuto[2,3], Anna Barbato[2], Veronica Romano[1], Maria Rosaria Ruocco[4], Alessandro Venuta[1], Antonino Iaccarino[1], Gaetano Calì[5], Antonello Accurso[6], Luigi Insabato[7], Nunzio Velotti[8], Elena Vigliar[1], Stefania Montagnani[1] and Alessandro Arcucci[1]

[1] Department of Public Health, University of Naples Federico II, 80131 Naples, Italy
[2] TIGEM, Telethon Institute of Genetics and Medicine, 80078 Naples, Italy
[3] Medical Genetics, Department of Translational Medical Science, University of Naples Federico II, 80131 Naples, Italy
[4] Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, 80131 Naples, Italy
[5] IEOS Istituto di Endocrinologia e Oncologia Sperimentale 'G Salvatore', National Council of Research, 80131 Naples, Italy
[6] Department of General, Oncological, Bariatric and Endocrine-Metabolic Surgery, University of Naples Federico II, 80131 Naples, Italy
[7] Anatomic Pathology Unit, Department of Advanced Biomedical Sciences, School of Medicine, University of Naples Federico II, 80131 Naples, Italy
[8] Department of Advanced Biochemical Sciences, University of Naples Federico II, 80131 Naples, Italy

Breast cancer (BC) is a heterogeneous mass characterized by an abnormal tumor microenvironment (TME). Breast cancer-associated fibroblasts (BCAFs), the most abundant stromal cells of the breast TME, support BC initiation and progression through interactions with BC cells. BCAFs are constitutively activated cells and express high levels of fibroblast activation and inflammation markers, such as α-smooth muscle actin (α-SMA) and cyclooxygenase 2 (COX-2), respectively. However, distinct BCAF populations with different levels of α-SMA have been detected in human BCs and correlate with BC aggressiveness. Furthermore, BCAF aggregates have been found in the blood circulation of metastatic BC patients. We isolated human primary BCAFs from invasive BC tissues and we analyzed, by Real-Time PCR, the expression of two important BCAF markers, FAP and SPARC (or osteonectin) in primary BCAFs and paraffiin-embedded BC tissues. This analysis showed no significant differences between the primary BCAFs and BC tissues for FAP and SPARC gene expression. Hence, these data indicate that our in vitro BCAFs can closely mimic the in vivo BCAFs. Then, we developed and characterized an in vitro stromal model consisting of human primary BCAFs grown in vitro as BCAF monolayers, BCAF aggregates, i.e. spheroids collected after 72h and 216h of 3D cell culture, and reverted BCAFs, that are fibroblasts grown as spheroids for 216h and then reverted to 2D cell adhesion growth. We firstly evaluated activation and inflammation markers of these BCAF cultures. Then, we analyzed the viability and the migratory capability of MCF-7 cells following treatment with conditioned media from the different BCAF cultures. We demonstrated that, after 216 h of 3D culture, BCAFs acquired an inactivated phenotype, associated with a significant reduction of α-SMA and COX-2 protein expression levels. The deactivated phenotype of BCAF spheroids at 216 h was further confirmed by the cytostatic effect exerted by their conditioned medium on MCF-7 cells. We further demonstrated that reverted BCAFs retained a less activated phenotype as indicated by α-SMA protein expression reduction compared with BCAF monolayers. Interestingly, reverted BCAFs displayed a reduced pro-tumorigenic phenotype as indicated by the anti-migratory effect exerted by their conditioned medium on MCF-7 cells. In

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conclusion, our results highlight the molecular and functional plasticity of primary BCAFs in response to different cell culture conditions. Furthermore, our study showed that the deactivation of BCAFs without drug treatment is possible and it could be a starting point to identify and develop new therapeutic strategies targeting BCAFs.

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Evaluation of the β-Caryophyllene effects alone or in combination with Ascorbic Acid and d-Glucosamine on inflamed Human Chondrocytes

Elisa Belluzzi[1,2], Elena Mattiuzzo[3], Alessia Faggian[3], Rina Venerando[4], Andrea Benetti[3], Pietro Ruggieri[2] and Paola Brun[3]

[1] Musculoskeletal Pathology and Oncology Laboratory, Department of Surgery, Oncology and Gastroenterology, University of Padova, 35128 Padova, Italy
[2] Orthopaedic and Traumatology Clinic, Department of Surgery, Oncology and Gastroenterology, University of Padova, 35128 Padova, Italy
[3] Department of Molecular Medicine, Histology Unit, University of Padova, 35121 Padova, Italy
[4] Department of Molecular Medicine, University of Padova, 35121 Padova, Italy
[5] Faculty of Medicine, University of Padova, 35100 Padova, Italy

Background: Osteoarthritis (OA) is a disabling disease affecting all joint tissues and particularly articular cartilage. Moreover, inflammatory process and mediators plays an important role in the initiation and progression of OA determining cartilage extracellular matrix degradation.

Aim*:* The aim of this study is to evaluate the effects of β-caryophyllene (BCP), a plant-derived sesquiterpene with anti-inflammatory and antioxidant properties, alone or in combination with ascorbic acid (AA) and d-glucosamine (GlcN) on human primary chondrocytes inflamed with macrophage-derived conditioned medium (CM).

Materials and Methods: Cartilage biopsies were obtained from OA patients and controls. Histological staining and immunohistochemistry for inflammatory markers were performed to characterize the tissues. Chondrocytes cell viability, gene expression of pro-inflammatory mediators, metalloproteinases (MMPs), collagen type II and aggrecan levels were analyzed in primary human chondrocytes isolated from OA patients and exposed to the CM of activated U937 monocytes and subsequently treated with BCP alone or in combination with AA and GlcN.

Results: Histology and immunohistochemistry analysis pointed out cartilage degeneration, fragmentation and fissuring in OA patients, associated with the presence of positivity to inflammatory molecules compared to controls. The macrophage-derived CM induced a decrease of chondrocyte viability and a significantly increase of inflammatory molecules and MMPs expression. When BCP was added to the inflamed cells, alone or in combination with AA and GlcN, there was an increase of cell viability, a decrease of gene expression of interleukin-1 beta, NF-κB1 and MMP-13 and an increase of ECM matrix molecules (aggrecan and type II collagen).

Conclusions: Low-doses of BCP are effective in counteracting the inflammatory effects determined by macrophage-derived CM stimulation on chondrocytes. Moreover, the combined used of BCP, AA and GlcN seems to have synergistic effects in protecting chondrocytes.

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Involvement of nucleic acids in experimental and pathological aortic valve calcification

Antonella Bonetti[1], Magali Contin[1], Maurizio Marchini[1], Fulvia Ortolani[1]

[1] Histology and Embryology, Department of Medicine, University of Udine, I-33100 Udine, Italy

Calcification onset in aortic valves has been largely debated, being increasingly relevant the pro-calcific role of cell-membrane-derived acidic phospholipids as part of the so called "calcium-phospholipid-phosphate complexes" [1]. Our previous studies on *in vivo* experimentally and pathologically calcified aortic valve leaflets as well as aortic valve interstitial cell (AVIC) pro-calcific cultures strengthened the cell-mediated triggering of valve calcification. Indeed, the degenerative process was found to depend on overall cytomembrane dissolution with lipid release and appearance of a phthalocyanine-positive acidic material (PPM) stuffing cell cytoplasm, followed by its spreading towards cell edges with formation of peripheral layers (PPLs) acting as major hydroxyapatite (HA) nucleators [2-4]. Here, additional involvement of anionic nucleic acids in valve mineralization has been investigated. Ultrastructural analyses of mineralizing cultured AVICs as well as those populating *in vivo* calcified valve leaflets showed ribosome detachment from degenerating rough endoplasmic reticulum *cisternae* and their embedding within both intracellular PPM and forming peripheral PPLs. Using antibodies against ribosomal RNA (rRNA), immunohistochemical analyses actually showed positivity at level of PPM/PPL material characterizing mineralized AVICs and cell-derived vesicular byproducts. Moreover, immunogold labelling reactions revealed gold particles to decorate PPM/PPLs, supporting the evidence that rRNA derived from ribosome degeneration contributes to the formation of such pro-calcific acidic material. Interestingly, nuclear DNA resulted as an additional HA nucleational site in mineralizing AVICs, as revealed by silver particle deposition onto nuclear chromatin after post-embedding von Kossa reactions for selective calcium-binding site visualization. In conclusion, the present results indicate that nucleic acids may contribute to AVIC mineralization both *in vivo* and *in vitro*, with their acidic nature promoting PPM/PPL capacity of HA nucleation. The obtained results are also consistent with previous finding that complete removal of nucleic acids during decellularization of native heart valves is of paramount importance to attain durable, calcification-free valve biosubstitutes [5].

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Soluble guanylate cyclase stimulation fosters angiogenesis and blunts myofibroblast-like features of scleroderma endothelial cells

Bianca Saveria Fioretto[1], Irene Rosa[1], Eloisa Romano[2], Dilia Giuggioli[3], Marco Matucci-Cerinic[2], Mirko Manetti[1]

[1] Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, Florence, Italy
[2] Department of Experimental and Clinical Medicine, Division of Rheumatology, University of Florence, Florence, Italy
[3] Department of Medical and Surgical Sciences for Children and Adults, Scleroderma Unit, University of Modena and Reggio Emilia, Modena, Italy

In scleroderma (systemic sclerosis, SSc), peripheral microvasculopathy and angiogenesis impairment advance in parallel with the development of tissue fibrosis orchestrated by myofibroblasts originating from different sources, including endothelial-to-myofibroblast transition (EndMT). Soluble guanylate cyclase (sGC) stimulation was found to counteract transforming growth factor (TGF)β-induced fibroblast-to-myofibroblast differentiation with significant antifibrotic effects in various experimental models of tissue fibrosis. Here, we investigated the effects of pharmacological stimulation of sGC on impaired angiogenesis and myofibroblast-like features of SSc dermal microvascular endothelial cells (SSc-dMVECs). To determine whether sGC stimulation affected cell viability and proliferation, five lines of SSc-dMVECs and five lines of healthy dermal MVECs (H-dMVECs) were challenged with the sGC stimulator MK-2947 and assayed by annexin V/propidium iodide flow cytometry and WST-1, respectively. To study angiogenesis and EndMT, MK-2947-treated SSc-dMVECs were subjected to wound healing and capillary-like tube formation assays, and analyzed for the expression of endothelial and myofibroblast markers by quantitative real-time PCR, immunoblotting and immunofluorescence. Cell contractile ability was investigated by collagen gel contraction assay. In other experiments, H-dMVECs were preincubated with MK-2947 before induction of EndMT through administration of recombinant human TGFβ or serum from SSc patients. MK-2947 treatment did not affect H-dMVEC viability/proliferation, while it significantly increased SSc-dMVEC proliferation, wound healing capability and angiogenic performance. After MK-2947 treatment, SSc-dMVECs exhibited significantly increased proangiogenic MMP9 and decreased antiangiogenic MMP12 and PTX3 gene expression. A significant increase in gene and protein expression of CD31 and vascular endothelial cadherin paralleled by a decrease in α-smooth muscle actin, S100A4, type I collagen and Snail1 myofibroblast markers was also found in MK-2947-treated SSc-dMVECs. Furthermore, stimulation of sGC with MK-2947 significantly counteracted the intrinsic ability of SSc-dMVECs to contract collagen gels and reduced phosphorylated-ERK1/2 protein levels. Finally, treatment with MK-2947 efficiently protected H-dMVECs against TGFβ- and SSc serum-induced EndMT. These findings demonstrate for the first time that pharmacological stimulation of sGC effectively ameliorates the angiogenic performance and blunts the myofibroblast-like profibrotic phenotype of SSc-dMVECs, thus providing new evidence for repurposing sGC stimulators for the treatment of SSc-related skin fibrosis and peripheral vascular manifestations.

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Combined histomorphometric and Fourier Transform Infrared Imaging (FTIRI) analyses to reveal Type I Collagen networking changes in osteoporotic bone

Caterina Licini[1], Guendalina Lucarini[1], Valentina Notarstefano[2], Mariangela Di Vincenzo[1], Andrell Hosein[1], Nada Daohuadi[1], Daniela Lamanna[1], Giorgia Cerqueni[1], Elisabetta Giorgini[2], Saverio Marchi[1], Monia Orciani[1] and Monica Mattioli-Belmonte[1].

[1] Department of Clinical and Molecular Sciences (DISCLIMO), Università Politecnica delle Marche, Via Tronto 10/A, 60126 Ancona, Italy
[2] Department of Life and Environmental Sciences (DISVA), Università Politecnica delle Marche, Via Brecce Bianche, 60100 Ancona, Italy

Getting insights on bone extracellular matrix (ECM), proteins' reciprocal interaction and their effects on bone mineralization and structure is important in normal and pathological tissue biology assessment and to finetune regenerative approaches. To this aim, we analyzed superimposed tissue sections of healthy (H) and osteoporotic (OP) bone, exploiting the combination of the Fourier Transform Infrared Imaging (FTIRI) technique with histomorphometry, Sirius Red staining, and immunohistochemistry, as well as Western Blotting. We studied Type I Collagen, mainly responsible for bone structural properties and its mineralization [1], and selected non-collagenous proteins (NCPs): Decorin (DCN), Osteocalcin (OCN), Osteopontin (OPN), Bone Sialoprotein-2 (BSP-2), Osteonectin (ONT) and Transforming Growth Factor beta (TGF-beta), which are differently implicated in bone ECM composition, mineral deposition, and cell-matrix interaction [2].

Focusing the attention on OP samples, we identified zones in which collagen distribution was superimposable to the H bone (OP+) and areas showing decreased and disordered collagen disposition (OP-). We evidenced how the alterations in OP bone tissue architecture were mainly associated with warped Type I Collagen structure and deposition, but not with changes in the total protein amount. Moreover, OP tissue exhibited modifications in DCN, OCN, OPN, and BSP-2 distribution as well as changes in their cooperative or antagonist role, suggesting the NCPs' deep impact on the collagen features in OP bone.

Our morphological combined methodology provides a comprehensive evaluation of the intricate bone ECM network and its role in bone homeostasis, a matter that is still not fully addressed in the literature. These findings are crucial for designing targeted clinical strategies for bone mass preservation in OP and/or developing new therapeutic approaches to bone diseases.

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The hepatic effects of melatonin in female cholestatic murine model

Romina Mancinelli[1], Ludovica Ceci[1], Lindsey Kennedy[2], Heather Francis[2], Shannon Glaser[3], Gianfranco Alpini[2], Paolo Onori[1] e Eugenio Gaudio[1]

[1] Department of Anatomical, Histological, Forensic Medicine and Orthopedic Sciences, Sapienza University of Rome, 00161, Rome, Italy
[2] Division of Gastroenterology and Hepatology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA
[3] Department of Medical Physiology, Texas A&M University, Bryan, TX 77807, USA

Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease of the bile duct system, characterized by ductular reaction (DR), biliary senescence, and cholestasis (1). Studies demonstrated that: (i) males are more prone to developing PSC compared to females, (ii) the hepatobiliary junctions have important role in chronic liver diseases. Indeed, reduction of tight junction increases paracellular permeability leading to progression of bile injury (2). The melatonin acts as protective factor in several cholestatic liver diseases. We previously found that: (i) cholangiocytes synthesize melatonin through the enzyme, aralkylamine N-acetyltransferase (AANAT), (ii) they express both MT1 and MT2 receptors; and (iii) melatonin ameliorates biliary damage/senescence and liver fibrosis, as well as clock gene mRNA expression in male bile duct ligated (BDL) and Mdr2$^{-/-}$ mice (model of PSC) through downregulation of MT1 but not MT2 (3). We aimed to understand the prolonged effects of melatonin in the modulation of biliary/liver phenotypes in a female model of PSC (Mdr2$^{-/-}$). For that reason, female WT and Mdr2$^{-/-}$ mice at 12 wk underwent melatonin treatment for 12 wk. We evaluated: (i) DR by IHC for CK19, biliary senescence by β-gal staining, and liver fibrosis by Fast Green/Sirius Red and Masson's trichrome; (ii) mRNA expression of fibrotic markers (TGFβ1, αSMA, FN1) in total liver by qPCR; (iii) inflammation by IHC for F4/80 and mRNA expression of inflammatory markers (Ccl1, Ccl2, Ccl5); and (iv) angiogenesis by IHC for VEGFA and double IF for CD31/CK19, and by qPCR for Anpt1/2 and VEGFA/C in total liver samples. At last, we evaluated the ductulus-canalicula-jucntions (DCJ) by IF of CK19 with BSEP. Prolonged administration of melatonin ameliorated liver inflammation and biliary senescence compared to control-treated mice. In addition, we observed that female Mdr2$^{-/-}$ mice have elevated DR that promotes significantly decreased of hepatobiliary junctions. This phenotype is reversed in female Mdr2$^{-/-}$ mice treated with melatonin. Parallel to these findings, we demonstrated reduced collagen deposition, decreased mRNA expression for fibrotic markers, and a reduction in liver angiogenesis. The beneficial effects of melatonin on DR, biliary senescence and fibrosis were associated with reduced liver angiogenesis and increased of DCJ. Long term effects of melatonin and could be considered a possible therapeutic compound in cholestatic diseases.

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Dr Jekyll and Mr Hyde: the bipolar nature of the epiphyseal plate

Mario Raspanti[1], Piero Antonio Zecca[1], Marina Protasoni[1] and Marcella Reguzzoni[1]

[1] Department of Medicine & Surgery, Insubria University, Varese

The epiphyseal plate is essentially a cartilage layer sandwiched between two bony segments at the end of long bones. On the epiphyseal side, the chondrocytes are small and rounded and form a stable, resting zone; toward the diaphysis, they gradually enlarge and blend into a proliferating area and then a hypertrophic zone, where they express type I and type X collagen easing the cartilage transition into bone. The molecular mechanisms underlying these processes are partially known (1), and these tissues' structural biology is even less documented (2).

In the present research, the epiphyseal plates from ovine proximal femoral epiphyses were investigated by histological sections, ground sections, scanning electron microscopy, thermal deproteination (3) and micro-tomography. Images were obtained with a Nikon Eclipse 300 photomicroscope, a FEI XL-30 FEG SEM and a Stratec XCT-Research SA+ microCT.

On the epiphyseal side, the chondrocytes are widely dispersed, rounded and stable; toward the diaphysis, they become progressively hypertrophic, and the interposed territorial matrix undergoes mineralization. This latter process takes place very actively, forming a continuous bony surface at the mineralization front but leaving behind a constellation of small cartilage islets interspersed in a delicate meshwork of cancellous bone. Both tissues are then remodelled together, as shown by frequent Howship lacunae. Matrix vesicles initiate the calcification process and, differently from other hard tissues, proceed to completion with no visible involvement of the collagen fibrils.

In stark contrast, the other interface of the epiphyseal plate shows no trace of an active ossification process. All techniques consistently confirm a mature, stable, wide-spaced cancellous bone that is visibly different from the bone left behind by the mineralization front on the diaphyseal side. The cartilage involvement is limited to impressions of chondrocytes lying on the very bone/cartilage interface.

The two sides of the epiphyseal plate show a surprisingly opposite structure, with endochondral ossification proceeding very actively on the diaphyseal face of the cartilage layer but not at all on the epiphyseal front. The blood and nutrient supply on the two faces seem equivalent. Yet even the two bony surfaces enclosing the cartilage are diametrically different from each other and the subchondral bone under the articular cartilage.

Other research is underway to elucidate the cause of such divergent behaviors occurring a few micrometers apart on the opposite faces of the same tissue.

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Extracellular lactic acidosis drives reprogramming of human subcutaneous adipocytes toward pathogenic myofibroblasts

Irene Rosa[1], Elena Andreucci[2], Bianca Saveria Fioretto[1], Eloisa Romano[3], Marco Matucci-Cerinic[3], Lido Calorini[2], Mirko Manetti[1]

[1] Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, Florence, Italy
[2] Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Section of Experimental Pathology and Oncology, University of Florence, Florence, Italy
[3] Department of Experimental and Clinical Medicine, Division of Rheumatology, University of Florence, Florence, Italy

Emerging evidence underlines that adipocytic progenitor cells/adipocytes may give rise to pathogenic myofibroblast-like cells both in solid tumors and in multiple forms of fibrosis. Lactic acidosis is commonly observed in the tumor microenvironment, as cancer cells and cancer-associated fibroblasts generate their energy through glycolysis with production of large amounts of lactic acid even in the presence of oxygen, a phenomenon known as aerobic glycolysis. A close association between aerobic glycolytic metabolism and myofibroblastic activation has also been reported in different fibrotic diseases. Based on these premises, the aim of our in vitro study was to investigate whether glycolysis-derived acidic microenvironment could be implicated in the adipocyte-to-myofibroblast transition (AMT) process. Human subcutaneous adipose tissue-derived stem cells (ADSCs) at low passage (passages 2–4) were cultured in adipocyte differentiation medium for 10 days to obtain cells displaying characteristic adipocyte morphology with accumulation of prominent intracytoplasmic lipid droplets by phase-contrast microscopy. Cells were subsequently cultured for 3 days in i) ADSC growth medium (pH 7.4), ii) a chemically induced acidic extracellular microenvironment obtained by 1N HCl administration directly in ADSC growth medium to reach pH 6.7 in the presence of 10mM lactate, or iii) ADSC growth medium with 10 ng/ml of recombinant human transforming growth factor β1 as positive control of AMT. Cell viability and proliferation were determined by annexin V/propidium iodide flow cytometry and MTT assay, respectively. The expression of adipogenic/adipocytic and myofibroblastic markers was assessed by quantitative real-time PCR, immunoblotting and immunofluorescence. Acidic conditioning did not alter cell viability and proliferation. The acidic microenvironment was found to induce the loss of the expression of the adipogenic/adipocytic markers FABP4, C/EBPα, PPARγ, adiponectin and perilipin in parallel with a significant upregulation of the mesenchymal/myofibroblastic markers α-smooth muscle actin (α-SMA), COL1A1 and COL1A2. Immunofluorescence analysis further revealed the loss of perilipin-coated cytoplasmic lipid droplets and the accumulation of intracellular type I collagen and expression of α-SMA–positive stress fibers characteristic of myofibroblasts in acidic cell cultures. This study reveals a previously unrecognized relationship of extracellular lactic acidosis and generation of pathogenic myofibroblast-like cells from adipocytic precursors that may have pathophysiologic implications in a variety of diseases characterized by metabolic reprogramming toward glycolysis.

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Histopathological abnormalities of the skeletal muscle tissue from a patient affected by a rare genetic neuromyopathy

Federica Scalia[1,2], Rosario Barone[1], Antonella Marino Gammazza[1], Francesca Rappa[1], Leila Noori[1], Filippo Macaluso[3], Fabrizio Lo Celso[4], Everly Conway de Macario[5], Alberto J. L. Macario[2,5], Francesco Cappello[1,2]

[1] Departmentof Biomedicine, Neuroscienceand Advanced Diagnostics (BIND),University of Palermo,90127 Palermo,Italy
[2] Euro-Mediterranean Instituteof Scienceand Technology (IEMEST), 90139 Palermo,Italy
[3] SMART Engineering Solutions & Technologies (SMARTEST) Research Center, eCampus University, Palermo,Italy
[4] Department of Physics and Chemistry - Emilio Segrè, University of Palermo, Palermo, Italy
[5] Departmentof Microbiology and Immunology,Schoolof Medicine,Universityof Maryland at Baltimore- Institute of Marine and Environmental Technology (IMET), Baltimore, MD 21202, USA

Many rare neuromuscular disorders are inherited and heterogeneous diseases difficult to identify and study. The histopathological approach, combined with molecular assays, is becoming fundamental to better understand the pathogenic mechanisms and to develo p new treatments. Likewise, the etiopathogenic role of molecular chaperones in neuromuscular disorders is constantly emerging: the defect of a gene encoding for molecular chaperones or chaperonins may lead to dysfunctional, or non-functional protein with a key role in nervous and muscle tissue. We grouped these disorders in a nosological entity named neurochaperonopathies (NCP) [1]. Hereditary and clinical features are established, but "how" and "how much" the lesions occur in the affected tissue is not known. Here, we report histological abnormalities of skeletal muscle from a young patient with a severe neuromyopathy carrying the homozygous c.670C>G p.(Leu224Val) mutation in the gene encoding the subunit 5 (CCT5) of the CCT chaperonin complex [2,3]. Disorganization and atrophy of the affected skeletal muscle tissue is shown. Many fibers from patient's tissue are in apoptosis and the CCT5 subunit is diversely distributed compared with normal skeletal muscle tissue. Modified localization and signal intensity of antibodies against several sarcomeric and extra-sarcomeric proteins were observed, such as for myosin and desmin protein. Furthermore, we also found weak signals of the antibodies against members of chaperones system (CS) which appear in precipitates with desmin. The reported tissue abnormalities may be related to denervation, malnutrition or inactivity but also to failure of CCT5 activity: through *in silico* analyses, we also show modification of the molecular anatomy of CCT5 p.(Leu224Val) variant. Our data contribute to elucidate the molecular mechanisms that lead to the patient's phenotype and pave the way to developing novel therapeutic approaches, such as chaperonotherapies, to replace or improve the defective molecular chaperone.

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Tessuto muscolare e connettivo

Seconda sessione

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Lymphatic vessels detection in subcutis and superficial fascia

Giovanna Albertin[1], Laura Astolfi[2], Raffaele De Caro[1], Carla Stecco[1]

[1] Department of Neuroscience (DNS), Section of Human Anatomy, University of Padova, Italy
[2] Bioacoustics Research Laboratory, Department of Neuroscience-DNS, University of Padova, 35129 Padova, Italy
I-APPROVE-International Auditory Processing Project in Venice, Department of Neurosciences, University of Padova, Santi Giovanni e Paolo Hospital, ULSS3 Serenissima, 30122 Venezia, Italy

Lymphedema is a clinical manifestation caused by the impaired lymphatic transport or by the chronic accumulation of interstitial fluid that leads adipose deposition, fibrosis, or persistent inflammation on subcutis (1). Today, lymphedema's therapies require a detailed understanding of the anatomy of subcutaneous lymphatic system (2), moreover, the exact localization of the collectors and their relationships with fascial layers have not been defined yet. The aim of this study is to investigate the distribution, density and organization of lymphatic vessels (LV) with reference to the layered conformation of the subcutaneous tissue, that consist in the superficial adipose tissue (SAT), deep adipose tissue (DAT) and superficial fascia (SF) (3, 4). With this purpose, the subcutaneous tissue of three adult voluntary patients was harvested during abdominoplastic surgery and stained with a specific marker for the endothelial cells of lymphatic vessels, the monoclonal antibody D2-40 (5).

On the papillary dermis has been highlighted a huge presence of LV, they run parallel to the skin surface and are embedded in the loose connective tissue. On SAT, only thin LV, with a mean diameter of 11.6±7.71 μm, were visible close to the fibrous septa (retinacula cutis, that connects the dermis to the deeper layers). In the DAT, the LV follow above all the blood vessel, they are thicker with a mean diameter of 22.5±12.77 μm. The SF exhibits the highest density of LV, with a mean diameter of 19.5±5.77 μm, they show a path parallel to the surface, where intertwining each other form a characteristic plexus.

With this study, for the first time, the different distribution of the lymphatic vessels in the various subcutaneous layers, and the existence of a new lymphatic plexus within the superficial fascia, was demonstrated, adding new information on the alterations involving the subcutaneous tissues and consequently opening up new perspectives for surgery and manual treatments.

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Pentraxin 3 in idiopathic inflammatory myopathies

Tiziana Annese[1,2], Marco Fornaro[3], Dario D'Abbicco[3], Fabio Cacciapaglia[3], Laura Coladonato[3], Loredana Lorusso[2], Mariangela Mastrapasqua[2], Mariella Errede[2], Daniela Virgintino[2], Florenzo Iannone[3], and Francesco Girolamo[2]

[1] Department of Medecine and Surgery, LUM University, Casamassima, Italy
[2] Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari, Italy
[3] Department of Emergency and Organ Transplantation, University of Bari, Italy

Pentraxin-3 (PTX3) plays a role in the regulation of innate immune responses and different inflammatory reactions decreasing complement deposition [1]. No data of PTX3 muscle expression and plasma levels in patients affected by idiopathic inflammatory myopathies (IIMs) have been reported. The aim of this pilot study is to localize PTX3 expression in normal and diseased muscle, to correlate with PTX3 plasma levels, and to evaluate whether PTX3 can be a biomarker of disease activity in IIMs.

Methods: Twenty patients affected by IIM, 10 Dermatomyositis (DM) and 10 Polymyositis (PM), compared to 10 patients with rheumatoid arthritis (RA) and 10 healthy blood donors (HDs) aged, sex and BMI matched were evaluated for PTX3 plasma levels. Histopathology and immunohistochemical (IHC) analyses were performed before the start of any treatment on muscle biopsies from this cohort of DM and PM patients, and 3 additional age-matched healthy subjects, used as controls. Demographic and clinical characteristics were recorded at the time of first evaluation. PTX3 levels were assessed using a commercially available enzyme-linked immunosorbent assay kit (Abcam). Disease activity in IIMs was assessed by Myositis Disease Activity Assessment Visual Analogue Scale (MYOACT), while disease activity score on 28 joints whith erythrocyte sedimentation rate (DAS28-ESR) was used for RA patients.

PTX3 was expressed in sarcoplasm of type 2 muscle fibers in HD, DM, and PM without statistical differences in the percentage of positive myofibers. Additional PTX3 was localized in $CD68^+$ dendritic cells in a subgroup of PM patients. Perifascicular areas of DM muscles deprived of capillaries show reduced $PTX3^+$ myofibers and increased complement activation.

Mean serum PTX3 levels were significantly higher in IIM patients than HDs (518±260 pg/ml vs 275±114 pg/ml, p=0.009), while no significant difference was observed compared to RA patients (383± 146 pg/ml, p=0.14). Linear regression analysis adjusted for age, sex and disease duration showed a direct correlation between PTX3 and CPK levels (β: 0.590) and MYOACT score (β: 0.759) in both DM and PM. No association between PTX3 levels and disease activity was found in RA. In conclusion, plasma PTX3 may be a possible biomarker of disease activity in IIMs, in which could play a protective role for type II myofibers from inflammation. However, its relevance deserves further study.

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Anatomy-inspired scaffolds for skeletal muscle regeneration: development and preclinical investigation of human acellular diaphragmatic matrices

Silvia Barbon[1 2 3], Elena Stocco[1 2 3], Martina Contran[1], Federico Facchin[4], Rafael Boscolo-Berto[1], Silvia Todros[5], Deborah Sandrin[6], Filippo Romanato[6], Piero Pavan[5], Veronica Macchi[1 2], Vincenzo Vindigni[2 4], Franco Bassetto[2 4], Raffaele De Caro[1 2 3], Andrea Porzionato[1 2 3]

[1] Section of Human Anatomy, Department of Neuroscience, University of Padova, Padova, Italy
[2] LifeLab Program, Consorzio per la Ricerca Sanitaria, Padova, Italy
[3] Foundation for Biology and Regenerative Medicine, Tissue Engineering and Signaling – TES, Onlus, Padova, Italy
[4] Plastic Surgery Unit, Department of Neuroscience, University of Padova, Padova, Italy
[5] Department of Industrial Engineering, University of Padova, Padova, Italy
[6] Department of Physics and Astronomy "G Galilei," University of Padova, Padova, Italy

Volumetric Muscle Loss (VML) is the traumatic/surgical loss of skeletal muscle with resultant aesthetic damage and functional impairment. Besides rehabilitative therapy, surgical intervention is required, and the gold standard strategy is based on the transposition of autologous muscular grafts/flaps to the damage site. However, comorbidity problems and suboptimal functional recovery are the main limitations of the current surgical approaches to VML treatment, which still needs to be improved. A therapeutic alternative may be represented by the preparation of bioengineered acellular scaffolds that promote muscle regeneration. In this work, the human diaphragm was decellularized and investigated for the development of biocompatible muscular allografts highly resembling the structural features of the target tissue to be repaired. To this end, human diaphragm was decellularized by four different detergent-enzymatic protocols involving 1) sodium dodecyl sulfate (SDS), 2) SDS + Tergitol^{TM}, 3) sodium deoxycholate and 4) Tergitol^{TM}. The resulting diaphragmatic matrices were characterized for the efficiency of the decellularization process (histology, DNA quantification), the preservation of specific microstructure, protein composition (histology, immunohistochemistry, glycosaminoglycan quantification, second harmonic generation-SHG imaging), mechanical properties, absence of cytotoxicity, ability to interact with cells and in vivo biocompatibility.

After decellularization, cells, DNA (<50 ng/mg of tissue), and muscle fibers were efficiently removed, while collagen, elastin, and glycosaminoglycan components were correctly preserved. The detergent-enzymatic treatments did not affect the expression of muscular matrix markers (Collagen I and IV, Laminin), while causing the loss of HLA-DR expression to obtain non-immunogenic allografts. SHG microscopy confirmed the preservation of the collagen component whereas the uniaxial tensile tests revealed that decellularization may lead to lower stiffness of the muscular matrix. Cytotoxicity assay measured 80-90% viability of adipose-derived stem cells grown by indirect co-culture with decellularized samples. Notably, scaffold repopulation studies highlighted that the acellular matrices are bioactive supports promoting cell adhesion and proliferation, while subcutaneous implant in BALB/c mice revealed that the decellularized diaphragm did not elicit a severe immune reaction by the recipient, integrating with the host tissues and presenting preliminary signs of angiogenesis.

Taken together, these results proved the high bioactivity and biocompatibility of acellular diaphragmatic scaffolds, paving the way to carry out in vivo transplantation of human acellular diaphragmatic matrices into the damaged muscle of VML animal models.

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From LINC complex to signaling pathways: the mechanosignaling network under mechanical strain

Camilla Evangelisti[1], Stefano Ratti[1], Antonietta Fazio[1], Elisabetta Mattioli[2,3], Giovanna Lattanzi[2,3], Alberto Di Martino[1,2], Cesare Faldini[1,2], Lucia Manzoli[1]

[1] Alma Mater Studiorum-University of Bologna, DIBINEM, Bologna, Italy
[2] IRCCS Istituto Ortopedico Rizzoli, Bologna Italy
[3] CNR Institute of Molecular Genetics - IGM-CNR - Unit of Bologna, Bologna Italy

The muscle-tendon unit (MTU) regulates motor function downstream a plethora of environmental chemical and mechanical signals. The musculoskeletal system is characterized by a high degree of heterogeneity in terms of cell population, cell density, and physical and mechanical properties. The precise arrangement and interplay of all these components guarantees the necessary strength and resistance for muscle to bone force transmission. Not surprisingly, the MTU is clearly affected in several diseases, such as muscular dystrophies and aged-related diseases.

The mechanical stress that MTU must sustain and the relative risky rupture at the junctional sarcolemma level are sensed by membrane receptors and transduced via mechanosignaling. In particular, external signals are transmitted through the cytoskeleton to the nuclear envelope via LINC (linker of nucleoskeleton and cytoskeleton) complex, which includes nesprins, SUN1/2, A-types lamins and emerin, that in turn interacts with different signaling pathways.

Our study is aimed at identifying the main molecular interactions between the LINC complex and signaling pathways that regulate mechanosignaling crosstalk under physiological mechanical strain conditions (uniaxial mechanical strain 0.5Hz, 10%, 4h), comparing young and old myogenic cells obtained from human derived samples.

We showed that, upon induction of mechanical strain, differentiating myoblasts modulate expression of A-type lamins, downregulate LINC complex proteins at the nuclear envelope and rearrange the muscle-specific intermediate filament desmin network. In this context, lamin A/C binding to histone deacetylase 2 (HDAC2) is modulated. As a consequence, p21, one of the main HDAC2 target genes, that is upregulated during myoblast differentiation, undergoes different modulation in cells from old individuals.

Then, we investigated signaling pathways linking changes in mechanical strain to LINC modulation, with particular focus on phosphoinositide 3-Kinase (PI3K)/Akt/mechanistic target of rapamycin (mTOR) pathway. Our data on primary myoblasts and myotubes showed that these cells, after uniaxial mechanical strain, can transduce physical stimuli into biochemical stimuli through PI3K/Akt/mTOR signaling pathway, activating the phosphorylation of Ser473Akt, indicative of mTORC2 inhibition, and activating the phosphorylation of Ser235/236 S6RP, an mTORC1 downstream substrate, especially in young samples when compared to old samples. On the contrary, total Akt and S6RP levels were unaffected.

These preliminary results pave the way to a more comprehensive understanding of mechanosignaling and signaling pathways under mechanical strain in skeletal muscle cells.

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Characterization of collagen content and turnover in skeletal muscles in a mouse model of Duchenne muscular dystrophy: in vivo and in vitro analysis

Matteo Giovarelli[1], Laura Brigida Cornaghi[2], Silvia Zecchini[1], Cecilia Barbieri[1], Ambra Nava[2], Luca Denti[3], Michele Sommariva[2], Francesca Arnaboldi[2], Nicoletta Gagliano[2]

[1] Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan, Italy
[2] Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy
[3] Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy

Skeletal muscles of patients affected from Duchenne muscular dystrophy (DMD) are characterized by a progressive skeletal muscle degeneration and weakness, associated to the deposition of collagen (COL), leading to muscle fibrosis.

Since muscular fibrosis is a striking characteristic of DMD, this study was aimed at evaluating skeletal muscles fibrosis in a mouse model of DMD, by analyzing COL content by morphological methods. Moreover, COL turnover pathways in cultured primary muscle fibroblasts were also analyzed.

Quadriceps (QD), tibialis anterior (TA), gastrocnemius (GC) and diaphragm (DF) skeletal muscles, obtained from young (1-month-old) and adult (5-months-old) healthy (wt) and dystrophic mice (mdx), were fixed and paraffin embedded. COL content was assessed by Sirius red staining and expressed as fibrosis index.

Primary muscle fibroblasts were obtained from the QD muscle. COL-I, COL-III and matrix metalloproteinases (MMP)-1 levels and MMP-2 activity were assessed, respectively, by Slot blot and SDS-zymography in cell culture supernatants. Gene expression for lysyl oxidase (LOX) and lysyl hydroxylase 2 (LH2b), and TIMP-1 and 2 were analyzed by real time PCR.

COL content resulted similar in all the considered muscles of wt young mice while, when we compared wt adult mice, it increased in QD ($p<0.01$ vs TA, $p<0.05$ vs GC) and in DF ($p<0.01$ vs TA and GC). In 1-month-old mdx mice, COL content was significantly increased only in DF compared to the other muscles ($p<0.05$ vs QD, $p<0.01$ vs GC, $p< 0.005$ vs TA), while, in adult mdx mice, COL significantly increased in DF ($p< 0.005$ vs QD, TA and GC), in QD ($p<0.05$ vs TA) and GC ($p<0.01$ vs TA).

The fibrosis index, in adult mdx muscles, resulted significantly correlated with the percentage of centronucleated muscle fibers, formed during regeneration following necrosis and increased in adult mdx mice compared to young mdx for all analyzed muscles, suggesting that the development of DMD-associated fibrosis and the extent of muscle degeneration are tightly related.

COL-I and COL-III secreted by wt and mdx fibroblasts remained unaffected in young and adult mice. LOX tended to be up-regulated, whilst LH2b mRNA levels were significantly increased in both young and adult mdx compared to wt mice. MMP-1 and 2 were not affected, but TIMP-1 and 2 increased in fibroblasts of 5-month-old mdx mice.

Overall, morphological analysis showed that COL content increased at a different extent in the different skeletal muscles in wt. Moreover, fibrosis was more evident in mdx mice in QD and DF, suggesting that these are the muscles mainly affected. Indeed, DF, playing a crucial role in breathing, becomes highly fibrotic also in humans.

Molecular analysis revealed that higher collagen cross-linking and MMPs inhibition could act as major players in the mechanisms leading to muscular fibrosis, differently contributing to COL accumulation in the progression of DMD. In fact, in young mice fibrosis seems be dependent on increased collagen-crosslinking. Conversely, in adult mice, both increased collagen cross-

linking and the inhibition of its degradation could act as major molecular mechanisms to furtherly favor the expansion and the persistence of the fibrotic remodeling of dystrophic muscles.

Considered as a whole, our results could contribute to the characterization of the progression of fibrosis in DMD and the underlying mechanisms in order to find new therapeutic targets to effectively prevent muscle fibrosis and its progression.

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Beneficial effects of boosting skeletal muscle metabolism by SIRT1 activator in DMD mouse model

Matteo Giovarelli[1], Silvia Zecchini[1], Emilio Clementi[1,2], Clara De Palma[3]

1 Department of Biomedical and Clinical Sciences "Luigi Sacco" (DIBIC), Universit`a degli Studi di Milano, via GB Grassi 74, 20157 Milan, Italy
2 Scientific Institute, IRCCS Eugenio Medea, Laboratory of Molecular Biology, via Don Luigi Monza 20, 23842 Bosisio Parini, Italy
3 Department of Medical Biotechnology and Translational Medicine (BioMeTra), Universit`a degli Studi di Milano, via L Vanvitelli 32, 20129 Milan, Italy

Duchenne muscular dystrophy (DMD) is a severe and relentlessly progressive myopathy caused by out-of-frame or nonsense mutations in the X-linked DMD gene. DMD is a complex disease and multiple approaches are needed to target pathological processes, both the underlying genetic mutations and the secondary complications. Despite several therapeutic options have been developed with good results, more effective treatment options are essential and may be generated through the definition of novel therapeutic targets. A muscular metabolic dysregulation is an established DMD feature with mitochondrial dysfunctions as one of the earliest deficits that arise from multiple cellular stressors. Among metabolic regulators, Sirtuin 1 (SIRT1) represents an intriguing candidate since it acts on different aspects of cellular metabolism, regulating energy homeostasis, mitochondrial biogenesis, and inflammation. SIRT1 overexpression represents an important counter-mechanism to alleviate the dystrophic phenotype and its pharmacological modulation could be relevant as well in DMD conditions. Consistently, SIRT1 activation by selective compound, i.e., SRT2104, has already been proven to reinforce muscular structure, mitochondrial functionality, and to reduce inflammation. SRT2104 has never been tested in muscular diseases; therefore, considering its metabolic and immunomodulatory effects, we tested SRT2104 as an attractive candidate for DMD treatment. Accordingly, in our preliminary data, long-term SRT2104 administration improved muscle force and stimulated oxidative capacity. This was paralleled by reduced fibrosis and inflammatory infiltrate and increased regeneration in mdx muscle. In conclusion our results demonstrate the efficacy of SRT2104 as a new SIRT1 activator in DMD and highlight that considering DMD also as a metabolic disease and treating it as such, could provide important therapeutic strategies additional to gene therapies.

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Phospholipase C delta 4 is a nuclear protein involved in cell proliferation in rhabdomyosarcoma cells

Sara Salucci[1], Anna Bartoletti Stella[2], Alberto Bavelloni[3], Roberta Fiume[1], Sabrina Burattini[4], Pietro Gobbi[4], Vittorio Sambri [2], Irene Faenza[1]

[1] Cellular Signalling Laboratory, Department of Biomedical and NeuroMotor Sciences (DIBINEM), University of Bologna, 40126 Bologna, Italy
[2] Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, 40126 Bologna, Italy
[3] Laboratory of Experimental Oncology, IRCCS Istituto Ortopedico Rizzoli, 40136 Bologna, Italy
[4] Department of Biomolecular Sciences (DISB), Urbino University Carlo Bo, 61029 Urbino, Italy

Rhabdomyosarcoma (RMS) is a highly malignant and metastatic pediatric cancer arising from skeletal muscle myogenic progenitors. The current classification defines five different histotypes, with embryonal (eRMS) and alveolar subsets being the most frequently observed in children <5 years and in adolescents, respectively[1]. The genetic alterations characterizing eRMS lead to uncontrolled cell growth and to the interruption of proper myogenic differentiation. In this context, PLC delta 4, identified as a nuclear protein in different cell types where it drives proliferative processes[2,3], could have a role in the increase of proliferative state in eRMS too. Curcumin, a phenolic yellow compound found in Curcuma Longa, has a wide range of biologic and pharmacological activities: anti-inflammatory, antidiabetic mellitus and antioxidant. Recently, the anticancer effect of curcumin has garnered considerable attention[4]. Our preliminary data showed curcumin ability to decrease PLC delta 4 and cyclin expression. Curcumin blocks eRMS cell proliferation by inducing apoptotic cell death. Downregulation of nuclear PLC delta 4 signaling by curcumin could be a new strategy for RMS treatment.

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Innervation of the superficial fascia

Carla Stecco[1], Caterina Fede[1], Lucia Petrelli[1], Raffaele De Caro[1]

[1] Dipartimento di Neuroscienze, Università degli studi di Padova

Introduction/Background: The superficial fascia has only recently been recognized as a specific anatomical structure in its own right anatomical entity, being first considered as included in the hypodermis. Furthermore, whereas it is actually recognized that the innervation of the deep/muscular fascia plays a key role in proprioception and nociception [1] [2], and there are studies highlighting the cell populations and the extracellular matrix characterization of the deep fascia [3], there are very few studies that have analyzed these characteristics in the superficial fascia.

Methods: Our group analyzed two different anatomical districts (abdomen and thigh), from cadavers, obtained from the 'Body Donation Programme' at the Institute of Anatomy, University of Padova, and from volunteers patients, undergoing elective surgery procedures at the Orthopaedic Clinic of Padova, with a research approved by the Institutional Ethical Committee (n.3722/AO/16). Each sample was processed for histological and morphometric analysis by Hematoxylin&Eosin, Weigert-Van Gieson for elastic fibers, Alcian Blue pH 2.5 for glycosaminoglycans. Furthermore, immuhistochemistry stainings were performed, with antibodies specific for extracellular matrix components (Collagen-I, Collagen-III, HABP-hyaluronic acid binding protein), and for nervous fibers (S100 antibody for the myelin-forming cells, PGP9.5 antibody as neuronal marker, Tyrosine Hydroxylase for autonomic innervation).

Results: The superficial fascia is the second most highly innervated tissue after the skin, with a density of 33.0 ± 2.5/cm2, and a mean nerve sizes of $19.1 \pm 7.2 \mu$m [4]. Free nerve endings innervate the tissue, and autonomic nerve fibers are present in the blood vessels, in the areas of vascularization and near adipocytes and in the connective tissue itself. Fibroblasts, myofibroblasts, mast cells are evident in the tissue. Finally, the elastic fibers are more abundant in the superficial fascia than the deep fascia, demonstrating that the superficial fasciae are more adaptable [5].

Conclusion: In the light of these findings is evident that the superficial fasciae have a clear and distinct anatomical entity, and that they should be considered according to their characteristics, innervation and vascularization to better understand their role in thermoregulation, exteroception and pain perception. The knowledge of the superficial fascia may improve grading and developing of different manual approach for treatments of fascial dysfunctions.

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An update on the expression of sarcoglycan sub-complex in adipose tissue

Giovanna Vermiglio[1], Antonio Centofanti[1], Natasha Irrera[2], Alessandra Bitto[2], Giacomo Picciolo[1], Debora Di Mauro[1], Saverio Cinti[3], Giuseppina Cutroneo[1]

[1] University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia
[2] University of Messina, Department of Clinical and Experimental Medicine, Messina, Italia
[3]Università delle Marche, Department of Experimental and Clinical Medicine, Ancona, Italia

The Sarcoglycan sub-complex (SGC) is a glycoprotein system that is expressed in several type of tissue such as the adipose tissue [1]; although that, only the role it plays in muscle tissue is known. Our previous immunofluorescence data have shown the expression of these proteins in mouse adipose tissue and an increased expression in brown adipocytes if compared to white adipocytes. The aim of the present study was to perform a quantification of sarcoglycan sub-complex expression both in white and brown adipocytes and during trans-differentiation from white to brown. To perform this study, we used culture of 3T3L1 cells that were induced to transdifferentiation using agonists of β3-receptors. After that, we have analysed the expression of SGC in white adipocytes and in brown adipocytes obtained by transdifferentiation by immunofluorescence technique, RT PCR and western blot. Our results showed that all SGC is expressed in adipocytes and its expression increase after trans-differentiation. These data support strongly the SGC is a protein complex that plays different role from role played in muscle tissue. It will be necessary to clarify the exact role of SGC in adipose tissue.

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Cellule staminali, istogenesi e differenziamento

Prima sessione

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A stem/progenitor cell population with liver regenerative potency is harbored in human duodenal submucosal glands

Guido Carpino[1], Vincenzo Cardinale[2], Antonio Franchitto[2], Diletta Overi[2], Lola Reid[3], Domenico Alvaro[2], Eugenio Gaudio[2]

[1] University of Rome "Foro Italico"
[2] Sapienza University of Rome
[3] University of North Carolina, NC, USA

Common hepato-bilio-pancreatic progenitors exist at an early stage of development in the primitive duodenum [1] and persists in the adult biliary tree [2]. Our aim was to identify and characterize putative endoderm progenitor cells with liver fate potency persisting in the adult human duodenal submucosal layer.

Human duodena were obtained from organ donors and were characterized by immunohistochemistry and immunofluorescence. Putative progenitor cells were isolated after removal of the mucosa layer. Cells were cultured on plastic or as organoids and were transplanted into severe combined immunodeficient (SCID) mouse livers.

In situ studies of human duodenum revealed that progenitor-like cells were endowed in duodenal submucosal glands, and they showed a unique phenotype distinguishable from intestinal crypt cells. The genetic signature studies indicated that duodenal submucosal (dSG) cells are closer to biliary tree stem cells and to definitive endodermal cells rather than adult hepatocytes. In vitro, human dSG cells demonstrated clonal growth, capability to form organoids, and ability to acquire functional hepatocyte traits. In vivo, transplanted cells engrafted into the livers of SCID mice, showed differentiation to mature hepatocytes, and were able to rescue hosts from liver damage by supporting repopulation of the murine liver.

In conclusions, a progenitor-like cell population with clonal growth and organoid formation capability is present in adult duodenum and endowed within submucosal glands; these cells have liver differentiation potency *in vitro* and *in vivo*. These cells can be isolated, do not require reprogramming, and thus represent a readily available cell source for regenerative medicine of the liver including autologous cell therapies.

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YAP/TAZ activity prevents tissue senescence and aging by controlling cGAS/STING in stromal cells [1]

Michelangelo Cordenonsi[1], Hanna Lucie Sladitschek-Martens[1], Alberto Guarnieri[1], Giulia Brumana[1], Tito Panciera[1], Francesca Zanconato[1], and Stefano Piccolo[1,2]

[1] Department of Molecular Medicine, University of Padua, Padua, Italy
[2] IFOM ETS, the AIRC Institute of Molecular Oncology

Ageing is intimately connected to induction of cell senescence, but why it is so remains poorly understood. A key challenge is the identification of pathways normally suppressing senescence, lost during ageing, and functionally relevant to oppose ageing. Here we connected the structural and functional decline of ageing tissues to attenuated function of YAP/TAZ, master effectors of cellular mechanosignaling. YAP/TAZ activity declines during physiological ageing in cells of the connective and smooth muscle tissues; recapitulating such decline through YAP/TAZ genetic inactivation in the same tissues leads to accelerated ageing. Conversely, sustaining YAP function rejuvenates old cells and opposes the emergence of ageing-related traits. Ageing traits induced by YAP/TAZ inactivation are preceded by induction of tissue senescence. This occurs because YAP/TAZ mechanotransduction suppresses cGAS/STING signaling, to the extent that STING inhibition strikingly prevents tissue senescence and premature aging-related tissue degenerations after YAP/TAZ inactivation. The findings demonstrate that YAP/TAZ regulation is causal to the aging process, as such linking mechanobiology to cGAS/STING, a pillar of innate immunity. Thus, sustaining YAP/TAZ mechanosignaling or inhibiting STING may represent promising approaches to limit senescence-associated inflammation and improve healthy ageing.

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Novel CAPE derivatives enhance DPSC bone differentiation

Viviana di Giacomo[1], Marwa Balaha[1,2], Monica Rapino[3], Agata Przekora-Kuśmierz[4], Paulina Kazimierczak[4] and Amelia Cataldi[1]

[1]Department f Pharmacy, University G d'Annunzio, Chieti-Pescara, Italy
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kafrelsheikh University, Egypt
[3] Genetic Molecular Institute of CNR, Unit of Chieti, University G d'Annunzio, Chieti-Pescara, Italy
[4] Independent Unit of Tissue Engineering and Regenerative Medicine, Medical University of Lublin, Lublin, Poland

Caffeic acid phenethyl ester (CAPE) is one of the most extensively investigated active components of propolis because of its numerous pharmacological activities, including anti-oxidant, anti-inflammatory, anti-viral, anti-fungal, and regenerative properties. Two different novel series of CAPE derivatives were designed, synthesized and their potential in bone regeneration was investigated. First, their effects on mesenchymal stem cell viability was evaluated: all the compounds showed no cytotoxicity and four of them appeared to stimulate cell proliferation at very low concentrations, in a manner similar to the reference compounds. Subsequently, Dental Pulp Stem Cells (DPSCs) were induced to osteogenic differentiation in presence of the most promising compounds (0-5 μM) and cell proliferation, Alizarin Red Staining (ARS), alkalyn phosphatase (ALP) activity and BMP2, SP7 and DSPP gene expression were evaluated at 0, 7, 14, 21 and 28 days.

DPSC exposed to all the compounds showed an increase in AR staining and in ALP activity at all the experimental times, and a modulation of the gene expression of the three well known markers of osteogenic differentiation. The adsorption of the compounds on a biomaterial synthesized for bone regeneration improved osteoblast adhesion and proliferation, as proved by preliminary results in confocal and stereoscopic microscopy.

Concluding, our novel synthesized CAPE derivatives appeared to positively regulate osteogenic differentiation, showing a promising potential in regenerative medicine.

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Inflammatory microenvironment licensing immunoregulatory function of dental pulp stem cells

Rosanna Di Tinco[1], Giulia Bertani[1], Giulia Orlandi[1], Alessandra Pisciotta[1], Laura Bertoni[1], Jessika Bertacchini[1], Paola Sena[1], Carlo Salvarani[2], Gianluca Carnevale[1]

[1] Department of Surgery, Medicine Dentistry and Morphological Sciences with Interest in Transplant, University of Modena and Reggio Emilia, Modena, Italy
[2] Rheumatology Unit, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy

Introduction & Aim: Mesenchymal stromal/stem cells (MSCs) have been widely characterized for their regenerative and immunomodulatory properties. Recent studies reported that MSCs can contribute to tissue regeneration by modulating inflammatory microenvironment suggesting how these properties are strictly related [1]. MSCs can be isolated from different adult tissues including dental pulp. Human dental pulp stromal/stem cells (hDPSCs) are characterized by multipotency and low immunogenicity, as well as by immunomodulatory properties exerted by different mechanisms (i.e., Fas/FasL pathway) after exposure to an inflammatory microenvironment [2,3]. Among these immunoregulatory mechanisms, PD-1/PD-L1 pathway seems to be an important effector in the suppression of immune system activation and its role is not fully understood in hDPSCs [4]. Therefore, the present study aimed to 1) investigate the downstream mechanism of a new immune-regulatory pathway, i.e., PD-1/PD-L1, in hDPSCs 2) how the inflammatory microenvironment might affect hDPSCs immunomodulatory potential.

Methods: Inflammatory microenvironment was created *in vitro* by the activation of peripheral blood mononuclear cells (PBMCs) isolated from healthy donors and rheumatoid arthritis (RA) patients with anti-CD3 and anti-CD28 antibodies. Direct and indirect co-cultures between hDPSCs and PBMCs were carried out to evaluate the activation of immunomodulatory checkpoints in hDPSCs and the inflammatory pattern in PBMCs through Real-Time PCR, Western Blot, Immunofluorescence and Immunohistochemistry analyses.

Results: Our data revealed that hDPSCs up-regulate PD-L1 and IL6 via both direct and indirect interaction-dependent mechanisms in response to exposure to CD3/CD28 co-stimulated PBMCs (aPBMCs). This ability is strictly related to hDPSCs stemness status. Moreover, as demonstrated by using a selective PD-L1 inhibitor, hDPSCs were able to activate compensatory pathways (i.e., Fas/FasL). At the same time, hDPSCs are able to control the inflammatory process by modulating pro-inflammatory cytokines expression in aPBMCs.

In the second step of our study, we investigate how PD-L1, IL6 and FasL expression might be affected by different inflammatory conditions mimicked by direct and indirect hDPSCs-aPBMCs co-cultures at different ratios and timepoints. Our data revealed that PD-L1 and IL6 mRNA levels show the same trend in both direct and indirect co-culture systems, regardless hDPSCs-aPBMCs ratio and timepoints. On the other hand, FasL mRNA levels were significantly up-regulated in hDPSCs only after a direct contact with aPBMCs, with an increasing trend across the different hDPSCs-aPBMCs ratios. The up-regulation of FasL mRNA levels in hDPSCs was also observed after indirect co-cultures only at later timepoints. Future studies aimed at deeply investigating the molecular mechanisms involved will corroborate these results.

Conclusions: Our results pointed out that DPSCs can modulate the inflammatory microenvironment by the activation of PD-1/PD-L1 pathway synergistically cooperating with other immune-regulatory pathways including Fas/FasL. These immunomodulatory properties are orchestrated by the inflammatory microenvironment. Based on this evidence, these properties might be functional in controlling the inflammatory milieu typical of autoimmune diseases, such as rheumatoid arthritis.

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Human neuromuscular junction on a chip: impact of stem cell derived extracellular vesicles on muscle atrophy and nerve health

Martina Gatti[1], Francesca Beretti[1], Manuela Zavatti[1], Maurilio Sampaolesi[2], Tullia Maraldi[1]

[1] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena 41125, Italy
[2] Department of Development and Regeneration, Translational Cardiomyology, KU Leuven, Leuven, Belgium and Department of Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza University of Rome

Neuromuscular junctions (NMJs) are specialized synapses, which are crucial for the communication between spinal lower motor neurons (MNs) and skeletal muscle. NMJs become vulnerable in degenerative diseases, such as muscle atrophy, where the impairment of NMJs results in muscle weakness. In this dramatic context, the crosstalk between the different cell populations fails, and the regeneration ability of the entire tissue is hampered. How skeletal muscle and NMJ send retrograde signals to motor neurons represents an intriguing field of research. In order to study the perturbation in NMJs occurring in muscle atrophy, a MN/myotube system was generated through commercially available microfluidic devices (Dittlau et al., 2021), and muscle atrophy was induced *in vitro* by treatment with dexamethasone (DEX), a synthetic glucocorticoid.

Recent works demonstrate paracrine actions of stem cells, including amniotic fluid stem cells (hAFSC), that stimulate adult myogenesis. In particular, extracellular vesicles (EVs) represent promising candidates for hAFSC-mediated muscle regeneration. Studies on the regulation of NMJs by EVs are still lacking, therefore, we treated healthy or atrophic systems of NMJs with hAFSC-derived EVs. The presence of EVs reduced morphological and functional defects induced by DEX.

A possible regulation of the redox balance has been reported to be affected in the NMJ area, but the specific role of oxidative stress and their sources in NMJ dysfunctions remains poorly understood. As a final aim of the study, we investigated how hAFSC-EVs can modulate the interplay between muscle and MNs through a ROS balance: oxidative stress, occurring in myotubes treated with DEX and thus involving neurites as well, was prevented by EV incubation.

In conclusion, we provided validated fluidically isolated system for studying human motor neuron and myoblast cell interactions in healthy and atrophic conditions, allowing the isolation of subcellular compartments for region-specific analyses, and demonstrated the efficacy of hAFSC-EVs in counteracting NMJ perturbations.

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Protective effect of pituitary adenylate cyclase-activating polypeptide in the cornea

Grazia Maugeri[1], Agata Grazia D'amico[2], Benedetta Magrì[1] and Velia D'agata[1]

[1] Section of Anatomy, Histology and Movement Sciences, Department of Biomedical and Biotechnological Sciences, University of Catania, 95100 Catania, Italy
[2] Department of Drug Sciences, University of Catania, 95125 Catania, Italy

The cornea is a transparent tissue covering the eyeball's anterior portion. It is mechanically strong, ensures protection and provides about 70% eye refractive power. Due to its direct connection with the external environment, the cornea is highly exposed to different types of insults, such as ultraviolet B (UV-B) radiations. For this reason, corneal injury is one of the main causes of blindness in the world. In the last years, many studies have shown the beneficial effects of pituitary adenylate cyclase activating peptide (PACAP) in different eye diseases. Some PACAP effects are mediated by the transactivation of epidermal growth factor receptor (EGFR) and/or stimulation of activity-dependent protein (ADNP). However, the role of PACAP on corneal endothelium and epithelium has not been investigated, yet. Therefore, first we evaluated the expression and the effect of PACAP on human corneal endothelial cells (HCECs). Our results have shown, for the first time, that PACAP and related receptors are expressed in HCECs (1). Moreover, the peptide maintained human corneal endothelium integrity after growth factors deprivation, through the phosphorylation of EGFR, which in turn promoted the MAPK/ERK1/2 signaling pathway activation (2). Second, we analyzed the expression and the role of ADNP on corneal epithelial cells exposed to UV-B radiations. Our results showed for the first time, ADNP expression in human and rabbit corneal epithelium. Furthermore, the treatment with ADNP mimicking peptide, NAP, decreases ROS production and JNK signaling pathway activation, by counteracting UV-B-rays-induced apoptotic cell death (3). Overall, these data suggested that PACAP or NAP might represent a valid strategy for the treatment of some corneal diseases.

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Inhibition of endolysosomal two-pore channel 2 (TPC2) affects the osteoclastogenesis by modulation of autophagy process

Azadeh Montaseri[1], Michela Rossi[2], Fioretta Palombi[1], Samantha Barbonari[1], Silvia Sideri[1], Anna Riccioli[1], Simonetta Petrungaro[1], Claudia Giampietri[1], Andrea Del Fattore[2], Antonio Filippini[1]

[1] Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Unit of Histology and Medical Embryology, Sapienza University of Rome, 00161 Rome, Italy
[2] Bone Physiopathology Research Unit, Genetics and Rare Diseases Research Division, Bambino Gesu` Children Hospital, IRCCS, 00167 Rome, Italy

Two-pore channels 2 (TPC2) are voltage-and ligand-gated cation channels located in the lysosome membrane [1]. TPC2 is associated with control of autophagy pathway by regulating the release of Ca^{2+} and Na^{+} and control of lysosomal pH [2]. Interestingly, autophagy has an important role in regulation of podosome and ruffled border formation and bone resorption activity by osteoclast [3]. Based on these, we investigated the interaction between TPC2 and the autophagy pathway during osteoclast differentiation. For this purpose, peripheral blood mononuclear cells (PBMCs) have been treated with naringenin (50μM, 100μM and 200 μM), as a pharmacological TPC2 inhibitor, for 2 weeks. We reported increased formation of giant and multinuclear osteoclasts with evident podosomes and F-actin ring by naringenin at 50μM while reduced osteoclastogenesis was seen after treatment of PBMCs with naringenin at 200μM. Furthermore, TPC2 inhibition by naringenin at high dose upregulated the expression of beclin1 and P62 and significantly reduced that of the LC3II/I, while TPC2 inhibition by naringenin at low concentrations did not change the expression of autophagy markers. We can conclude that TPC2 inhibition by naringenin has a bi-phasic effect on osteoclastogenesis and can impede the autophagy completion by targeting the release of Na^{+} and Ca^{2+} from lysosomes which subsequently modifies the lysosomal pH and prevents the lysosome-autophagosome fusion.

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Effects of dynamic shear stress on the biological properties of pericyte-like cells from human dental pulp in an inflammatory microenvironment

Alessandra Pisciotta[1], Giulia Bertani[1], Rosanna Di Tinco[1], Laura Bertoni[1], Giulia Orlandi[1], Paola Sena[1], Michele Signore[2], Ruggero de Maria[3], Jessika Bertacchini[1], Gianluca Carnevale[1]

[1] Department of Surgery, Medicine Dentistry and Morphological Sciences with Interest in Transplant, University of Modena and Reggio Emilia, Modena, Italy
[2] RPPA Unit, Proteomics Area, Core Facilities, Istituto Superiore di Sanità, Rome, Italy
[3] Institute of General Pathology, Università Cattolica del Sacro Cuore, Facoltà di Medicina e Chirurgia, Rome, Italy

Human dental pulp stem cells represent a mesenchymal stem cell niche localized in the perivascular area of dental pulp and are characterized by low immunogenicity and immunomodulatory/anti-inflammatory properties (1). Pericytes are mural cells that surround the endothelium of small vessels. They regulate numerous functions including vessel growth, stabilization and permeability (2). Pericytes localized in the head, dental pulp, thymus and the outflow tract of the aorta originate from the neural crest. It is well established that pericytes have a tight cross-talk with endothelial cells in neo-angiogenesis and vessel stabilization (3). These processes are regulated by different factors including the microenvironment and flow shear stress modifications. The aim of this study was to evaluate the effects of a pulsatile unidirectional flow in presence or not of an inflammatory microenvironment on the biological properties of pericyte-like cells isolated from human dental pulp (hDPSCs).

Human DPSCs were cultured under both static and dynamic conditions with or without peripheral blood mononuclear cells pre-activated with anti-CD3 and anti-CD28 antibodies (aPBMCs). Pulsatile unidirectional flow shear stress was generated by using a specific peristaltic pump. The evaluation of the angiogenic potential and inflammatory properties of hDPSCs was carried out through Reverse Phase Protein microarrays (RPPA), confocal immunofluorescence and western blot analyses.

Our data showed that hDPSCs expressed the typical endothelial markers, that were upregulated when cultured with an endothelial induction medium (VEGF, Tie2, Angpt1, eNOS). The endothelial commitment was further confirmed by the ability of hDPSCs to form tube-like structures. RPPA analyses revealed that these properties were modulated when a pulsatile unidirectional flow shear stress was applied to hDPSCs. Moreover, the expression of immune-modulatory molecules, such as PD-L1, was downregulated and in parallel the pro-inflammatory molecule NF-kB was upregulated, when compared to standard static culture conditions. In particular, this data was confirmed by creating an inflammatory microenvironment. Our results highlighted that a further reduction of immune-modulatory properties was induced in hDPSCs. This evidence was strengthened by the detection of upregulated levels of expression of pro-inflammatory cytokines in aPBMCs.

In conclusion, the application of a pulsatile unidirectional flow shear stress induced a modulation of immuno-modulatory/inflammatory properties of dental pulp pericyte-like cells.

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Bone marrow morphology in myeloproliferative neoplasms: tracking fibrosis by CCR2 expression on CD34$^+$ cells

Giulia Pozzi[1], Cecilia Carubbi[1], Giuliana Gobbi[1], Prisco Mirandola[1], Marco Vitale[1,2], Elena Masselli[1,2]

[1] Department of Medicine and Surgery (DiMeC), University of Parma, Parma, Italy
[2] Parma University Hospital, AOU-PR, Parma, Italy

Bone marrow fibrosis is a central pathological feature of MPN contributing to an impaired microenvironment (the so called "bad soil") favoring malignant over normal hematopoiesis (1). Megakaryocytes (MKs) play a crucial role in this process by the overproduction of a plethora of pro-inflammatory cytokines that favor the proliferation of the malignant clone by perturbing the bone marrow microenvironment (2, 3). In MPN, higher grade of bone marrow fibrosis dictates a more severe disease stage with dismal prognosis and higher risk of leukemic evolution (4, 5). Therefore, accurate patient allocation into different disease categories and timely identification of fibrotic transformation are mandatory for adequate treatment planning, including bone marrow transplant for eligible patients. Diagnostic strategy still mainly relies on clinical/laboratory assessment and bone marrow histopathology, which, however, requires an invasive procedure and frequently poses challenges also to expert hemopathologists (6, 7).

Here we tested the diagnostic accuracy of the detection, by FCM, of peripheral blood/bone marrow CCR2$^+$CD34$^+$ cells to discriminate among MPN subtypes with different degrees of bone marrow fibrosis. We found that detection of CCR2 on MPN CD34$^+$ cells has a very good diagnostic accuracy for the differential diagnosis between "true" ET and prePMF (AUC 0.892, $P<0.0001$), and a good diagnostic accuracy for the differential diagnosis between prePMF and overtPMF (AUC 0.817, $P=0.0089$). Remarkably, in MPN population, the percentage of CCR2-expressing cells parallels the degree of bone marrow fibrosis. In 12 ET/PV patients with a clinical picture suggestive for transition into spent phase, we demonstrate that only patients with confirmed sMF (i.e., bone marrow fibrosis ≥ 2 at histopathology) showed significantly higher levels of CCR2$^+$CD34$^+$ cells as compared to those with only mild increased of reticulin fibers, suggesting that the expression of this receptor on hematopoietic progenitors can effectively track fibrotic changes in MPNs. Overall, FCM CCR2$^+$CD34$^+$ cells detection can be envisioned in support of conventional bone marrow morphology at the time of disease diagnosis in compelling clinical scenarios, with the great advantage of being extremely rapid (with reports available in few hours). For patients in follow-up, its role can be conceived as an initial patient screening (for subsequent bone marrow biopsy) when disease evolution is suspected.

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Mesenchymal stem cell-conditioned medium promotes vascularization of nanostructured scaffold transplanted into nude mice: a morphological study

Borgese M.[2], Barone L.[1], Gornati R.[1], Reguzzoni M.[2]

[1] University of Insubria, Department of Biotechnology and Life Science
[2] University of Insubria, Department of Medicine and Surgery, Laboratory of Human Morphology

Human adult mesenchymal stem cells have mainly been studied in the past decade due to their multilineage differentiation and potential use in many cell-based therapies. However, in the last few years, there has been a growing body of evidence suggesting that the role of hMSCs in tissue regeneration is mainly due to their secretion of pro-angiogenic, anti-apoptotic and antiinflammatory factors known as a paracrine effect. The increasing evidence showing the potential of hMSC secretome has led to the acknowledgement that the use of hMSC conditioned medium may represent a valid alternative to the use of stem cells, overcoming the main obstacles related to cell sample handling survival and rejection. Accordingly, this study focuses on the characterisation and in vivo application of hMSCs conditioned medium (CM). To this aim, hMSCs have been isolated from two different sources, adipose tissue (hASCs) and dental pulp (hDPSCs). Although hASCs have been largely studied, very few is known about hDPSCs. Therefore, hDPSCs have been characterised by FACS, qPCR and immunofluorescence up to their 30th passage to confirm their stemness maintenance over long culture. hASCs and hDPSCs CMs, obtained after 72h of starvation in both normoxic and hypoxic conditions, have been concentrated and characterised by ELISA to evaluate the effect of hypoxia on the release of proangiogenic factors. To compare the pro-angiogenic potential of hMSC secretome vs the cells, the hASCs and hDPSCs CMs, obtained in normoxic conditions, have been mixed with a collagen scaffold, INTEGRA® Flowable Wound Matrix, and grafted in BALB-C nude athymic mice for 28 days. Even though an exhaustive characterisation of the conditioned culture medium, which also includes the microvesicle fraction, is still in progress, the data obtained demonstrated that Integra® FWM associated with CM showed the same efficiency as Integra® FWM related to cells in promoting cellular invasion and capillary growth. This encourages the cell-free approach for damaged tissue regeneration.

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IL-1 Associated Post Senescence Reprogramming Impairs Response To EGFR Neutralization

Donatella Romaniello[*1,2], Valerio Gelfo[*1,2], Federica Pagano[1], Enea Ferlizza[1], Michela Sgarzi[1], Martina Mazzeschi[1], Alessandra Morselli[1], Carmen Miano[3], Moshit Lindzen[4], Gabriele D'Uva[3] Francesco Fazi[5], Luca Tamagnone[6], Karim Rihawi [7], Andrea Ardizzoni [1,7], Yosef Yarden[4] and Mattia Lauriola[1,2]

[1] Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, 40126, Italy
[2] Centre for Applied Biomedical Research (CRBA), Bologna University Hospital Authority St Orsola-Malpighi Polyclinic, Bologna, Italy
[3] National Laboratory of Molecular Biology and Stem Cell Engineering, National Institute of Biostructures and Biosystems (INBB), Bologna, Italy
[4] Department of Biological Regulation, Weizmann Institute of Science, Re- hovot, Israel
[5] Centre Department of Anatomical, Histological, Forensic and Orthopaedic Sciences, Sapienza University of Rome, 00185, Rome, Italy
[6] Istituto di Istologia ed Embriologia, Universita` Cattolica del Sacro Cuore, 10168 Rome, Italy
[7] Medical Oncology, IRCSS Azienda-Ospedaliero Universitaria di Bologna, Via Albertoni 15, Bologna, 40138, Italy
[*] Equal contribution

Tumor heterogeneity and cell plasticity, guided by drug pressure, play both an important role in generating multiple escaping routes, thus establishing resistance to therapy. Pro-inflammatory environments often trigger cellular stress activating senes- cence programs. For long time senescence has been considered the conclu- sion of the life-cycle with a permanent growth arrest in normal cells. Several intrinsic or extrinsic stimuli, characterized by the secretion of cytokines or chemokines have been reported involved in this process, referred as senes- cence-associated secretory phenotype, SASP. Interestingly, recent findings suggested that in tumor cells, senescence is a dynamic process activated also by therapeutical agents (namely therapy-induced senescence, TIS). During this time, defined as "pseudo-senescence, tumor cells, surrounded by pro- longed SASP environment are reprogrammed to re-enter the cell cycle (post- senescence) becoming newly tumorigenic and promoting tumor relapse [1- 5].

Previously, in our laboratory, we reported a striking correlation between re- duced sensitivity to CTX and increased expression of IL-1 [5-7]. Moreover, gene expression profile of 1700 CRC patients reveled that high IL-1 receptor 1 (IL-1R1) level is associated with poor survival [6]. Finally, the aim of this work was to explore the mechanism employed by IL-1 to orchestrate CTX resistance in mCRC. By using two CRC cell lines under CTX treatment we proved that IL-1 harness an escaping mechanism, known as "pseudo-senes- cence", to drive cells into cell cycle arrest. Later on, a chronic IL-1 exposure proved to re-program CRC cells into a post-senescence state, becoming highly proliferative, thus acquiring stemness properties and resistance to CTX, with a tendency toward the drug-addiction [8]. This model was vali- dated by using a recombinant decoy able to sequester IL-1 from the medium (TRAP IL-1). Indeed, in CTX sensitive cells, TRAP IL-1 inhibited CTX ac- tivity by restoring cell growth. On the contrary, sequester IL-1 in CTX re- sistant cells, blocked cell proliferation [13]. Moreover, a syngeneic engraft- ment in C57BL/6N (immunocompetent mice) of engineered MC38 cells ex- pressing both high murine EGFR and murine TRAP IL-1 displayed an im- pressive low growth tumor rate when compared with control transfected with murine EGFR. In conclusion, pharmacologically targeting IL-1 may repre- sent a viable strategy to restore CTX efficacy in CRC patients.

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Implication of cellular senescence in osteoarthritis. A study on equine synovial fluid mesenchymal stromal cells

Gabriella Teti[1], Valentina Gatta[1], Francesca Chiarini[2,3], Alessandra Ruggeri[1], Mirella Falconi[4]

[1] Department of Biomedical and Neuromotor Sciences, University di Bologna, Bologna, Italy
[2] CNR-National Research Council of Italy, Institute of Molecular Genetics, Unit of Bologna, 40136 Bologna, Italy
[3] IRCCS Istituto Ortopedico Rizzoli, Bologna, 40136, Italy
[4] Department of Experimental, Diagnostic and Specialty Medicine - DIMES

Osteoarthritis (OA) is the most common degenerative orthopedic disease in humans. It is described as a chronic degenerative disease marked by the loss of articular cartilage, joint remodeling, and changes in the synovial membrane associated with inflammation. Senescence is a natural cellular response to stressors including telomere dysfunction, DNA damage, and oncogene activation. It is characterized by arrested cellular proliferation and the induction of a distinctive secretory phenotype (SASP). Beneficial in certain conditions, the accumulation of senescent cells has been implicated in the pathophysiology of many diseases associated with aging, including OA.

Although the cell therapy based on the application of mesenchymal stem/stromal cells (MSCs) and/or their secretome represents a promising tool for OA treatment, the recent discovery that synovial MSC preparations isolated from OA patients contain many senescent cells that inhibit cartilage regeneration limits their clinical application. However, it is not fully understood how the senescence of synovial MSCs can contribute to the OA development. Therefore, in this study we aim to characterize and compare synovial fluid MSCs (sf-MSCs) isolated from OA joints with healthy sf-MSCs investigating the senescence hallmarks and how they affect the chondrogenic potential of OA-MSCs.

Sf-MSCs were isolated from tibio-tarsal joints of healthy and diseased horsed with established diagnosis of OA with an age ranging from 8 to 22 years. Cells were cultured in vitro and characterized for cell proliferation assay, cell cycle analysis, ROS detection assay, ultrastructure analysis, evaluation of senescent markers such as $p21^{CIP}$ and $p16^{INK4}$ with the aim of highlighting the presence of senescence phenotype in cells derived from OA joints. To evaluate the influence of OA on chondrogenic differentiation, sf-MSCs isolated from diseased joint were stimulated in vitro for up to 21 days with chondrogenic factors, the expression of chondrogenic markers was checked and compared to healthy sf-MSCs (h-MSCs).

Results clearly showed an arrest of cell cycle in combination with upregulation of the senescent markers $p21^{CIP}$ and $p16^{INK4}$, reduced autophagy and increased ROS production in cells isolated from OA joints, demonstrating a senescent state. Furthermore, OA MSCs showed a reduced ability in synthetizing proteoglycans while the ability to express collagen II is upregulated, suggesting the involvement of OA MSCs in developing a fibrotic joint environment.

In conclusion, our results demonstrate the presence of senescent sf-MSCs which could have a key role in OA onset and development and articular aging.

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Cellule staminali, istogenesi e differenziamento

Seconda sessione

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The CXCR1/CXCR2 inhibitor Reparixin Alters the Development of Myelofibrosis in the *Gata1*^{low} Mice

Francesca Arciprete[1], Paola Verachi[2], Francesca Gobbo[3], Fabrizio Martelli[4], Andrea Martinelli [5], Ronald Hoffman[7,9], Anna Rita Migliaccio [1,7,8], Maria Teresa Massucci[9], Laura Brandolini[9], Cristina Giorgio[10] , Marcello Allegretti [10], Giuseppe Sarli[2] and Maria Zingariello[1]

[1] Department of Medicine, Campus Biomedico, Rome, Italy
[2] Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum University, Bologna, Italy
[3] Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy
[4] National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy
[5] Center for animal experimentation and well-being, Istituto Superiore di Santà, Rome, Italy
[6] Myeloproliferative Neoplasm Research Consortium
[7] Division of Hematology/Oncology, Tisch Cancer Institute and Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[8] Altius Institute for Biomedical Sciences, Seattle, WA, USA
[9] Dompé Farmaceutici Spa R&D, L'Aquila, Italy
[10] Dompé Farmaceutici Spa R&D, Napoli, Italy

A major role for human (h)CXCL8 (interleukin-8) in the pathobiology of myelofibrosis (MF), the most severe of Philadelphia chromosome-negative myeloproliferative neoplasms, has been suggested by observations indicating that MF megakaryocytes express increased levels of hCXCL8 and that plasma levels of this cytokine in MF patients are predictive of poor patient outcomes (1,2). Here, we demonstrate that, in addition to high levels of TGF-β, the megakaryocytes from the bone marrow of the *Gata1*^{low} mouse model of myelofibrosis express high levels of murine (m) CXCL1, the murine equivalent of hCXCL8, and of its receptors CXCR1 and CXCR2. Using these data as a foundation, we tested the hypothesis that treatment with the CXCR1/R2 inhibitor Reparixin cures myelofibrosis in Gata1^{low} mice. Treatment with Reparixin in aged-matched *Gata1*^{low} mice demonstrated reductions in bone marrow and splenic (not shown) fibrosis. Of note, the levels of fibrosis detected using two independent methods (Gomori and reticulin staining) were inversely correlated with plasma levels of Reparixin. Immunostaining of marrow sections indicated that the bone marrow from the Reparixin-treated group expressed lower levels of TGF-β1 than those expressed by the bone marrow from vehicle-treated mice while the levels of mCXCL1, and expression of CXCR1 and CXCR2, were similar to that of vehicle-treated mice. Moreover, immunofluorescence analyses performed on bone marrow sections from *Gata1*^{low} mice indicated that treatment with Reparixin induced expression of GATA1 while reducing expression of collagen III in megakaryocytes (Figure 1). These data suggest that in *Gata1*^{low} mice, Reparixin reduces fibrosis by reducing TGF-β1 and collagen III expression while increasing GATA1 in megakaryocytes. Our results provide the preclinical rationale for the evaluation of Reparixin, alone and in combination with current JAK inhibitor, therapy for the treatment of patients with myelofibrosis in a clinical trial under development from the Myeloproliferative Neoplasm Research Consortium.

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The protective role of PTX3 during pregnancy under inflammatory conditions at the time of placentation

Rita Bonfiglio[1§], Francesca Palone[1§], Barbara Bottazzi[2], Marina Sironi[2], Cecilia Garlanda[2], Luisa Campagnolo[1], Alberto Mantovani[2], Antonietta Salustri[1] and Antonella Camaioni[1]

[1] Department of Biomedicine and Prevention, University of Rome Tor Vergata, 00133 Rome
[2] Humanitas Clinical and Research Center-IRCCS, Milan, Italy
[§] These authors contributed equally

PTX3 is a well-known innate immune molecule involved in the modulation of inflammatory response[1]. Nevertheless, although the high susceptibility to abortion of pregnant females complicated by inflammatory conditions, the role of PTX3 in this field is still unclear[2,3].

Thus, the effect of the administration of recombinant human PTX3 (rhPTX3) on a Lipopolysaccharide (LPS)-induced abortion model, has been evaluated by using both *Ptx3* null mutant and WT pregnant 129/Sv mice.

Pregnant mice were treated with LPS ± rhPTX3 at 9.5 dpc and were sacrificed at 13.5 dpc to assess the embryonic resorption rate. Decidual levels of both the endogenous and the rhPTX3 were measured by ELISA at 1.5h post-treatment, whereas the expression of genes involved in the inflammatory response, i.e. *iNos* and *Ptsg2,* was evaluated by Real Time PCR at 6h post-treatment.

Data here reported demonstrate, for the first time, that PTX3 exerts a protective effect in pregnancies exposed to inflammatory stimuli during placentation. We found that *Ptx3* null mice resulted significantly more sensitive to LPS-induced abortion than WT mice. Accordingly, exogenous administration of rhPTX3 to *Ptx3* null pregnant mice counteracted the harmful effect of LPS by significantly increasing the number of healthy embryos and of pups at birth. These insights were further confirmed by the results obtained on WT pregnant mice in which rhPTX3 treatment, i.e. a surplus of PTX3, conferred an additional protection against LPS- induced abortion. Noteworthy, we found endogenous PTX3 at the maternal-fetal interface in WT mice in physiological conditions and we showed that the exogenous rhPTX3 was able to penetrate efficiently into the decidua of both *PTX3* null and WT pregnant mice. Furthermore, exogenous rhPTX3 administration significantly reduced the LPS-induced increase of *iNOS* and *Ptsg2* transcript levels in decidua, providing evidence that rhPTX3 contributes to dampen the inflammatory state at the maternal-fetal interface.

All together, these results suggest that in early pregnancies challenged by an inflammatory state, PTX3 in decidual tissues exerts a local protective effect significantly improving pregnancy outcome.

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Mir-192-5p Expression in MDS progenitor cells during Response to Azacitidine and Lenalidomide Therapy

Alessia De Stefano[1], Sara Mongiorgi[1], Stefano Ratti[1], Antonio Curti[2], Lucia Manzoli[1], Lucio Cocco[1] and Matilde Y Follo[1]

[1] Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy
[2] IRCCS Azienda Ospedaliero-Universitaria di Bologna - Istituto di Ematologia "Seràgnoli", Bologna, Italy

BCL2 expression is dysregulated in hematopoietic stem cells and can be regulated by miRNAs, i.e. small non-coding RNAs that act on the epigenetic machinery. The expression of miRNAs is linked to cancer development and could give a proliferative clonal advantage to Myelodysplastic Syndromes (MDS) cells and thus be related to leukemic progression. miRNA profiles are studied to identify new prognostic factors or therapeutic targets[1]. High-risk MDS are now treated with hypomethylating agents, like Azacitidine (AZA), alone or in combination with other drugs, such as Lenalidomide (LEN)[2].

This study included 26 high-risk MDS patients treated with AZA and LEN every 4 weeks. All samples came from the IRCCS Hematology "L e A Seràgnoli", Bologna, Italy, where also the clinical data are being collected. miRNA expression was assessed on patients' cells extracted at baseline and during the therapy, at the 4th (T4) and 8th (T8) cycle of therapy. Results were then validated by Real-Time PCR, also used to examine the gene expression, and miRNA targets were studied by dual Luciferase assay.

For our analyses, we considered 10 patients as responders (R, showing response within T4 and maintaining it at T8), 10 losing response (LR, showing response within T4 and losing it at T8) and 6 non-responders (NR, never showing a response). Paired analysis between R and NR patients showed a statistically significant up-regulation of miR-192-5p between T0 and T4, hinting at a relevant role for this miRNA during AZA+LEN response. Real-Time PCR analyses confirmed the modulation of miR-192-5p and an altered expression of BCL-2 (target of miR-192-5p, proven in vitro by dual Luciferase assays). Furthermore, Kaplan-Meier analyses sowed an association between high levels of miR-192-5p at T4 and the overall survival (OS) ($p=0.08$) and leukemia-free survival (LFS) ($p=0.04$) in our MDS cases. More interestingly, this correlation was stronger ($p=0.03$) in R, as compared with LR and NR.

All in all, this study shows that AZA+LEN therapy in MDS affects the expression of miR-192- 5p in MDS progenitor cells, whose high level at T4 is associated with higher OS and LFS in responder patients. Moreover, we showed that miR-192-5p specifically targets and inhibits BCL-2, hinting at a regulation of MDS proliferation and apoptosis. Additional studies, to be performed in a larger cohort of MDS patients, are needed to confirm these data, as well as better understand the molecular mechanisms and the prognostic relevance of miR-192-5p in AZA+LEN therapy.

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Human fetal membrane-mesenchymal stromal differentiation into functional spinal motor neurons

Giulia Gaggi[1,2], Andrea Di Credico[1,2], Massimo Corsini[3], Riccardo Di Pangrazio[3], Paride Giovannini[3], Barbara Ghinassi[1,2] and Angela Di Baldassarre[1,2]

[1] Department of Medicine and Aging Sciences, "G D'Annunzio" University of Chieti- Pescara, 66100 Chieti, Italy
[2] Reprogramming and Cell Differentiation Lab, Center of Advanced Studies and Technology, University "G d' Annunzio" Chieti-Pescara, Italy
[3] Sapienza University of Rome, 00185 Rome, Italy

Human Fetal Membrane-Mesenchymal Stromal cells (hFM-MSCs) are a stem cell population that can be easily isolated after delivery from the amniochorionic membrane of placentas. As all the perinatal stem cells, they might represent a potential good candidate for the regenerative medicine since their use does not rise any ethical issues, they are not tumorigenic and have low immunogenicity. We previously reported that hFM-MSCs share some epigenetic characteristics with induced pluripotent stem cells. Here, we investigated whether the hFM- MSCs can give rise to ectodermal-derived lineages such as spinal motor neurons (MNs). hFM- MSCs were driven toward the MN differentiation by the sequential exposure to specific factors that first inhibit the TGFβ/BMP signaling and then activate the retinoic acid and sonic hedgehog pathways. During the differentiation steps, a gradual gene and protein upregulation of early and late of MN markers (PAX6, HB9, ISL1, the vesicular acetylcholine transporter and neurofilament L) was detected. The electrical maturation of the MNs was monitored by Multi-Electrode Array technology and after 5 weeks the characteristics of the spontaneous electrophysiological activity (spikes and bursts) were recorded and analysed. Finally, the microscopical analysis evidenced that, when co-cultured with myotubes, differentiated MNs were able to create functional neuromuscular junctions. Indeed, many areas of robust skeletal muscle cell contractions were observed after 7 days. In conclusion, our data demonstrated the hFM-MSCs can overcome the mesenchymal restriction, differentiating into cells displaying morphological, phenotypical and functional features of MNs.

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Cross-talk between autophagy and apoptosis in an experimental *in vitro* model of cholangiocarcinoma

Claudia Giampietri[1], Simonetta Petrungaro[1], Valerio de Franchis[1], Antonio Filippini[1] and Eugenio Gaudio[1]

[1] Department of Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza University, Rome, Italy

Apoptosis plays a crucial role in cancer, representing a tumor suppressor pathway. Conversely the role of autophagy in cancer cells is still largely debated. Autophagy may be impaired during the initial steps of cancer development, promoting cancer onset, while it may increase within tumor mass, where it can represent a survival strategy for cancer cells exposed to metabolic and hypoxic stress conditions. In the present work we analyzed the crosstalk between autophagy and apoptosis in cell cultures of human cholangiocarcinoma cell line (HuCCT1) and healthy cholangiocyte cell line (H69). By Western blot experiments in HuCCT1 cells we found higher levels of the anti-apoptotic c-Flip proteins as compared to H69 consistently with the lower apoptosis level observed in cancer cells. We also found that autophagy induction, obtained through nutrient starvation or rapamycin, led to c-Flip proteins down-regulation-in in HuCCT1 but not in H69 suggesting that autophagy promotion may represent a trigger for HuCCT1 apoptosis. Furthermore autophagy induction in HuCCT1 does not promote cell proliferation as evaluated through PCNA Western blot experiments. Additional experiments are ongoing to elucidate the effects of caspase activity on autophagy in our experimental models. It has been previously shown that caspase activity is able to interfere with autophagy, promoting cleavage of Beclin 1 (1), which therefore loses its ability to induce autophagy (2). We are currently investigating in our *in vitro* model if caspase inhibitors may promote autophagy of cholangiocarcinoma cells.

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Characterization of Epidermal Growth Factor Like Domain 7 (EGFL7) expression and its potential involvement in endometrial receptivity

Valentina Lacconi[1], Micol Massimiani[1,2], Fabio La Civita[1] and Luisa Campagnolo[1]

1 Section of Histology, Dept of Biomedicine and Prevention, University of Rome "Tor Vergata"
2 Saint Camillus International University of Health Sciences, Rome, Italy

Implantation of the embryo into the endometrium is one of the most critical events in human reproduction, occurring in a specific period of the menstrual cycle called the "window of implantation" (WOI). NOTCH signaling is one of the pathways involved in the regulation of endometrial receptivity by inducing decidualization, a process during which the uterine stromal cells undergo a mesenchymal to epithelial transition, acquiring a secretory phenotype (Afshar et al, 2012; Su et al, 2015). Previous studies demonstrated that NOTCH pathway is controlled, at least in part, by the secreted factor EGFL7. Data from our laboratory demonstrated that EGLF7 is expressed by the mouse blastocyst and by mouse and human trophoblast cells, in which it regulates migration and invasion by activating the NOTCH1 receptor (Campagnolo et al, 2005; Massimiani et al, 2015). To further clarify the role of EGFL7/NOTCH pathway in the regulation of endometrial receptivity, we used the Human Endometrial Stromal cell line (HESCs) which expresses low levels of endogenous EGFL7 in basal conditions and primary endometrial stromal cells obtained from biopsies from fertile women and women experiencing RPL (recurrent pregnancy loss). HESC and cell cultures from fertile women respond to EGFL7 supplementation by inducing the expression of NOTCH target genes, while cells derived from RPL patients did not. Induction of decidualization showed an up-regulation of EGFL7 expression in HESC and endometrial stromal cells from fertile women, in association with the up-regulation of the decidual markers PRL and IGFBP1. Decidualized cell cultures from RPL patients, instead, showed low levels of PRL and IGFBP1 and no up-regulation of EGFL7. In order to elucidate the role of EGFL7 in the decidualization process, we performed knock down experiment in HESC and observed that reduced expression of EGFL7 reflects in down-regulation of the decidual markers expression (PRL and IGFBP1), similarly to what observed in RPL patients. Our data suggest that EGFL7 is a new player regulating the modifications occurring in the endometrium in preparation to implantation and that its downregulation might be associate to implantation defects.

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The Effect of Carvacrol in Cardiomyocytes induced with LPS-G: Anti-Inflammatory Pathway Explorations

Guya Diletta Marconi[1], Luigia Fonticoli[2], Ylenia Della Rocca[2], Francesca Diomede[2], Jacopo Pizzicannella[3], Oriana Trubiani[2]

[1] Department of Medical, Oral and Biotechnological Sciences, University "G d'Annunzio" Chieti-Pescara, Via dei Vestini, 31, 66100 Chieti, Italy
[2] Department of Innovative Technologies in Medicine & Dentistry, University "G d'Annunzio" Chieti-Pescara, Via dei Vestini, 31, 66100 Chieti, Italy
[3] Ss Annunziata Hospital, ASL 02 Lanciano-Vasto-Chieti, 66100 Chieti, Italy

Carvacrol (CAR), is a phenolic compound found in some essential oils which possesses different biological activities, such as anti-inflammatory and antioxidant activities. Our work aimed at analyzing the response of HL-1 cardiomyocytes to an inflammatory stimulus triggered by lipopolysaccharide from *Porphyromonas gingivalis* (LPS-G), alone or in co-treatment with CAR, to evaluate the potential protective role of CAR in the inflammatory progression through modulation of the TLR4/NFκB/NALP3/IL-1β pathway and ROS production. In an *in vitro* model, HL-1 cardiomyocytes were treated with LPS-G alone or in co-treatment with CAR. We studied the anti-inflammatory effect of CAR by the reduction in TLR4, NFκB, NALP3, and IL-1β expression using immunofluorescence staining. Western blot analysis also confirmed the modulation of the TLR4/NFκB/NALP3/IL-1β pathway. ROS results established the protective effects of CAR. Our outcomes insinuate that CAR may offer an important protection role against inflammatory stimulus induced by LPS-G, implicating the suppression of the TLR4/NFκB/NALP3/IL-1β signaling pathway.

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Kisspeptin regulates sperm differentiation by affecting cannabinoid receptors expression and distribution in vivo

Marianna Marino[1], Raffaella D'Auria[1], Elena Mele[2], Grazia Maria Giovanna Pastorino[3], Francesca Felicia Operto[3], Andrea Viggiano[1], Rosaria Meccariello[2*], Antonietta Santoro[1*]

[1] Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno, Italy
[2] Department of Movement Sciences and Wellbeing, Parthenope University of Naples, Italy
[3] Child and Adolescent Neuropsychiatry Unit, Medical School University of Salerno, Italy
*equally senior authors

Kisspeptin (Kp) system, including Kp-10 -the cleavage product of Kiss1 precursor - and the GPR54 G protein-coupled receptor, represents the main central gatekeeper of the hypothalamus pituitary gonadal axis and a peripheral modulator of spermatogenesis and sperm function [1]. The role of Kp on post-testicular maturation and fertilization is not yet fully understood hence, we carried out in vivo experiments on peripubertal male rats (n=4/group, PND45) injected (i.p.) with Kp-10 for three weeks. Subsequently caput and cauda SPZ were obtained from epididymal tracts and then processed for spermiogram and immunofluorescence (IF) analysis. Testes were also collected to evaluate the expression rate of miRNAs notably involved in the control of spermatogenesis progression at puberty. The treatment with Kp-10 did not affect SPZ vitality neither in caput nor in cauda, but induced a marked increase of sperm motility (about 2-fold induction). IF analysis showed that in epididymis caput and cauda Kiss1R was localized in myoid cells all around the epididymal tubules, whereas in SPZ from caput and cauda, a different expression pattern of the Kiss1R was observed. In fact, in SPZ from the epididymis caput, Kiss1R was located in the ventral and inferior region of the sperm head, corresponding to the region joining the sperm tail to the elongated nucleus. Instead, in the cauda, Kiss1R was detected mainly at the level of the ventral part of the perinuclear theca in the apical region of the sperm head (the perforatorium). Double IF for Kiss1R and MSJ-1 (a marker of acrosome region) corroborated this localization with MSJ-1 surrounding Kiss1R in the perforatorium. Surprisingly, in Kp-10 treated rats, we noted a change in the location of the Kiss1R that appears, not only in the sperm head, but also at the level of the flagellum both in caput and cauda SPZ. Since also the endocannabinoid system plays a role in male reproduction [2], cannabinoids receptors CB1 and CB2 expressions were evaluated in Kp-10 treated rats. Of note, the treatment with Kp-10 markedly reduced CB1 and CB2 receptors expressions in SPZ from epididymis caput. Particularly, CB1 remains expressed only at the level of the region docking sperm tail to the nucleus while CB2 expression was completely blocked. These evidences indicate that the CB receptors could be inhibited by Kp suggesting a possible crosstalk between the two systems. The observed morphological-functional results in SPZ, were accomplished by Kp-10 dependent effects on the expression rate of miR-132, miR-145 and let-7a in the testis. In conclusion, the in vivo modulation of Kp signaling at puberty affects sperm maturation in rats modulating the distribution of Kiss1R and cannabinoid receptors in epididymal SPZ and the expression of miRNAs controlling male gamete formation in testis.

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Nuclear shape instability evoked by lamin A deregulation promotes metastases in Ewing sarcoma

Francesca Paganelli[1,2], Tommaso Balestra[3], Cristina Capanni[2,3], Antonietta Fazio[1], Pol Arnau Romero[4], Maria Cristina Manara[5], Lorena Landuzzi[5], Stefania Petrini[6], Camilla Evangelisti[1], Pier-Luigi Lollini[7], Alberto M Martelli[1], Giovanna Lattanzi[2,3], Katia Scotlandi[5], Carla Palumbo[8], Francesca Chiarini[2,3]

[1] Alma Mater Studiorum, University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italy
[2] CNR Institute of Molecular Genetics "Luigi Luca Cavalli-Sforza", Unit of Bologna, Bologna, Italy
[3] IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy
[4] University of Barcelona, Barcelona, Spain
[5] IRCCS Istituto Ortopedico Rizzoli, Experimental Oncology Laboratory, Bologna, Italy
[6] Confocal Microscopy Core Facility, Research Center, Bambino Gesu' Children's Hospital IRCCS, Rome, Italy
[7] Alma Mater Studiorum, University of Bologna, Department of Experimental, Diagnostic and Specialty Medicine, Bologna, Italy
[8] University of Modena and Reggio Emilia, Department of Biomedical, Metabolic and Neural Sciences, Section of Human Morphology, Modena, Italy

Ewing sarcoma (EWS) is the second most common bone cancer in children and adolescents, in which the presence of metastases at the onset remains the most clinically relevant adverse prognostic factor [1].

The nuclear shape, its size, and the chromatin conformation are well-known features of cancer, but their contribution to malignant progression remains poorly understood [2].

Lamin A, which is the main constituent of the nuclear lamina, has a crucial role in maintaining the integrity of the nuclear structure and constitutes a platform for the binding of chromatin and transcription factors and is involved in the connection between nucleus and cytoskeleton (i.e. through LINC complex proteins interactions), essential for mechano-signaling [3]. In addition to its structural role, lamin A is also able to interact with specific chromatin domains and therefore is able to modulate different intracellular signaling [4]. For these reasons, lamin A alterations have potential consequences for nuclear shape and genome instability, tumor progression, and metastatization [5].

We investigated how nucleo-cytoskeleton dynamics is influenced by lamin A modulation in EWS settings, due to our first observation that EWS patients with low *LMNA* expression had a significant worse 5-years overall survival. We also found that *LMNA* levels were significantly lower in metastatic lesions compared to primary tumors.

What is really fascinating is that in metastatic EWS patient samples low or undetectable lamin A protein levels were linked to a significantly more marked nuclear misshaping, highlighting that nuclear shape instability, induced by low/absent levels of lamin A, was related to a more aggressive EWS phenotype.

The reduction/absence of lamin A led to dysmorphic nuclei in EWS cancer cells, causing a severe mislocalization of the LINC complex, thus disrupting nucleo-cytoskeleton interactions, with a corresponding gain in malignant properties, which resulted in increased invasiveness in EWS *in vitro* studies.

Lamin A overexpression or its accumulation by a statin-based pharmacological treatment allowed us to reconstitute a functional nucleo-cyotoskeleton interplay, which led to a significant downregulation of the ROCK2 kinase, a crucial driver of EWS cell migration, and to a reduced nuclear retention of YAP, a well known negative prognostic factor in EWS [6,7].

We identified lamin A as a favorable mediator of nuclear shape stability in EWS and we demonstrated that its overexpression significantly impaired metastatic burden *in vivo*.

The impact of lamin A was particularly evident against liver metastases that resulted significantly smaller and reduced in the number. Moreover, lamin A upregulation was able to induce a more EWS differentiated phenotype (i.e. expression of neural markers such as *NEF-H* and b3-tubulin). EWS is a very undifferentiated tumor likely due to overexpression of CD99 protein, which prevents terminal neural differentiation, and *EWS-FLI1*, which induces aberrant cell differentiation [8,9]. These data suggest a role for lamin A in addressing cells toward a more differentiated state despite the presence and activity of either CD99 or *EWS-FLI1*.

Our study characterizes a novel mechanism coupling the modulation of nuclear structure, mediated by lamin A, with EWS metastatic phenotype. We also identified statins, which are already employed in clinical practice, as a tool capable of reconstituting functional mechanosignaling pathways, resulting in reduced EWS metastatic potential, while triggering neural differentiation.

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MET-YAP1 interplay at the foundation of perinuclear actin fibers remodeling

Michela Sgarzi[1], Martina Mazzeschi[1], Donatella Romaniello[1], Valerio Gelfo[1], Alessandra Morselli[1], Enea Ferlizza[1], Spartaco Santi[2], Balaźs Győrffy[3,4], Mattia Lauriola[1]

[1] Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy
[2] Institute of Molecular Genetics, National Research Council of Italy, Bologna, Italy
[3] Semmelweis University Dept of Bioinformatics and 2nd Dept Of Pediatrics, Budapest, Hungary
[4] TTK Cancer Biomarker Research Group, Institute of Enzymology, Budapest, Hungary

The perinuclear actin cap was recently described as a cytoskeletal structure, engaging a subset of apical contractile actin bundles known as stress fibers and involved in a number of biological functions, such as regulation of the nuclear shape and cellular motility[1]. Activated mesenchymal-to-epithelial transition factor receptor (MET) have been related to an altered actin remodeling, but no data are available on receptor-mediated actin cap assembly and the overall nuclear architecture organization. In a cell system characterized by a hyper-activation of MET signalling, we found an aberrant rearrangement of the cellular actin caps, which crashed into patches localized in the proximity of the nucleus and associated to an enhanced 3D nuclear organization measured as nuclear height. Conversely, upon CRISPR/Cas9 MET ablation, cells display a symmetric alignment of the actin stress-fibers with an overall improved organization of the perinuclear actin cap, resulting in a squamous flattened cell morphology associated with compressed nuclei. Of note, as previously reported[2], the presence of a functional actin cap enabled MET-KO cells to acquire persistent and directed cell motility, while reducing cells' speed. Moreover, MET-KO cells dramatically lost the capability to grow and form spheroids when cultured in anchorage- independent conditions, both in matrigel or in low attachment, suggesting an increased differentiation status. A pathway enrichment analysis performed by comparing WT and KO RNA sequencing data, unveiled the contribute of multiple pathways associated with actin cytoskeleton remodeling and cell shape regulation. Moreover, genetic cascades entangled in the response to mechanical stimuli were also aberrantly affected by MET constitutive activation. In line, the co- transcriptional activator YAP1, which plays a major role in cell mechanosensing and focal adhesions/actin stabilization[3,4] appeared the culprit of the genetic reassembling of KO cells. YAP1 was shown to localize primarily in the nucleus in the KO model, where it exerts a higher co-transcriptional activity compared to WT cells, as measured by YAP1-responsive luciferase reporter assay. In line, ligand- mediated MET activation in normal epithelial cells prompted YAP1 cytoplasmic translocation and reduced YAP1 activity.

Taken together, our results demonstrate a new mechanism of MET-mediated actin cytoskeleton rearrangement responsible for meandering random migration, which requires YAP1 inactivation.

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Mitochondrial trafficking in the tumour microenvironment: impact on breast cancer progression and drug resistance

Virginia Tirino[1], Vitale Del Vecchio[1], Luigi Mele[3], Marcella La Noce[1], Claudia Prisco[1], Sameer Kumar Panda[1], Ibone Rubio Sanchez Parajes[1], Vincenzo De Falco[1], Vincenzo Desiderio[1], Gianpaolo Papaccio[1]

[1] Department of Experimental Medicine, University of Campania "Luigi Vanvitelli" via L Armanni 5, 80138, Naples, Italy

Mitochondria are subcellular organelles, vertically inherited from the maternal side, which are involved in many physiological cell processes. Recent studies demonstrated that mitochondria can also be transferred horizontally from a donor to an acceptor cell, in physiological and pathological conditions. This mechanism is defined as "Mitochondrial transfer" (MT). Many evidence show that Adipose Stem Cells (ASCs) can migrate into the tumour microenvironment and interact with cancer cells to promote tumour growth. This is particularly relevant in breast cancer (BC), where a large amount of adipose tissue is in close proximity to the growing tumour. In this study, we aimed to investigate this mechanism in crosstalk between ASCs and breast cancer cells and evaluate how MT affects the phenotype of cancer cells. We demonstrated that the MT occurs in co-cultures from patient derived or immortalised ASCs to MCF-7, through cell-to-cell adhesion and/or Tunnelling Nanotubes (TNTs) formation. The MT resulted differently modulated after treatment with Cytochalasin B, an actin polymerization inhibitor, or CCCP an oxidative phosphorylation uncoupler. By using a new protocol called MitoCeption (MC) we were able to isolate mitochondria from ASCs, and subsequently force the uptake and retention into the MCF-7. The acceptor cells were more viable, after treatment with chemotherapy and ROS agents, but surprisingly less able to form colonies. Moreover, in MCF-7 subjected to MC we also observed several metabolic changes, in particular an increased respiratory capacity linked to the ATP production in normoxia, and a significant proton leaking in hypoxia condition. On the contrary, the MCF-7 migration capacity and their "stemness" features were not modified by the MC.

Here, we demonstrated with advanced in vitro technologies the MT from ASCs to MCF-7. We dissected the functional effects induced by the exogenous mitochondria engulfment in MCF-7, especially referring to the drug resistance, antioxidant activity, and metabolism. On the basis of our results, we could hypothesize new therapeutic approaches for the BC treatment.

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Santiago Ramòn y Cajal: From the "Neuron", the benchmark of contemporary knowledge, to the "Connectome", the map of the brain

Demetrio Milardi[1], Francesco Bonanno[2], Filippo Cavallaro[2], Yan Abu Ahmad[3], Simona Pergolizzi[4], Giuseppina Rizzo[1], Fabio Trimarchi[1], Giuseppe Anastasi[1]

[1] Department of Biomedical, Dental Sciences and Morphological and Functional Images, University of Messina, Italy
[2] Department of Clinical and Experimental Medicine, University of Messina, Italy
[3] Department of Adult and Childhood Human Pathology "Gaetano Barresi", Medical School, University of Messina, Italy
[4] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences

Santiago Ramòn y Cajal (1852-1934) was a multi-faceted man: he was a doctor, a philosopher, a researcher and an artist. In 1906 he shared the Nobel Prize in Medicine with Camillo Golgi for their work on the structure of the nervous system, even if he did not agree with Golgi's idea that the neurons are interconnected like a network.

Cajal is not only a man. He is the personification of an idea, he is a philosophical, spiritual and political leader who brings together millions of people. Cajal was the face of a different Spain, that rarely is represented, a Spain that is revolutionary and unconventional. Santiago Ramòn y Cajal was a multi-talented man, a visionary who restructured the concept of histology and anatomy. He was a figure who made the jump in science to overcome the ancient ideals and concepts described in histology and anatomy texts. He gave life to anatomy with his illustrations and he made this discipline clear for everyone. From the outset of Cajal's studies in 1888, we have witnessed the beginning of a new era in neuroscience characterized by the tracing of the first point-to-point connectivity maps of the nervous system. A paradigm shift in neuroscience and in our way to conceive the human brain has taken place in the 21th century, when the current research introduced the term "connectome" to refer to a complete map of the neural structural and functional connections within the brain.

I'll guide you across a historical journey, unfolding how the past and the development of neuroimaging techniques have led to the beginning of the huge international projects.

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The contribution of morphological disciplines to gender medicine

Ilaria Campesi[1], Maria Alessandra Sotgiu[1], Pasquale Bandiera[1], Vittorio Mazzarello[1], Alessio Pirino[1], Andrea Montella[1]

[1] Department of Biomedical Sciences, University of Sassari, Sassari, Italy

Gender medicine is a branch of medicine that studies the biological and sociocultural differences between males and females of all ages (from intrauterine life to old age) and the influence of these factors on the state of health and disease, as well as on the response to pharmacological therapies (1).

The purpose of gender medicine is consequently to guarantee the diagnostic-therapeutic appropriateness by making possible treatments tailored to the individual needs (2).

The World Health Organization, in a document that illustrates European health policies, indicates gender as a key element for health promotion aimed at developing diversified therapeutic approaches for women and men. To reach this greater appropriateness, however, it is necessary to orient health interventions, build specific paths, organize training processes, and direct research in this field.

The evidence that sex is a key factor on the individual predisposition towards some pathologies presupposes the existence of cellular, tissue, morphological, physiological, and pathophysiological differences.

The plan for the application and dissemination of gender medicine of the Italian Ministry of Health, prepared following the " Legge 11 gennaio 2018 n. 3", aims to provide a coordinated and sustainable direction for the dissemination of Gender Medicine through the diffusion, training, and indication of health practices that in research, prevention, diagnosis, and treatment take into account the differences deriving from gender, to guarantee the quality and appropriateness of the services provided by the National Health Service (SSN) in a homogeneous way throughout the national territory.

Precision medicine, therefore, requires great attention to inter-individual variability. Both in scientific experiments and clinical practice, the rigorous evaluation of sex and gender differences allows the pursuit of better overall health, avoiding systematic errors that generate results with poor scientific and applicative validity.

In this sense, a significant contribution can derive from the intelligent application of experimental morphological studies to the clinic and the consequent training activities carried out from a sex and gender perspective, deepening the clinical applications of in-depth knowledge of individual anatomy.

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Learning enhancers to improve sensitive learning: the Utibilius Project

Riccardo Ruffoli[1], Beatrice Casini[1], Gianfranco Natale[1], Francesco Fornai[1,2]

[1] Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Via Roma 55, 56126 Pisa, Italy
[2] IRCCS Neuromed, Via Atinense 18, 86077 Pozzilli, Italy

The current student population includes persons with disabilities (e.g., hypovision, hypoacusia or deafness) and/or specific learning disorders (LsD), i.e. a set of heterogeneous disorders that may impair the ability to read, write, calculate, listening and express fluently. The Italian laws define the compensatory and dispensatory tools to be currently used to guarantee their equal rights and equity in the course of educational processes. This includes the higher education level provided both during short- and long-term University courses. The aim of this report is to focus on the roles and tools which are required by Academic institutions to promote the dissemination of appropriate learning enhancers. These should be intended as tools enabling access and full proficiency of learning subjects intended as in person lecture as well as interactive software to be used on demand. In fact, evidence exists that appropriate tools and context in the use of learning enhancers provide a significant improvement of learning efficacy, which is most effective in sensitive learning conditions such as those occurring in students facing specific disabilities and/or LsD.

For this purpose, our group developed the Utibilius Projects, i.e. a set of tools named and aimed to the dissemination of proper methodologies (good practices) which ameliorate the learning process for all students, and specifically those with disabilities and LsD. In particular, we created a set of tools such as PowerPoint presentations and videoclip aimed to produce adequate editing and dedicated fonts. These include the accessibility checker use in Microsoft 365 Office, the application of Screen Readers, and the development and use of conceptual and mental maps.

To this aim, as a pilot analysis a survey was conducted in the Medical faculty at the University of Pisa to assess the amount of awareness concerning the presence of students with disabilities including LsD who are actually present in various courses. At the same time, a preliminary assessment was carried out in the medical faculty concerning the awareness of what learning enhancers are and which potential they may express when properly used as a seminal part of educational tools.

The answers received represent only a small sample of the faculty (about 12%). Nonetheless, the faculty members who answered the questions declared to possess non-sufficient level of awareness about the occurrence and the potential use of learning enhancers. This includes the knowledge of primary tools designed at fostering the access to teaching material. Often, who answered the questions considers the access to teaching material to be adequate. It is remarkable that, at the same time some of them, admitted they never checked.

Most respondents admitted to possess a poor knowledge about the existence of accessibility checkers in Microsoft applications, the use of dedicated fonts and editing rules that improve the didactic material usability for LsD subjects. Most of them ignored about the existence and use of screen readers. Finally, the majority of respondents admitted not to know the difference between a conceptual map and a mental map and, of course not to ever used these maps. It is remarkable that most survey participants wished to know more about the most appropriate methods and practical approaches to use learning enhancers to improve accessibility and proficiency of learning material. This knowledge was required to be delivered mostly through PowerPoint presentations and/or tutorial videos.

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In conclusions the dissemination and the implementation of appropriate learning enhancers for increase the accessibility and usability of the didactic material represent a crucial point in the academic education. This is essential to guarantee learning equity for all persons, especially for students with disabilities and/or LsD, and to ensure an appropriate and equal academic education for all individuals, that meet their expectations and attitudes.

Tecnologie innovative

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Investigation of the expression of miRNA extracts from spent blastocyst culture media at the sixth day: a preliminary analysis

Alice Conti[1], Laura Benedetti[1], Claudia Omes[2], Veronica Tomasoni[2], Davide De Marchi[3], Flaminia Aliberti[1], Gabriella Cusella[1], Gabriele Ceccarelli[1]

[1] Department of Public Health, Experimental Medicine and Forensic, Human Anatomy Unit, University of Pavia, Pavia, Italy
[2] Center for Reproductive Medicine - Obstetrics and Gynecology Unit, Woman and Child Health Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[3] Department of Electrical, Computer and Biomedical Engineering, University of Pavia, Pavia, Italy

Infertility, defined by the failure to achieve a pregnancy after 12 months of regular, unprotected sexual intercorses, is a complex disorder that impacts negatively on couples, with additonally economic and social implications on society [1]. Among the treatments offered to infertile couples, those utilizing Assisted Reproductive Technology (ART) are associated with the highest rate of success [2]. Currently, in ART laboratories, when timelapse technologies or PGT (Pre-implantation Genetic Testing) procedures are not avaible, the assessment of the quality and the competence of embryos selected for transferred in uterus is performed based on statical morphological observations conducted by the clinical embryologist. For this reason, basic reasearch tries to find diagnostic evaluation methods that can help embryologists in making this choice, avoiding invasive methods. In this scenario, it has been demonstrated by several groups that miRNAs are selectively secreted from the developing embryos into the culture media and thus have strong potential as biomarkers of embryo developmental competence [3]. Hence, we comprehensively characterized the profile of three different miRNAs secreted by human pre-implantation embryos in spent culture media (hsa-miR-661, hsa-miR-372-5p, hsa-miR-21-5p) at sixth day and investigated whether secreted miRNAs can be used as predictive markers of clinical outcomes in *in vitro* fertilization (IVF) cycles. In addition, a bioinformatics analysis was performed to identify any pathways up/down-regulated by the miRNAs analyzed. Preliminary results demonstrated that there is a higher expression of the three miRNAs considered in spent culture media of embryos not progress to the blastocyst stage and classified as non-evolved or degenerate (NE/DG) with respect to cleavage embryos developed to blastocyst stage (BLOK), in line with the literature. Bioinformatic analysis showed that especially hsa-miR-661 is strictly associated with pathway that regulate cell shape and the morphogenesis of the epithelial sheet. Further analysis will be necessary to explore more in detail this noninvasive embryos selection and its impact on IVF procedures outcome.

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Targeting the Adaptive Molecular Landscape in Prostate Cancer: Dissection of the novel AKT/miR-145/RAS Circuit

Valentina Serafin[1,2*], Manuela Zavatti[3*], Luca Braglia[3], Silvia Bresolin[2,4], Sandra Marmiroli[3]

[1]Department of Surgery, Oncology and Gastroenterology, University of Padova and [2] Pediatric Research Institute Città Della Speranza, Padova
[3] Cellular Signaling Unit, Section of Human Morphology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia
[4] Haemato-Oncology Laboratory, Department of Woman and Child Health, University of Padova
*equal contributors

Aberrantly active PI3K/AKT signaling is regarded as a main therapeutic target due to its role in tumorigenesis (1, 2). It is therefore not surprising that a number of drugs targeting either of the key components of the pathway have been trialled for both solid and hematological malignancies. However, the results of clinical studies with such PI3K or AKT inhibitors have been so far disappointing, mainly because of the rapid emergence of resistance triggered by interruption of negative feedback circuits or perturbation of pathway homeostasis (2, 3). On this ground, the dissection of resistance mechanisms may lead to more effective therapeutic approaches as well as to the identification of markers characterizing patient subgroups that will derive maximal benefit from PI3K/AKT targeting, and are therefore very important to the success of future clinical trials.

In this study, we found that pharmacological inactivation of AKT in PC3 prostate cancer cells leads to down-regulation of a tumor suppressor microRNA, miR-145-5p and, in turn, to a dramatic increase (~20 folds) in the expression of one of its target genes, namely the oncogene KRAS. Remarkably, KRAS is also a well known upstream activator of PI3K/AKT. Interestingly, levels of miR-145 are known to discriminate between patients with benign prostatic hyperplasia and prostate cancer, and miR-145 loss increases risk for localized to metastatic disease progression. Moreover, low expression of miR-145 is part of a miRNA signature to predict poor survival of PC patients. Confirmation that the observed drop of miR-145-5p triggers an increase of RAS, detected both in terms of mRNA and protein, was obtained using PC3 cells engineered by us to transiently silence the 145-5p guide strand of miR-145 following exposure to doxycycline. Interestingly, we demonstrated that not only silencing but also pharmacological inactivation of AKT leads to RAS overexpression. Furthermore, through a phosphoprotein array we were able to confirm that such burst of RAS expression elicits reactivation of both the PI3K/AKT and the ERK cascades. From a mechanistic point of view, therefore, this study provides the rationale for a not yet described adaptive resistance mechanism, in which the inactivation of AKT down-regulates miR-145-5p, which, in turn, increases RAS expression and reactivates PI3K signaling, thus limiting treatment efficacy, at least in a cell culture model of prostate cancer. These results are particularly relevant in light of recently published (NCT04493853; NCT03072238; NCT02525068) as well as ongoing (NCT04737109; NCT03673787) clinical trials, based on the combination of androgen deprivation therapy with AKT inhibitors (namely capivasertib or ipatasertib) (4), as they might explain the rapid emergence of resistance in some patients. Thus, our data warrant further investigation and validation in metastatic, hormone-sensitive prostate cancer, such as the well known TRAMP mouse model.

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Multimodal machine learning algorithms associated with neuroimaging for early detection of alzheimer's disease

Enea Traini[1], Giulio Nittari[1], Gopi Battineni[1], Mohmmad Amran Hossain[1], Ciro Ruocco[1], and Francesco Amenta[1*]

[1] Clinical Research, Telemedicine and Telepharmacy Centre, School of Pharmaceutical Sciences and Health Products, University of Camerino, via Madonna delle Carceri, 9, 62032, Camerino (MC) Italy

Adult-onset dementias are a main health issue worldwide. The most common and well-known form of these dementias is Alzheimer's disease (AD). In early stages of AD, people experience memory problems often referred to the age-related physiological reduction of cognitive capabilities. Detailed cognitive testing is not performed routinely in the elderly and therefore AD in early stages can be undetected.

Appropriate methods are required to develop strategies for early detection of AD. The conventional approach is based on cognitive testing. However, this is not accurate enough and should be integrated with reliable biomarkers to allow a definitive diagnosis. Increasing evidence suggests that markers extracted from Magnetic Resonance Imaging (MRI) may allow a correct diagnosis of AD. Brain scans provide useful information about brain morphology and allow to track the progression of neurodegeneration. Voxel-based morphometry (VBM) is a volumetric MRI-based method used for clinical diagnosis of AD. VBM shows brain neuro-structural changes that can be used as an important marker in AD assessment.

Non-invasive methods such as computer aided diagnosis systems (CADS) have been proposed as a complement of imaging techniques to improve AD diagnostics. CADS with good performance can provide accurate information of early stages of brain neurodegeneration. Computer methods in neuroscience represent a useful way to use huge and complex datasets, but at the present there is no standardized computer aided approach in early detection of AD based on neuroimaging data. The potentialities of six different supervised classifiers for identifying reliable CADS using MRI obtained from subjects diagnosed for AD at different stages are presented. The gradient boosting algorithm outperforms other models with 97.58% of accuracy and therefore can represent a suitable companion to MRI for identifying AD in early stages.

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Production and characterization of a decellularized extracellular matrix (dECM) from porcine pre-pubertal tunica albuginea

Francesca Mancuso[1], Iva Arato[1], Martina Alunni Cardinali[2], Mario Calvitti[1], Catia Bellucci[1], Cinzia Lilli[1], Debora Puglia[3], Anna Donnadio[4], Alessandro di Michele[2], Consuelo Fabi[1], Maurizio Mattarelli[2], Francesca Luzi[3], Elena Eugeni[1], Daniele Fioretto[2], Giovanni Luca[1,5]

[1]Department of Medicine and Surgery, University of Perugia
[2]Department of Physics and Geology, University of Perugia
[3] Department of Civil and Environmental Engineering, University of Perugia
[4]Department of Pharmaceutical Sciences, University of Perugia
[5]International Biotechnological Center for Endocrine, Metabolic and Embryo-Reproductive Translational Research (CIRTEMER), Department of Medicine and Surgery, University of Perugia

Fertility preservation for prepubertal male patients undergoing gonadotoxic therapies, potentially depleting spermatogonial cells (1), is an expanding necessity.

The construction of testicular organoids (2) seems promising to create the right supportive environment for the development of spermatogonial stem cells (SSCs).

A wide variety of proposals is available, including models relying on extracellular matrix (ECM) which is known to play an important role in modulating cell adhesion, signalling, migration, proliferation and three-dimensional array and has been proposed as a biologic scaffold for tissue engineering applications (3).

The ECM is a meshwork of both structural and functional proteins assembled in unique tissue-specific architectures. It has been demonstrated that ECM-based materials have been used in a wide variety of tissue engineering and regenerative medicine approaches to tissue reconstruction (4).

We aimed to assess the production and characterization of decellularized ECM (dECM) from porcine pre-pubertal tunica albuginea.

Molecular biology techniques, i.e. genomic DNA and total RNA analysis, were performed along with microscopy and spectroscopic imaging, i.e. histo-morphological evaluation, transmission electron microscope (TEM) and scanning electron microscope (SEM) analysis, rheological analysis, correlative Brillouin and Raman microspectroscopy (BRamS), and ATR-FTIR spectroscopy, in order to obtain a complete evaluation of the morphology and the mechano-chemistry of each step of the dECM production process, from the original tissue to the final product.

We obtained a dECM in both dry powder and hydrogel form, with a good preservation of both structural and functional components. Spectroscopical analysis disclosed a high level of purity in terms of nucleic acids content, confirmed also by molecular assays and histological staining, and the presence of a conspicuous mesh of fibrous proteins, proteoglycans and glycosaminoglycans.

Our study represents a basepoint for the development of three-dimensional composite scaffolds aimed to restore the testicular *in vitro* spermatogenesis.

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Anatomical and functional custom-made restoration techniques with Direct Metal Laser Forming technology

Piero Antonio Zecca[1], Marina Protasoni[1], Marcella Reguzzoni[1] and Mario Raspanti[1]

[1] University of Insubria, Department of Medicine and Surgery, Laboratory of Human Anatomy and Morphology

In orthopedic and prosthetic craniomaxillofacial reconstructive surgery, the need to replace portions of anatomic bony tissue following the loss of the parts above for any injury or surgical resection attributable to another pathological cause is often required. In these patients, both morphological and functional aspects might be evaluated, leading to the evolution of the techniques used in the industrial manufacturing. During the past few years, scientific research in this area has been oriented towards using and implementing materials that would allow an active role of the bone surrounding the implant. In this regard, histological studies have shown that the porous surfaces of the grafts seem to have a wider interaction with bone cells, while retaining structural and mechanical properties similar to the surrounding bone. In particular, a range of porosity between 100 and 700 μm was shown as ideal for the purposes. The main problem regarding the morphological structure of the implant is linked to the mechanical alterations that the graft undergoes to present a suitable surface for the integration; it must avoid being excessively weakened by too numerous pores, and conventional construction methods of these systems do not seem to have satisfied the needs.

The aim of this research is to explore the use of Rapid Prototyping technology. Among the different systems, the Direct Metal Laser Forming (DMLF) technology allowed the building of structures of controlled porosity through a process of melting where a laser beam, controlled by a computer, can join tiny cells of metals, then creating the design of three-dimensional (3D) object layer by layer. This study shows how engineered grafts interact with the bone and their mechanical properties could help produce better, more efficient surgery. The novelty of this research is that bone structures can be produced with carefully controlled physical and mechanical properties, so that their strength and bone in-growth characteristics can be customized and optimized for the specific location being treated.

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Neuroscienze

Prima sessione

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Cognitive impairment in multiple sclerosis: study of early transcriptome changes in the prefrontal cortex in a murine model of the disease

Adinolfi A.[1], Pieraccioli M.[2], Di Sante G.[3], Tredicine M.[4,5], Camporeschi C.[4,5], Ria F.[4,5], Guerra M. [1], Cesari E.[2], Corvino V.[1], Sette C[1,2]., Geloso M.C.[1]

[1] Sezione di Anatomia Umana, Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, Roma, Italia
[2] GSTEP-Organoids Core Facility, Fondazione Policlinico Agostino Gemelli IRCCS, Roma, Italy
[3] Sezione di Anatomia Umana, Clinica e Forense, Dipartimento di Medicina e Chirurgia, Università degli studi di Perugia, Perugia, Italia
[4] Sezione di Patologia Generale, Dipartimento di Medicina e Chirurgia Traslazionale, Università Cattolica del Sacro Cuore, Roma, Italy
[5] Global Health Research Center, Università Cattolica del Sacro Cuore, 00168 Roma, Italy

Mounting evidence points to the presence of primitive neuronal damage in MS, occurring independently from demyelination and involving grey matter structures involved in cognitive processes, like the prefrontal cortex (PFC). An early event in such neuronal damage is represented by synaptic dysfunction, which likely contributes to the cognitive impairment (CI) that is characteristic of both MS and its related experimental models (1).

The functional features of the synapse are fine-tuned by the regulation of expression, splicing and activity of synaptic proteins. In this regard, we previously showed that in EAE SJL mice, an experimental model of relapsing remitting MS, splicing of *Nrxn1-3* genes is modulated in response to inflammation in the PFC. *Nrxn1-3* encode for presynaptic proteins that play an important role in cognition. We found that altered splicing of *Nrxn1-3* was positively correlated with the local levels of the pro-inflammatory cytokine IL-1b and it was associated with CI in behavioural tasks of EAE mice (2).

To investigate more globally the EAE-associated transcriptome changes, we have now sequenced RNA samples isolated from the PFC of CTRL (n=4) and EAE (n=7) mice, sacrificed at the acute phase of disease [14-16 days post immunization; Clinical Score and Signs = 2-2.5]. Unbiased clusterization of EAE mice based on their overall transcriptomic signature highlighted two subgroups. Heat map representation of the top differentially-regulated (DR) genes (n=100) indicated the presence of a subgroup showing higher differences with the CTRL samples and the other displaying lower or intermediate changes. Since functional annotation highlighted cytokine-mediated pathways and inflammatory response as significantly enriched among DR genes of both EAE subgroups, we then stratified EAE mice into EAE-L (low immune-reaction/inflammation) and EAE-H (high immune-reaction/inflammation).

EAE-H and EAE-L groups exhibited, respectively, 1524 and 841 DR genes. Importantly, some of the genes exclusively regulated in EAE-H mice (e.g *TNFRSF1A*) (3), displayed strong relevance for MS-related CI. We also investigated whether the two EAE phenotypes differentially affected the neuronal compartment, using the Cell type-Specific Expression Analysis (CSEA) tool (4). Interestingly we obtained overlaps with different CNS cell types for both EAE-H and EAE-L mice. However, while upregulated genes in EAE-L mice essentially overlapped only with the microglial cell type, those upregulated in EAE-H mice revealed also astrocytic features. Since microglia represents the first line of defense within the CNS, which is then followed by astroglia activation (5), our data are consistent with a more advanced stage of inflammation in the EAE-H subgroup. Moreover, genes that are downregulated in the EAE-H group showed multiple highly significant overlaps with cortical neurons, interneurons and oligodendrocytes, including genes directly involved in cognitive functions.

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These categories were much less represented among the genes downregulated in the EAE-L PFC.

Collectively, our results indicate that high levels of inflammation exert a pronounced impact on neuronal gene expression. Moreover, they suggest that the increasing inflammatory status in the EAE brain gradually induces expression of genes related to different features of the disease, which possibly represent suitable targets or biomarkers for different clinical stages of MS patients.

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The morphological features of rat brain vascular pericytes (RBVP) during toxic insult and their role in blood-brain barrier characterization

Jacopo J. V. Branca[1], Donatello Carrino[1], Ferdinando Paternostro[1], Claudio Nicoletti[1], Massimo Gulisano, Alessandra Pacini[1]

[1] Department of Experimental and Clinical Medicine, Section of Human Anatomy, University of Florence, Florence, Italy

Pericytes are fibroblast-like cells that cover the endothelial cells surface by their extensive cytoplasmic elongation [1]. Together with other cell types of central nervous system, pericytes are included in the so-called neuro-vascular unit, thus having a pivotal role in the blood-brain barrier (BBB) formation since they are essential both for BBB function and development [2].

The aim of our study is to investigate the morphological and molecular alteration of rat brain vascular pericyte (RBVP) cell line during cadmium toxic insult, by the treatment at increasing concentrations (0.1-100 μM) and time (8-16-24 hours) in starvation conditions.

Preliminary results demonstrated that cadmium acetate change the cytoplasmic structure highlighted by α-Smooth Muscle Actin, F-actin and Vimentin. Furthermore, the toxic stimulation leads to mitochondrial dysfunction thus increasing the oxidative stress in a time and dose-dependent manner.

Finally, when pericytes are co-cultured with brain endothelial cells, the toxic insult seems to be more deleterious increasing the BBB alteration, maybe due to alterations in pericytes architecture.

In conclusion, the data showed that the cadmium toxicity on RBVP cell line occurred at low concentration and such alterations might reflect on BBB function dysregulation thus decreasing its pivotal role on the brain parenchyma protection.

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New pathogenetic mechanisms beyond the neurocentrical view for chemotherapy-induced peripheral painful neuropathy: focus on the microvascular network in the central and peripheral nervous system of rats

Antonio Zippo[1], Eleonora Pozzi[2], Laura Monza[2], Virginia Rodriguez-Menendez[2], Elisa Ballarini[2], Annalisa Canta[2], Alessia Chiorazzi[2], Cristina Meregalli[2], Alberto Bravin[3], Guido Cavaletti[2], Valentina Alda Carozzi[2]

[1] Institute of Neuroscience, Consiglio Nazionale delle Ricerche, Monza (Italy)
[2] Experimental Neurology Unit, School of Medicine and Surgery, University of Milan-Bicocca, Monza (Italy)
[3] Physics Department, University of Milan-Bicocca, Milan (Italy)

Chemotherapy-induced painful peripheral neuropathy (CIPPN), with paresthesia, numbness, dysaesthesia, sensory ataxia and neuropathic pain ranks among the most common dose-limiting toxicity of several widely used anticancer drugs. The only effective option to prevent severe and permanent neurological damages of chemotherapy is to reduce or withdraw the anticancer treatment, impacting, however, on the patients' oncological outcome. Beside peripheral neurons, for several years considered the only reasonable target for CIPPN study, the recent evaluation of the microvascular angiogenesis in the somatosensory pathway reveals other important details on pain development and chronicization [1, 2]. In order to elucidate the correlation between chronic pain and vascular alterations in CIPPN, we evaluated the microvascular network in central and peripheral nervous compartments of rats exposed to chronic paclitaxel (PTX) or cisplatin (CDDP) treatments.

Fourty-eight rats were enrolled and treated with PTX 10 mg/kg once a week for 4 weeks, or CDDP 2 mg/kg twice a week for 4 weeks, or with vehicles as internal controls [3]. Animals were tested for neurophysiological abnormalities and pain before and after the treatments. Post-mortem samples of S1 cortex, L4-5 spinal cord, L4-5 Dorsal Root Ganglia (DRG) and peripheral nerves were analyzed at synchrotron radiation resources by X-ray Phase-Contrast Tomography (XPCT) Imaging and processed for quantitative and morphological analyses of microvascular structures. Complementarily, morphometrical, neuropathological and immunohistochemical evaluations were performed.

PTX and CDDP-treated rats developed a painful sensory axonopathy and a painless mild sensory neuronopathy, respectively. XCPT analysis, evaluating the vascular density, vessel diameter and tortuosity and the number of branch points, demonstrated consistent differences between the drugs: PTX increased the vascular density (putative angiogenesis) in all target sites whereas CDDP did not. Moreover, vessel diameters were significantly reduced in the DRG and S1 of PTX group. The tortuosity decreased in DRG and S1 of PTX group and in the L4/5 spinal cord of CDDP group. The number of branch points decreased in L4/5 of the PXT group. These events were confirmed by the positivity of vessel neogenesis to tomato lectin staining.

Evidence of vascular neo-genesis at capillary level was found in rats with painful symptoms derived from PTX, less evident in CDDP. These results can shed light on new pathogenetic mechanisms and potential novel therapeutic approaches for CIPPN.

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Overexpression of human alpha-synuclein induces VGF early changes in both blood and brain of rats

Cristina Cocco[1], Elias Manca[1], Barbara Noli[1], Giulia Corda[1], Sara Corsi[1], Maria Antonietta Casu[2], Roberto Frau[1], Manolo Carta[1], Valeria Sogos[1], Pardon Marie-Christine[3], Aurora Milia[1], Loris Deplano[1], Giuseppina Bassu[1]

[1] Department of Biomedical Sciences, University of Cagliari, 09042, Monserrato, Italy
[2] CNR-Institute of Translational Pharmacology, 09010, Pula, Cagliari, Italy
[3] University of Nottingham Medical School, Queen's Medical Centre, NG7 2UH, Nottingham, UK

We have previously shown that peptides containing the C-terminal end (LHRP) of the rat VGF protein possibly produced in the GABAergic neurons of the striatum, are reduced in the axon terminals of the substantia nigra (SN) of unilateral 6-hydroxydopamine lesioned rats. Similarly, peptides containing the C-terminal end (LRRP) of the human VGF protein were decreased in the blood of Parkinson's disease (PD) patients, at the time of diagnosis. To investigate whether VGF peptides might be modulated in the early stage of nigrostriatal neurodegeneration, we aimed to set up a PD animal model resulting in moderate dopaminergic degeneration based on the overexpression of the human αlfa-synuclein (α-syn) gene. Rats received a unilateral injection into the SN of the adeno-associated virus vector (AVV) expressing the human phosphorylated α-syn (p-alfa sin; group 1; n=13) or the green fluorescent protein gene (group 2; n=13) while rats without any treatment were used as further controls (group 3: n=6). In order to investigate if VGF changes are peculiar for Parkinson's disease, we studied also Huntington disease (HD) mice (n=7 vs. 5 controls). Antibodies to LHRP peptides but also to other peptides i.e. NAPP and TLQP (namely with the first 4 amino acid sequences) were used for immunohistochemistry, enzyme immunosorbent assay (ELISA) and western-blot (WB). Inclusions of the p-αlfa syn were observed in the SN of the AVV treated sides only, in parallel with a loss of dopaminergic immunolabelled cell bodies (~30%; $p<0.05$) and a VGF staining decrease for LHRP peptides (~25%; $p<0,05$), while the other VGF peptides were undetectable in any brain section irrespective of the treatment. In the striatum of AVV-treated rats, a lower expression of the p-αlfa syn compared to the SN, was observed in lesioned sides, and accordingly, both TH- and LHRP- immunostaining were not decreased. In plasma, a VGF reduction was seen for LHRP, TLQP, and NAPP peptides ($p<0.05$; decrease of ~50%, ~40% and ~30%, respectively, lesioned vs. controls) while using the HD mouse, neither plasma or brain revealed a VGF decrease. In extracts of normal striatum used for WB, the three VGF antibodies immunolabeled bands corresponding to the entire VGF protein (615aa, 70kDa), TLQP-62, (62 aa, 8kDa) and NAPP-129, (129 aa, 15kDa), while further studies will be done using WB with striatum and plasma of the different groups. In conclusion, VGF peptides are suggested as potential PD biomarkers.

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Microenvironmental changes and immune escape during the glioblastoma time course

Ciro De Luca[1], Ivana Allocca[1], Raffaella Cirillo[1], Giovanni Cirillo[1], Michele Papa[1,2] and Assunta Virtuoso[1]

[1] Laboratory of Morphology of Neuronal Network and Systems Biology, Department of Public Medicine, University of Campania "Luigi Vanvitelli", 80138 Napoli, Italy
[2] SYSBIO Centre of Systems Biology ISBE, ITALY, University of Milano-Bicocca, 20126 Milano, Italy

Glioblastoma multiforme (GBM) is the most prevalent and malignant primary brain tumor, characterized by heterogeneous clinical presentation, parenchymal diffusion, and tumor-host interactions. The reliability of translationally valid models has hindered research on GBM for decades. An in vivo model should replicate the immune escaping strategies of glioma cells from the onset towards the bulk formation and long-distance spreading.

GL261 glioma cells were injected into the right striatum of C57Bl/6 syngeneic mice that were sacrificed after 7, 14, and 21 days (7D, 14D, 21D). In vivo tumor development was monitored using 3D tomography with fluorescent 2-DeoxyGlucosone (DG).

We aimed to characterize the GBM time course with an immunocompetent syngeneic host focusing on neuroinflammation and extracellular matrix (ECM). We used morphological and semiquantitative analyses of protein expression to unravel novel aspects of early GBM pathogenesis. These data were confronted with a database of human GBM transcriptomics to support the translational validity of the model.

Our results showed the evolution of the GBM from the parenchymal seeding toward bulk formation and dissemination. The GBM bulk was surrounded by a glial scar by 14D, with an impaired microglial/macrophagic recruitment and reduced antigen-presenting functions. The changes in ECM proteins and protease were more expressed during the dissemination phase. Although the analyzed morphological and functional expression of proteins were not always paired with the human transcriptomics data, the mismatch offered intriguing cues for further discussions.

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The S100B protein as a therapeutic target for multiple sclerosis processes

Gabriele Di Sante[1], Chiara Camponeschi[2], Maria De Carluccio[2,3], Susanna Amadio[4], Maria Elisabetta Clementi[5], Beatrice Sampaolese[5], Cinzia Volonté[4,6], Anna Maria Stabile[1], Desirée Bartolini[1], Alessandra Pistilli[1], Maria Tredicine[2], Francesco Ria[2,7], Mario Rende[1], Fabrizio Michetti[3]

[1] Department of Surgery and Medicine, Institute of Human, Clinical and Forensic Anatomy, Piazza L Severi 1, 06125 Perugia, Italy
[2] Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy
[3] Department of Neuroscience, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy
[4] IRCCS Santa Lucia Foundation, Via del Fosso di Fiorano 65, 00143 Rome, Italy
[5] Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" SCITEC-CNR, Largo Francesco Vito 1, 00168 Rome, Italy
[6] National Research Council, Institute for Systems Analysis and Computer Science, Via dei Taurini 19, 00185 Rome, Italy
[7] Department Laboratory and Infectious Diseases Sciences, Fondazione Policlinico Universitario, A Gemelli IRCCS, Largo Agostino Gemelli 1–8, 00168 Rome, Italy

S100B is a calcium-binding protein mainly concentrated in astrocytes. Its levels in biological fluids are recognized as a reliable, even predictive, biomarker of active neural distress. Mounting evidence now points to S100B as a Damage-Associated Molecular Pattern protein which, when released at high concentration, triggers tissue reaction to damage in various disorders (1,2).

A number of correlative evidence proposes that S100B high levels may play a promoting role also in multiple sclerosis (MS) (3-5). We showed that in the relapsing-remitting experimental autoimmune encephalomyelitis (EAE) mouse MS model, the inhibitor of S100B activity pentamidine (PTM) ameliorates clinical scores and neuropathologic-biomolecular parameters (6).

Also, arundic acid (AA), an inhibitor of astrocytic S100B synthesis, in the chronic EAE mouse model, by the evaluation of clinical scores and neuropathologic-molecular analysis, induced lower severity compared to the vehicle-treated mice, particularly in the early phase of disease onset (7). A significant reduction of astrocytosis, demyelination, immune infiltrates, pro-inflammatory cytokines expression and enzymatic oxidative reactivity in the AA-treated group was observed, indicating the participation of S100B in neuroinflammatory processes, reasonably as an astrocytic activity. The active participation of astrocytes in S100B-induced MS processes is currently studied.

This scenario proposes S100B as a therapeutic target for MS, as for different neural disorders appearing to share some common pathogenic features, reasonably attributable to neuroinflammation (1,2).

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Mitochondria as targets of methamphetamine toxicity

Michela Ferrucci[1], Gloria Lazzeri[1], Stefano Puglisi-Allegra[2], Paola Lenzi[1], Francesco Fornai[1,2]

[1] Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, via Roma 55 - 56126 Pisa (Italy)
[2] IRCCS Neuromed, Via Atinense 18 - 86077 Pozzilli (IS), Italy

Methamphetamine (METH) is a psychostimulant, which is neurotoxic for monoamine neurons and mostly dopamine (DA) containing neurons [1]. The selective toxicity of METH is based on the selective uptake by the presynaptic transporters, namely the DA transporter (DAT), the norepinephrine transporter (NET) and serotonin transporter (SERT) [2]. Once in the neuron, a further step which produces a selective toxicity is the binding of METH with the vesicular monoamine transporter type-2 (VMAT-2). At this level, METH blocks the DA storage, and it disrupts the protonic pump which keeps DA as a polar weak base which cannot spread out the vesicle [3]. In this way, a massive DA efflux takes place in the cytosol [4]. Such an effect is concomitant with a competitive inhibition of monoamine oxidase type-A (MAO-A), which oxidizes DA [5]. Thus, massive amount of DA undergoes self-oxidation, which alters protein structures [6]. Such a specific mechanism of action is likely not to be solely responsible for METH-induced neurotoxicity. In fact, growing evidence shows that mitochondrial functions is affected at multiple levels of the respiratory chain [7]. Thus, in the present study we investigated the morphological aspects of mitochondrial alterations under the effects of METH exposure. Light (both MitoTracker red and MitoTracker green) and transmission electron microscopy (both plain ultrastructural morphometry and immuno-gold stoichiometry) allowed to detail a number of deleterious effects of METH on mitochondria. The effects of METH alter mitochondrial crests, matrix and size, while impairing mitophagy and the biogenesis of mitochondria. These effects are induced quickly following METH administration and persist over time due to the epigenetic regulation of proteins, which are involved in mitochondrial turnover. The METH-induced mitochondrial damage is dose-dependent (from 10 μM up to 1000 μM) and it correlates significantly with METH-induced neurotoxicity as shown by H&E, Trypan blue, and Fluoro-Jade B.

The present data lead to re-consider the molecular mechanisms of METH and provide a novel scenario to explain the persistency of METH-induced alterations of neural activity.

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Alpha-Synuclein oligomers in skin biopsy: a reliable biomarker in GBA- associated Parkinson's disease

Claudia Novello[1], Samanta Mazzetti[1,2], Milo Basellini[3], Alessandra Calogero[1], Rossana Asselta[5], Letizia Straniero[5], Valentina Ferri[2,4], Federica Garri[2,4], Emanuele Cereda[6], Gianni Pezzoli[2,4] and Graziella Cappelletti[1]

[1] Department of Biosciences, University of Milan, Milan, Italy
[2] Fondazione Grigioni per il Morbo di Parkinson, Milan, Italy
[3] Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain
[4] Parkinson Institute, ASST "Gaetano Pini/CTO", Milan, Italy
[5] Department of Biomedical Sciences, Humanitas University, Milan, Italy
[6] Clinical Nutrition and Dietetics Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Parkinson's disease (PD) has been recently redefined as a multisystem disorder [1]. The search of a peripheral biomarker for PD is crucial for diagnosis in alive patients and improve patient stratification for clinical trials. In this field, alpha-synuclein oligomers have been localized in the central nervous system and indicated as 'a new hope' in the field of synucleinopathies [2]. To date, emerging evidence proves the existence of alpha-synuclein-related pathology also in the peripheral nervous system [3]. Using skin biopsy, which constitutes a simple and minimally invasive model to detect alpha- synuclein-related pathology in living patients, we demonstrated the significant increase in alpha- synuclein oligomers in idiopathic PD patients compared to healthy controls [4]. The good sensitivity, specificity and positive predictive value (82%, 86% and 89%, respectively) of the proximity ligation assay score (PLA score) we reported, endorses the hypothesis that alpha-synuclein oligomers could be used as a reliable diagnostic biomarker for PD.

The aim of the present study was to search for alpha-synuclein oligomers in skin biopsies obtained from a PD-GBA cohort (n=22) compared to age- and sex-matched healthy subjects (n=22) and GBA carrier not affected by PD (n=5). Indeed, being GBA a major risk factor for PD, the patients have well-defined disease duration and GBA mutations. Our results indicate that PLA can distinguish PD- GBA patients from age- and sex-matched control subjects ($P<0.0001$). We obtained a high sensitivity (92%) and a good specificity (85%). In addition, PLA score was negative in all GBA carriers which are not affected by PD (n=4; mutation L444P). Furthermore, there was a better correlation with disease duration in PD-GBA (Spearman's rank 0.45) if compared with idiopathic PD (Spearman's rank 0.27; [4]). Finally, PLA score was not related to the different genetic mutations.

All together, these data reinforce the value of PLA score as a reliable biomarker for genetic forms of PD. Likewise, this approach could be useful to shed light on novel neuropathological aspects of PD.

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TRPV1, BDNF and trkB mediate the anti-inflammatory effect of beta-caryophyllene in the Rat Cerebral Cortex after hypoperfusion/reperfusion

Maria Pina Serra[1], Marianna Boi[1], Antonella Carta[1], Elisabetta Murru[1], Gianfranca Carta[1], Sebastiano Banni[1], Marina Quartu[1]

[1]Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, 09042 Monserrato, Italy

The bilateral common carotid artery occlusion followed by reperfusion (BCCAO/R) is a model to study early hypoperfusion/reperfusion-induced changes in biomarkers of the tissue physiological response to oxidative stress and inflammation (1-4). In this study, by means of immunochemical assays, we investigate if a single dose of beta-caryophyllene (BCP), administered before the BCCAO/R, can modulate the TRPV1, BDNF, and trkB receptor in the brain cortex; the glial markers GFAP and Iba1 were also examined. Frontal and temporal-occipital cortical regions were analyzed in two groups of male rats, sham-operated and submitted to BCCAO/R. Six hours before surgery, one group was gavage fed a dose of BCP (40 mg/per rat in 300 μL of sunflower oil), the other was pre-treated with the vehicle alone. Western blot analysis showed that, in the frontal cortex of vehicle-treated rats, the BCCAO/R caused a TRPV1 decrease, an increment of trkB and GFAP, no change in BDNF and Iba1. The BCP treatment caused a decrease of BDNF and an increase of trkB levels in both sham and BCCAO/R conditions while inducing opposite changes in the case of TRPV1, whose levels became higher in BCCAO/R and lower in sham conditions. Present results highlight the role of BCP in modulating early events of the cerebral inflammation triggered by the BCCAO/R through the regulation of TRPV1 and the BDNF-trkB system.

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Choline alphoscerate and thioctic acid activity on brain injury in spontaneously hypertensive rats

Seyed Khosrow Tayebati[1], Proshanta Roy[2], Ilenia Martinelli[1], Daniele Tomassoni[2], Vincenzo Bellitto[1], Francesco Amenta[1].

[1] Clinical Research Centre, School of Medicinal and Health Products Sciences, University of Camerino, 62032 Camerino, Italy
[2] School of Biosciences and Veterinary Medicine, University of Camerino, 62032 Camerino, Italy

Spontaneously hypertensive rats (SHR) represent a model of hypertension and vascular brain injury [1]. Several studies have shown that cerebrovascular changes in SHR may mimic brain vascular disorders of hypertensive individuals. Hypertension represents a risk factor for the development of cerebrovascular disease and cognitive impairment [2] and contributes to the physiopathology of cerebrovascular alterations. This leads to an increased production of reactive oxygen species, cholinergic pathways dysfunction, and neuroinflammation. In SHR, significant brain atrophy, a reduction of white matter volumes, and blood-brain barrier (BBB) dysfunction correlated with gliosis and neuroinflammation.

The present study was designed to investigate if treatment with choline alphoscerate (a-GPC) and (+)-thioctic acid [(+)-TIO], alone or in association, could induce neuroprotection in SHR brain. a-GPC is a cholinergic precursor enhancing cholinergic neurotransmission and countering cognitive impairment associated with cerebrovascular damage. (+)-TIO, the natural eutomer of TIO, has been shown to display antioxidant effects in the brain.

SHR 24-weeks old were treated with a-GPC and (+)-TIO alone or in combination. Age-matched Wistar Kyoto (WKY) rats were used as normotensive controls. Different brain areas were collected for Western blot and immunohistochemistry analysis of neuronal, glial, BBB, and inflammatory markers.

After four weeks of treatment a-GPC and (+)-TIO alone or in association slightly reduced systolic blood pressure values. Western blot and immunohistochemistry showed that a-GPC restored the expression of neuronal nuclei protein. The two compounds alone or in association did not prevent the downregulation of synaptic proteins. a-GPC and (+)-TIO countered astrogliosis and decreased the level of tumor necrosis factor-alpha. An increase of the BBB markers, aquaporin-4 and glucose transport-1, partially restored by the two compounds was noticeable in SHR.

Our results indicate that treatment with a-GPC and (+)-TIO elicits a neuroprotective activity. These data may have a pharmacological relevance and suggest that the two compounds, although they are not anti-hypertensive drugs, could represent a new perspective strategy to prevent hypertension-associated cerebrovascular injury.

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Sviluppo, mantenimento e riparazione della placca neuromuscolare

Marina Boido[1], Roberta Schellino[1], Stefan Hettwer[2], Jan W. Vrijbloed[3], Ruggero G. Fariello[3], Alessandro Vercelli[1]

[1] Dipartimento di Neuroscienze, Neuroscience Institute Cavalieri Ottolenghi, Università di Torino
[2] Neurotune AG, Schlieren, Switzerland
[3] PharmaFox Therapeutics AG, Möhlin, Aargau, Switzerland

Le giunzioni neuromuscolari (placche motrici) sono sinapsi periferiche specializzate, che consentono la trasmissione tra il terminale del nervo motorio e le fibre muscolari scheletriche. I meccanismi che portano all'innervazione muscolare coinvolgono un grande numero di molecole (acetilcolina e suoi recettori, agrina, MuSK, ecc.), che giocano un ruolo cruciale nella formazione/mantenimento delle placche.

In caso di malattie neuromuscolari o durante l'invecchiamento, le placche possono andare incontro a difetti morfo-funzionali, rappresentando un importante target terapeutico.

Ad esempio, i topi affetti da atrofia muscolare spinale (una malattia genetica dei motoneuroni) mostrano anomalie strutturali delle placche e una riduzione del 50% dei livelli di espressione di agrina nei muscoli, rispetto ai controlli. La somministrazione di agrina biologica NT-1654 ha avuto esiti benefici sulle fibre muscolari e sulle NMJ, ritardando la diminuzione delle prestazioni motorie.

Per quanto riguarda l'invecchiamento, la sarcopenia è una malattia progressiva del muscolo scheletrico che colpisce gli anziani. Alla ricerca di nuovi composti per prevenirla e curarla, abbiamo testato una nuova proteina, ActR-Fc-nLG3, dimostrando la sua efficacia nell'aumentare la massa muscolare e nel sostenere l'innervazione nei topi anziani.

Lo studio approfondito dei difetti delle placche aiuterà a definire terapie sempre più efficaci.

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Seconda sessione

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Dissociating temporally segregated segments of the human arcuate fasciculus: when structure meets function

Gianpaolo Antonio Basile[1], Alberto Cacciola[1], Tommaso Marino[2], Giuseppina Rizzo[2], Fabio Trimarchi[1], Giuseppe Santoro[1], Demetrio Milardi[1]

[1] Department of Biomedical, Dental Sciences and Morphological and Functional Images, University of Messina, Messina, Italy
[2] Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

Traditionally considered as a key structure involved in language processing, repetition of verbal information and complex syntax generation, the arcuate fasciculus (AF) is an associative white matter pathway connecting inferior frontal and temporal cortices. Despite being one of the most well-known and studied white matter bundles of the human brain, the anatomical and functional organization of this pathway is still a matter of debate. While earlier anatomo-clinical models of language processing considered the AF as a unique entity, recent evidence has highlighted the importance of distinct tract segments, each with its specific functional relevance in language comprehension and production [1]. Different anatomical subdivisions of the AF have been proposed [2], based either on task-based functional MRI or clinical-pathological correlations in post-stroke patients with aphasia. However, the evidence supporting these models remains largely speculative.

In the present work, we employed track-weighted dynamic functional connectivity (tw-dFC), a recently developed hybrid imaging technique, to analyze structure and function of the AF jointly and in a model-free, unsupervised way. By mapping time-windowed functional connectivity, sampled from resting-state functional MRI, back on the underlying white matter anatomy, reconstructed by tractography, we were able to gather information on spontaneous functional activity of the AF in a large cohort (n=214) of healthy subjects. Data-driven clustering allowed the identification of anatomically and functionally dissociable AF segments, which are arranged according to a dorso-ventral and medio-lateral topographical organization, showing similar functional activity over time. Our results provide the first independent, data-driven evidence for morpho-functional segregation in the AF and may contribute to shed new light on the anatomy and organization of this clinically relevant white matter tract.

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White matter pathways support the dynamic nature of functional brain connectivity

Alberto Cacciola[1], Gianpaolo Antonio Basile[1], Victor Nozais[2,3], Giuseppe Santoro[1], Ludovico Magaudda[1], Giuseppe Pio Anastasi[1]

[1] Department of Biomedical, Dental Sciences and Morphological and Functional Images, University of Messina, Messina, Italy
[2] Groupe d'Imagerie Neurofonctionnelle, Institut des Maladies Neurodégénératives-UMR 5293, CNRS, CEA, University of Bordeaux, Bordeaux, France
[3] Brain Connectivity and Behaviour Laboratory, Sorbonne Universities, Paris, France

The spatial organization of functional connectivity is likely to stem in part from the underlying anatomy of white matter circuits, suggesting that the system-level properties of functional brain networks can be explained by the underlying anatomical connectivity [1]. However, the contribution of structural connectivity to functional connectivity dynamics has yet to be elucidated. Herein, to shed new light on this complex topic, we applied track-weighted dynamic functional connectivity (tw-dFC), which is a powerful tool integrating structural, functional, and dynamic connectivity into a unified framework of analysis [2]particularly in understanding the dynamics of the structural/functional connectivity relation. Structural and functional connectivity are most often analysed independently of each other. Track-weighted functional connectivity (TW-FC. Independent component analysis applied to the tw-dFC data identified well-recognizable, anatomically meaningful maps of white matter bundles linking nodes of gray matter functional networks and showing consistent fluctuations in functional connectivity at their endpoints. We also found high correlation between resting-state and task-based white matter networks, providing complementary evidence to the hypothesis that regions intrinsically connected in the resting state are more easily recruited together during tasks. Unsupervised clustering of functional network connectivity (i.e., the "co-fluctuation" of the mean functional connectivity between pairs of components) disclosed the intrinsic functional organization of white matter components into associative, sensorimotor and visual clusters. Finally, co-fluctuations of functional connectivity between tw-dFC components could predict individual cognitive performance. In conclusion, dynamic fluctuations in functional connectivity are supported by specific, anatomically defined white matter bundles. Altogether our results provide a hybrid white matter atlas revealing novel evidence on the relationship between structural connectivity and functional activity.

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Effects of repetitive transcranial magnetic stimulation (rTMS) on matrix metalloproteases levels and cognitive measures in MCI patients

Giovanni Cirillo[1], Roberta Pepe[2], Sabrina Esposito[2], Francesca Trojsi[2], Michele Papa[1]

[1] Division of Human Anatomy, Laboratory of Morphology of Neuronal Networks & Systems Biology, Department of Mental and Physical Health and Preventive Medicine, University of Campania "Luigi Vanvitelli, Naples, Italy
[2] I Division of Neurology and Neurophysiopathology, Department of Medical, Surgical, Neurological, Metabolic and Aging Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy

Matrix metalloproteases (MMPs) are a large family of endoproteases involved in the degradation of the extracellular matrix (ECM). MMPs modulate synaptic plasticity and neurotrophins levels and control neuroinflammation and glial reaction. These mechanisms are involved in the pathogenesis of many neurodegenerative disorders, including dementias and mild cognitive impairment (MCI), a condition of cognitive decline that might progress to Alzheimer's disease (AD). Evidence has demonstrated an increase of circulating MMPs in MCI and AD patients that correlate with the neurodegenerative process. To date, no effective pharmacological treatments are available to slow or prevent the transition from MCI to AD, thus boosting the application of non-invasive brain stimulation (NIBS) techniques. Repetitive transcranial magnetic stimulation (rTMS) has provided therapeutic effects in several neuro-psychiatric disorders; however, mechanisms of long-term efficacy are largely unknown. It is conceivable that long-term effects reflect modulation of neural activity and brain function beyond the stimulation period at different levels, from the cellular/molecular to the brain network activity.

To study the neurobiological effects of rTMS and its efficacy on cognitive functions, we selected a group of patients (n=27) affected by MCI that received daily/4 weeks high frequency (10 Hz) rTMS or sham stimulation, bilaterally, over the dorsolateral prefrontal cortex (DLPFC). At baseline (T_0), 4 weeks after rTMS (T_1) and 6 months after the end of the stimulation (T_2), we collected cognitive test data and measured the plasmatic levels of different MMPs (MMP-1, MMP-9, MMP-10) and their endogenous inhibitors (TIMPs) (TIMP-1, TIMP-2). Compared to the sham group, we report a significant reduction of MMP-1, 9 and 10 plasmatic levels paralleled by an increase of TIMPs at T_2. Moreover, these data strongly correlate with the improvement of cognitive measures 6 months after rTMS.

In conclusion, we provide evidence of a long-term neurobiological effect of rTMS on MMPs system, that is paralleled by cognitive clinical improvement.

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COVID-19 Neuropathology: Evidence for SARS-CoV-2 invasion of Human Brainstem Nuclei

Aron Emmi[1,7], Stefania Rizzo[2], Luisa Barzon[3], Michele Sandre[4,7], Elisa Carturan[2], Alessandro Sinigaglia[3], Silvia Riccetti[3], Mila della Barbera[2], Rafael Boscolo-Berto[1], Patrizia Cocco[5], Veronica Macchi[1,7], Angelo Antonini[6,7], Monica De Gaspari[2], Cristina Basso[2], Raffaele De Caro[1,7*], Andrea Porzionato[1,7]

[1]Institute of Human Anatomy, Department of Neuroscience, University of Padova, Italy
[2]Department of Cardio-Thoracic-Vascular Sciences & Public Health, University of Padova, Italy
[3]Department of Molecular Medicine, University of Padova, Padova, Italy
[4]Department of Neuroscience, University of Padova, italy
[5]Pathology and Histopathology Unit, Ospedali Riuniti Padova Sud, Padova, Italy
[6] Movement Disorders Unit, Neurology Clinic, Department of Neuroscience, University of Padova
[7] Center for Neurodegenerative Disease Research (CESNE), University of Padova

Objectives: Neurological manifestations are common in COVID-19, the disease caused by SARS-CoV-2. Despite reports of detection of SARS-CoV-2 in the brain and cerebrospinal fluid of COVID-19 patients, it's still unclear whether the virus can infect the central nervous system, and which neuropathological alterations can be ascribed to viral tropism, rather than immune-mediated mechanisms. In the present study, we assess the neuropathological alterations occurring in COVID-19 subjects deceased during the acute stages of the disease and compare these findings with the neuropathological alterations occurring in pneumonia / respiratory failure patients.

Materials: 24 COVID-19 patients and 18 sex- and age-matched controls who died due to pneumonia / respiratory failure were included in the study. Clinical and demographical data was retrospectively obtained. The brain was sampled and underwent extensive neuropathological sampling, with particular focus on the brainstem.

Methods: Immunoperoxidase and immunofluorescent staining for 20 different antibodies (CD3, CD20, CD61, CD68, TMEM119, HLA-DR, pTAU, Beta-Amyloid, Beta-III Tubulin, GFAP, ACE2R, TMPRSS2, etc) was performed to assess the neuropathological alterations and two SARS-CoV-2 specific antibodies were employed to assess viral tropism. RT-PCR analyses for SARS-CoV-2 genomic sequences was performed to complement these findings. Morphometrical evaluation of brainstem regions of interest was performed to asses microglial activation and density.

Results: Aside from a wide spectrum of neuropathological alterations, including hypoxic/ischemic damage, perivascular lympho-monocytic cuffing and other aspecific findings, SARS-CoV-2-immunoreactive neurons were detected in the vagal nuclei of the medulla and in the substantia nigra of 5 COVID-19 subjects. Viral RNA was also detected by real-time RT-PCR. Quantification of reactive microglia revealed an anatomically segregated pattern of inflammation within affected brainstem regions, and was higher when compared to controls.

Discussion: These findings suggest that while acute fatal COVID-19 is characterized by neuropathological alterations, such as hypoxic / ischaemic damage, microthromboses and microgliosis, mediated mainly by systemic infection and the ongoing cytokine storm, SARS-CoV-2 neurotropism is a possible, yet not frequent, consequence of infection without immediately appreciable neuropathological alterations, at least in the acute phases of the disease.

Conclusions: these findings indicate the need to further investigation of the role of SARS-CoV-2 neurotropism in the general population, not only in severe / fatal COVID-19 cases, including whether SARS-CoV-2 neurotropism may determine specific neuropathological sequelae in cases of prolonged infection or in patients suffering from the long-term effects of COVID-19.

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Locus Coeruleus anatomical features in humans: an MRI approach

Filippo Sean Giorgi[1], Alessandro Galgani[1,2], Franco Lombardo[3], Nicola Martini[3], Gloria Tognoni[2], Francesco Fornai[1,4]

[1]Human Anatomy, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy
[2] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
[3] Neuradiology Unit, "Fondazione G Monasterio - CNR/Regione Toscana", Pisa, Italy
[4] Unit of Neurobiology of Movement Disorders, IRCCS INM Neuromed, Pozzilli, Isernia, Italy

The locus coeruleus (LC) is the main noradrenergic nucleus of the brain, providing innervation to the whole cortical mantle and many subcortical structures. LC plays a key role in sleep-wake cycle, and in several cognitive functions including attention, novelty orienting and memory encoding. Histopathological studies have shown that LC is altered in neurodegenerative diseases, and this may be relevant pathophysiologically, as LC integrity exerts neuroprotective effects in models of neurodegeneration. In some previous reports, LC abnormalities have been found also in aged healthy controls; however, recent *post-mortem* studies performed profiting from unbiased stereology did not confirm LC involvement during normal aging.

Recently, magnetic resonance imaging (MRI) with specific T1-weighted sequences for neuromelanin has been used to evaluate in humans the integrity and the anatomical features of LC [1].

Here we describe different approaches we used to assess *in vivo* in humans, by 3.0 Tesla MRI, the LC features, and in particular to provide an estimate of neuronal density (LC intensity), LC volume, and its rostral-caudal extension. In particular, the features of post-acquisition analysis based on native-space-[2] and template-space-[3] derived approaches are reported.

Both methods show that during physiological aging there is not a significant alteration of LC anatomical features: this finding may support the hypothesis that LC degeneration is specific to the pathophysiology of neurodegenerative diseases.

This idea is further supported by our data obtained in subjects affected by mild cognitive impairment and dementia. In fact, we showed that both native-space- and template-space-based LC analysis confirm a marked LC alteration (both in terms of cell density estimation and of nucleus length/volume) in patients with dementia compared with age-matched controls. Furthermore, by the template-based approach, we found that an alteration of specific features of LC also correlates with the proneness to convert to dementia in subjects with mild cognitive impairment after prolonged follow-up. In particular, this was the case for the rostral part of the left LC.

In conclusion, the neuroanatomical tools we used to explore LC features *in vivo* in humans not only might help to assess physiological variations of LC (e.g. gender-related ones, and correlation with sleep or cognitive features) but might even represent a non-invasive *in vivo* biomarker which, together with other ones, may help to predict the evolution of neurodegenerative disorders in subjects with initial disease symptoms.

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Phospholipase C b1 and Phospholipase C g1: key regulators of Glioblastoma aggressiveness and progression

Maria Vittoria Marvi[1], Sara Mongiorgi[1], Giulia Ramazzotti[1], Irene Neri[1], Lucia Manzoli[1], Nikos Tapinos[2], Stefano Ratti[1], Lucio Cocco[1]

[1]Cellular Signalling Laboratory, Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy
[2] Laboratory of Cancer Epigenetics and Plasticity, Brown University, Rhode Island Hospital, Providence Rhode Island, United States of America

Several studies have shown the importance of lipid signaling and phospholipases (PLCs) in the regulation of different mechanisms in the central nervous system as well as in the pathogenesis of human glioblastoma. Glioblastoma is the most aggressive and lethal brain tumor in adults. Nowadays, standard treatment for this tumor includes surgical resection followed by radiation and chemotherapy but, despite these therapeutic approaches, tumor recurrence is inevitable. Therefore, a better understanding of the molecular mechanisms underlying tumor transformation could help to find new successful targeted therapeutic strategies. Our recent works suggested a potential role of PLCβ1 and PLCg1, the two most abundant PLC isoforms in the brain, in regulating the phenotypic characteristics of glioblastoma. It was demonstrated that PLCb1 gene expression correlates with glioma's grade, and it is lower in glioblastoma samples compared to healthy individuals[1]. PLCb1 silencing, in both immortalized and primary cell lines, led to increased cell migration and invasion and induced the upregulation of mesenchymal markers and metalloproteinases. Cell proliferation, through increased Ki-67 expression, and the main survival pathways, as b-catenin, ERK1/2 and Stat3 pathways, were also affected by PLCb1 silencing[1]. On the contrary, data collected on patients' biopsies and engineered cell models, suggested a strong correlation between PLCg1 expression and the acquisition of a more aggressive tumor phenotype[2]. Finally, this trend was deepened using patient derived glioma stem cells (GSCs), which are usually responsible for aggressiveness, resistance, and recurrence in glioblastoma. GSCs analysis confirmed that PLCg1 silencing led to transformations in gene expression and phenotype, highlighting the importance of further investigating phospholipases as potential targets in the development of new therapeutic strategies for glioblastoma.

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The role of astrocytes in Autosomal-dominant leukodystrophy (ADLD)

Stefano Ratti[1], Isabella Rusciano[1], Sara Mongiorgi[1], Irene Neri[1], Alessandra Cappellini[1], Pietro Cortelli[2,3], Lucio Cocco[1], Giulia Ramazzotti[1], Lucia Manzoli[1]

[1] Cellular Signalling Laboratory, Department of Biomedical and NeuroMotor Sciences (DIBINEM), University of Bologna, Bologna, Italy
[2] Department of Biomedical and NeuroMotor Sciences (DIBINEM), University of Bologna, Bologna, Italy
[3] IRCCS Istituto delle Scienze Neurologiche di Bologna, UOC NeuroMet, Bologna, Italy

Autosomal Dominant Leukodystrophy (ADLD) is a rare and fatal adult-onset neurodegenerative disorder affecting central nervous system myelination. It is characterized by Lamin B1 (LMNB1) gene alterations[1], but the molecular mechanisms behind it are still unclear. Considering the role that oligodendrocytes, astrocytes and Leukemia Inhibitory Factor (LIF) have in myelination, this work aims to analyze the alterations in different cell populations. Morpho-functional aspects of primary cells from ADLD patients and engineered cellular models overexpressing LMNB1 have been analyzed. Results showed that ADLD patients' cells and astrocytes overexpressing LMNB1 display nuclear alterations not present in oligodendrocytes. Moreover, LMNB1 accumulation in astrocytes induces a reduction in LIF and LIF-R levels, leading to Jak/Stat3 and PI3K/Akt axes downregulation. Administrating exogenous LIF, the toxic effects induced by LMNB1 accumulation may be partially reverted in astrocytes but not in oligodendrocytes[2]. In addition, LMNB1 overexpression induces GSK3β inactivation, but not the upregulation of β-catenin targets resulting in a reduction of astrocyte survival. Moreover, LMNB1 accumulation affects proliferation and cell cycle progression with PPARγ and p27 increase and Cyclin D1 decrease, reducing cell viability and causing apoptosis and cytotoxicity. Interestingly, astrocytes overexpressing LMNB1 show increased immunoreactivity for both GFAP and vimentin together with NF-kB nuclear translocation and c-Fos increase, suggesting astrocytes reactivity and substantial cellular activation . Moreover, ADLD patients' cells display the activation of proinflammatory mechanisms and the increase of reactive oxygen species. Finally using 3D nanofiber myelination assay, preliminary data demonstrated that primary OPCs are unable to produce myelin when grown with primary astrocytes transfected with LMNB1.These data demonstrate that LMNB1 accumulation leads to cell sufferance, probably related to the induction of astrocytes reactivity that could be associated to ADLD pathology. Our studies, for the first time, hint at a pivotal role of astrocytes in ADLD.

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CD44 isoforms distribute differentially in CNS areas during EAE and is regulated by TLR2 in CD4+ cells during acute episodes of Multiple Sclerosis

Anna Maria Stabile[1], Alessandra Pistilli[1], Francesco Ria[2], Maria Tredicine[2], Matteo Lucchini[3], Camilla Moliterni[2], Assunta Bianco[3], Massimiliano Mirabella[3], Desirée Bartolini[1], Mario Rende[1], Gabriele Di Sante[1]

1 Department of Surgery and Medicine, Institute of Human, Clinical and Forensic Anatomy, Piazza L Severi 1, 06125 Perugia, Italy
2 Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy
3 Department of Neuroscience, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy

Mycobacterium Tuberculosis-derived products determine trafficking properties of antigen-specific T cells through its interaction with TLR2. Here, we report that Mtb products and TLR2 polymorphism together regulate CD44 expression on antigen specific, activated T cells, in vivo. TLR2 ligands modified the role of CD44 in trafficking of activated CD4+ T cells, shifting their migration capability depending on the mouse strain (1-3). CD44 represent a highly specific marker of bone marrow-derived cells infiltrating the CNS and anti-CD44 monoclonal antibody treatment blocks the development of EAE. Such a treatment could not be extended to human MS because of severe side effects. We previously reported that distribution of CNS infiltrates varied between EAE in the SJL and C57Bl/6 mice (1, 2, 4), with the forebrain being selectively infiltrated in the SJL strain. Thus, infectious (pathogenic or commensal) agents can modulate the trafficking properties of (self-reactive) T cells, leading to exacerbation of acute autoimmune diseases (3, 5).

Here we report that mRNA specific for isoform v9-v10 of CD44 (associated to the SJL/J variant of TLR2) was enriched in the forebrain of SJL EAE, suggesting that CD44 v9-10 isoform drove specifically the localization of infiltrating T cells in the forebrain. By performing in silico modelling, we found that CD44 v9-10 isoform displayed a reduction of the solvent accessible surface with respect to CD44 v8-10 and CD44s. TLR2 stimulation increased the ability of CD44 to bind stably to osteopontin, which may be responsible for the enrichment of CD44 v9-10 -positive cells in the forebrain. Thus, interaction with selected isoform(s) of CD44 and specific (extra-cellular matrix) components of the CNS may represent an innovative target for MS therapy.

Therefore, we examined the expression of CD44 variant isoforms in cells from the CSF of 34 MS patients. We observed that the MS patients could be divided in two subgroups based on the expression of two CD44 variants (namely, v7 and v8), either in CD44v7high/low or CD44v8high/low. CD44v7 (but not v8) was highly expressed in CSF cells from MS patients with gadolinium-enhanced (GE+) lesions confirming the relevance of our observations in the EAE model also in human pathology. Next, we assessed if TLR2 could modulate the repertoire of CD44 v7 and v8 in human activated CD4+ cells. We therefore, compared the expression of mRNA for CD44v7 and v8 upon TLR2 and TCR stimulation in CD4+, activated T cells from MS patients with GE+ lesions, MS patients without GE+ lesions and normal donors. The data showed that CD44s was upregulated (albeit mildly) in all samples. CD44v8 was upregulated only in MS patients. CD44v7 was upregulated only in cells from MS patients with GE+ lesions. Collectively, our data show that TLRs represent a key pathway through which pathogens or commensals of viral and bacterial origin modify trafficking of antigen-specific T cells. The presence of GE+ lesions associated with the ability of TLR2 to drive the production of CD44v7,

which in turn appears enriched in CSF cells. It will require further studies to explain how this ability is “transiently” wired in T cells.

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POSTER

Anatomia clinica e forense, anatomia per immagini, ingegneria tessutale e medicina rigenerativa

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Keratoconus: the possible involvement of inflammatory cytokines in its pathogenesis. An experimental study

Marco Artico[1], Vincenzo Scorcia[2], Marcella Nebbioso[1,3], Giuseppe Soda[4], Alessandra Serraino[5], Benedetta Giordano[5], Alessandra Micera[6], Samanta Taurone[1,6]

[1] Department of Sense Organs, "Sapienza" University of Rome, Rome, Italy
[2] Department of Ophthalmology, University Magna Græcia, Catanzaro, Italy
[3] Ophthalmology Unit, "Sapienza" University of Rome, Rome, Italy
[4] Department of Molecular Medicine, "Sapienza" University of Rome, Rome, Italy
[5] Division of Neurosurgery, Department of Human Neurosciences, "Sapienza" University of Rome, Rome, Italy
[6] IRCCS – Fondazione Bietti, Rome Italy

Keratoconus (KC) is generally described as a non-inflammatory disease, characterized by thinning in the central region of the cornea with consequent tissue degradation producing impaired visual acuity. In our experimental study, we analyzed the presence and implications of several inflammatory cytokines in the corneal tissues of patients suffering from keratoconus by immunohistochemical analysis. The analysis showed increased levels of inflammatory factors in the pathological tissues compared to controls, confirming that KC cannot be considered an entirely non-inflammatory pathology and that its etiopathogenesis includes different chronic inflammatory events. In the light of these results, the classification of KC as an inflammatory pathology or as a pathology related to inflammation might be useful in directing future research on the development of effective anti-inflammatory therapies that pharmacologically target the inflammatory mediators contributing to the development and progression of the disease.

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Biomimetic scaffolds based on oxidized polyvinyl alcohol and functionalized chitosan sponges for peripheral nerve regeneration

Silvia Barbon[1,2], Elena Stocco[1,2], Elena Zeni[3], Leonardo Cassari[3], Rafael Boscolo-Berto[1], Marta Confalonieri[1], Antonio Gloria[4], Teresa Russo[4], Veronica Macchi[1,2], Monica Dettin[3], Raffaele De Caro[1,2], Andrea Porzionato[1 2]

[1] Section of Human Anatomy, Department of Neuroscience, University of Padova, Padova, Italy
[2] LifeLab Program, Consorzio per la Ricerca Sanitaria, Padova, Italy
[3] Department of Industrial Engineering, University of Padova, Padova, Italy
[4] Institute of Polymers, Composites and Biomaterials, National Research Council of Italy, Naples, Italy

Tissue engineering strategies represent a reference point for preclinical research aiming to address the complex requirements of peripheral nerve repair. In particular, efforts are being made towards the optimization of biomaterials which can be fabricated as tubular scaffolds, or neuroguides, to enhance both structural and functional regeneration of the damaged nerve. Among these materials, the polysaccharide chitosan (ChS) and the synthetic polymer oxidized polyvinyl alcohol (OxPVA) have been shown to possess biomimetic properties which make them promising candidates for the development of innovative devices for neural repair. This work explores the fabrication of biohybrid scaffolds made of chitosan sponges functionalized with the self-assembling-peptide EAK +/- binding of laminin-derived adhesive sequences (IKVAV or YIGSR) and then combined with OxPVA by physical cross-linking. The chitosan-based sponges (ChS; ChS+EAK; ChS+EAK-IKVAV; ChS+EAK-YIGSR) were first fabricated as discoidal, white matrices showing a spongy-like macroscopic appearance and a microporous ultrastructure characterized by scanning electron microscopy (SEM). When mechanically assessed by compression tests, the ChS sponges showed a behavior similar to a flexible foam. Nevertheless, EAK addition to chitosan decreased the compressive modulus from 40.4 ± 3.1 kPa (ChS) to 31.1 ± 2.1 kPa (ChS+EAK) and the maximum stress from 16.8 ± 1.0 kPa (ChS) to 11.9 ± 0.6 kPa (ChS+EAK). These parameters were furtherly affected by the binding of the laminin-derived sequences, with the compressive modulus decreasing to 30.9 ± 1.9 kPa (ChS+EAK-IKVAV) and 30.6 ± 2.0 kPa (ChS+EAK-YIGSR) and the maximum stress being reduced to 7.3 ± 0.4 kPa (ChS+EAK-IKVAV) and 7.2 ± 0.4 kPa (ChS+EAK-YIGSR). After the fabrication of the biohybrid composites, SH-SY5Y cell seeding was performed to validate their bioactivity. Cell viability evaluation by MTT assay highlighted that the different composite scaffolds assured for a comparable cell growth at the earliest end-point (72 hours), whereas a significantly higher cell proliferation was sustained by OxPVA/ChS and OxPVA/ChS+EAK in the long term (7 days). Ultrastructural analysis of seeded scaffold surface demonstrated homogeneous cell distribution onto the different supports, detecting the presence of colonies at 72 hours from seeding, which became thicker clusters of cells at 7 days; this suggests an increased proliferation rate over time. As the final validation of scaffold biocompatibility, biohybrid supports were implanted for 14 days into the subcutaneous tissue of BALB/c mice. Histological and immunohistochemical investigations of the explanted samples revealed no severe immune response towards the composite grafts, with moderate lympho-monocyte infiltration (CD3- and F4/80-positive cells) at the graft-host interface. Overall, this in vitro study demonstrated the high bioactivity and biocompatibility of OxPVA/chitosan composite scaffolds, which turn out to be prospective candidates for in vivo applications into animal models of peripheral nerve injury.

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Extracellular Heat Shock Proteins in cancer theranostics: from bench to bedside

Celeste Caruso Bavisotto[1,2], Radha Santonocito[1], Giuseppa D'Amico[1], Francesca Rappa[1], Antonella Marino Gammazza[1], Everly Conway De Macario[3], Alberto J.L. Macario[3], Fabio Bucchieri[1], Claudia Campanella[1], Francesco Cappello[1,2]

[1] Department of Biomedicine, Neurosciences and Advanced Diagnostics, Section of Human Anatomy, University of Palermo, 90127 Palermo, Italy
[2] Euro-Mediterranean Institute of Science and Technology (IEMEST), 90139 Palermo, Italy
[3] Department of Microbiology and Immunology, School of Medicine, University of Maryland at Baltimore-Institute of Marine and Environmental Technology (IMET), Baltimore, MD 21202, USA

Cancer diagnosis includes a variety of tests to arrive to effective therapy choice and the correct patient monitoring. Among these, molecular analyses are typically performed on tissue acquired through a biopsy at diagnosis. However, tumors are highly heterogeneous and sampling in its entirety is challenging. Furthermore, it is well acknowledged that the early detection of cancer is critical to reducing patient mortality and increasing survival (1). In the past decade, a deal of attention has been paid to the use of liquid biopsy for the early diagnosis of tumors, and in particular plasmatic extracellular vesicles (EVs), as non-invasive platforms carrying molecules that provide clues regarding their origin, making it possible to obtain signatures from tissue-specific origins (2). Heat shock proteins (Hsps) are key molecules in cancer events, constitutively expressed at high levels and could furthermore be induced by the response to cancer-induced stress, representing important cancer hallmarks. Hsps and their regulatory molecules, such as microRNAs (miRNAs), can be released by EVs, thus are able to modulate the tumor microenvironment as well as long-distance intercellular communication and metastatization (3, 4).

We studied tumor samples obtained from a series of patients affected by different cancer type, focusing in particular on the role of extracellular Hsps and their miRNAs regulators, released by EVs. We propose extracellular Hsps as early cancer biomarkers, to be studied from liquid biopsy thought a set of standardized methods. Furthermore, extracellular Hsps can be considered as targets for modern and effective anticancer therapies.

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Liquid flow in natural derived Hydroxyapatite scaffold: experimental observations, biological outcome and CFD analysis

Jessika Bertacchini[1], Elisabetta Salerno[2], Giulia Orlandi[1], Claudio Ongaro[3], Alessandro d'Adamo[3], Andrea Ruffini[4], Gianluca Carnevale[1], Barbara Zardin[3], Diego Angeli[5]

[1] Department of Surgery, Medicine, Dentistry and Morphological Sciences with Interest in Transplant, Oncology and Regenerative Medicine, University of Modena and Reggio Emilia, Via del Pozzo 71, 41125 Modena, Italy
[2]CNR-NANO S3 Research Center on nanoStructures and bioSystems at Surfaces, via Campi 213/A, I-41125 Modena, Italy
[3] DIEF-Engineering Department "Enzo Ferrari", Via Pietro Vivarelli 10, 41125, Modena, Italy
[4] Institute of Science and Technology for Ceramics (ISTEC), National Research Council (CNR), Via Granarolo 64, 48018, Faenza, Italy
[5] Department of Sciences and Methods for Engineering, University of Modena and Reggio Emilia, Via Amendola 2, 42122 Reggio Emilia, Italy

In the field of bone tissue engineering, a remarkable interest is drawn by the development of three-dimensional cultures to study the bone cell proliferation under conditions as similar as possible to *in vivo* ones, e.g. by artificially producing mechanical stresses promoting a biological response (mechanotransduction). In this regard, a promising candidate is the fluidic shear stress, which governs the delivery rate of nutrients to the growing cells and that can be easily controlled in perfusion reactors. However, the introduction of 3D scaffolds complicates the direct measurement of the generated fluidic shear stress on the adhered cells inside the solid matrix, thus jeopardizing the complete potential of using multi-dimensional matrices. In our study we present a description of the fluidic behavior of the porous scaffold B-HA (1,2) and the preliminary observations about the modifications induced by different fluid shear stresses applied to osteoblast cells cultured on B-HA surface (3). The natural origin of B-HA makes this scaffold peculiar for its geometry that it is neither predictable nor fully customizable. For this reason, there is the need to characterize the behavior of both the B-HA scaffold and the surrounding cells, to understand the mechanisms that controls the mineralization process of cultured cells under static and dynamic conditions. For this broad purpose, our experimental workflow comprised three distinct phases: **1)** characterization of B-HA scaffold properties, useful to design a fluid perfusion system (bioreactor) and to define the fluid shear stresses acting on the cells under dynamic conditions. To this aim, the flow resistance, and the morphological features of a number of B-HA samples were analyzed. **2)** Assembling of a simple and inexpensive bioreactor, aimed at exposing the cells cultured in the B-HA scaffold to physiologically relevant flow conditions. **3)** Study of the morphological and biological modifications of osteoblast cells attached to B-HA, as induced by the flow. Although these experimental methods are reliable, they can be excessively time consuming, especially for the detailed characterization of the fluid flow behavior and consequent shear stress estimation. Therefore, the authors are focusing on Computational Fluid Dynamics (CFD), an effective tool able to investigate the complex relationship between the hydrodynamic environment and the regeneration of engineered tissues

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Age-related differences on nociceptive fibers in the knee joint: pilot study in two dissected specimens

Elisa Borsani[1,2,3], Stefania Castrezzati[1], Lorena Giugno[1], Flavio Azzola[4], Giovanni Bonaspetti[4], Francesca Bonomini[1,2,3]

[1] Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[2] Interdepartmental University Center of Research "Adaption and Regeneration of Tissues and Organs (ARTO)", University of Brescia, Brescia, Italy
[3] Italian Society for the Study of Orofacial Pain (Società Italiana Studio Dolore Orofacciale – SISDO), Brescia, Italy
[4] Department of Orthopaedics and Trauma Surgery, Istituto Clinico S Anna, Brescia, Italy

The multimodal pain control regimens represent a great clinical intervention for the optimization of therapies. In this regard, the age-related knowledge of the distribution and the density of nerve fibers, and in particular the nociceptive fibers, in the knee structures could influence and address pharmacological therapies and surgical and/or injection techniques.

The aim of this work was to dissect, isolate and analyze knee joint structures from a morphological and immunohistochemical point of view examining the distribution and density of nociceptive nerve fibers.

The anatomical study was conducted on two cadavers. In particular, on the right knee joint of two males at the age of 58 and 90 years.

Specimens were stored (- 20°C), defrosted before the dissecting session, and analyzed at the Anatomical Training Centre of the University of Brescia. The samples of capsule, ligaments, menisci, and infrapatellar fat pad have been evaluated immunohistochemically for PGP9.5 (Protein Gene Product 9.5), a marker for all the nerve fibers, and for CGRP (Calcitonin gene-related peptide) and SP (substance P) for nociceptive nerve fibers.

The results showed that the examined structures presented differences in the distribution and density of nerve fiber markers also in relation to the age of the subjects proposing new and wide studies to support the hypothesis that during aging there is a process of progressive reorganization of nerve endings.

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Abnormal communication between the internal spermatic and colic veins: evidence of an asymptomatic anatomical variation in living

Rafael Boscolo-Berto[1], Roberto Vezzaro[2], Andrea Porzionato[1], Veronica Macchi[1], Raffaele De Caro[1]

[1] Institute of Human Anatomy, Department of Neurosciences, University of Padova, Padova, Italy
[2] Radiology Unit, Pescara Hospital, Italy

Antegrade sclerotherapy is an invasive therapy used in the treatment of male varicocele. Among the possible, albeit rare, consequences are the so-called ischemic colitis, as reported since the first adoption of this treatment choice into urological clinical practice. The pathogenetic explanation relies on the presence of an atypical portal-systemic communication between the internal spermatic vein and the colic veins, which represent an infrequently reported anatomic variant.

By reviewing 100 consecutive CT scans performed with contrast medium for non-urological diagnostic indications, one case was detected with a significant anatomical variation consisting of a vascular communication between the internal spermatic and the colic veins as part of the portal-systemic anastomoses.

Although the prevalence of this anomaly is not negligible according to the relevant literature, only a few clinical cases reporting ischemic colitis following the antegrade sclerotherapy of the internal spermatic vein were published to date, and no modern CT-image of the anatomical variation is readily available.

For the first time, we demonstrated by modern CT scan the anatomical variation underlying a rare but devastating consequence of the antegrade sclerotherapy of the internal spermatic vein. This reference allows a better understanding of the anastomotic pathway between the anatomical planes. Furthermore, in the case of suspected post-procedure consequences, this CT reconstruction provides a comparison image that helps the radiologist formulate the diagnosis.

Once again, these findings underline that the knowledge of vascular anomalies involving the internal spermatic vein is of utmost importance to raise scientific awareness and prevent possible devastating consequences of the antegrade sclerotherapy for varicocele. Progress in the medical field and increased medical-legal awareness have supported the ripening of clinical anatomy and forensic clinical anatomy, whose multidisciplinary represents the strength.

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Autonomic Nervous System: a cadaveric study

Giannicola Carrozzo[1], Francesca Chiarini[2,3], Ester Orsini[1], Marilisa Quaranta[1], Giulia Adalgisa Mariani[1], Desirée Martini[1], Simone Lodi[1], Anna Maria Billi[1]

[1] Department of Biomedical and Neuromotor Sciences, Human Anatomy Section, University of Bologna
[2] CNR Institute of Molecular Genetics Luigi Luca Cavalli-Sforza, Bologna,
[3] IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy

The present study aims to compensate for the poor iconography of the macroscopic anatomy of the Autonomic Nervous System (ANS), from the emergence of the nerves up to the abdominal ganglia.

Furthermore, it could be very useful to understand the pathophysiology of diseases like PAF (Pure Autonomic Failure), a neurodegenerative disorder of the ANS characterized by orthostatic hypotension and other signs. It might be also helpful for providing images to plan surgical intervention such as sympathectomy, an irreversible procedure during which at least one sympathetic ganglion is removed, for example in extreme cases of hyperhidrosis, Raynaud's syndrome or neuropathic pain.

The study was carried out on an embalmed cadaver from the Body Donation Program of the ACChiSM Center, division of the Department of Biomedical and Neuromotor Sciences of the University of Bologna.

Dissection began by opening the posterior cranial fossa to show the brain stem and the emergence of the lower cranial nerves.

Upon the posterior surface of the pharynx, next to the sympathetic chain and the superior cervical ganglion, Hering's nerve and superior cardiac nerves were identified. The numerous anastomoses, mainly between the superior cervical ganglion and vagus nerve, were preserved.

In the neck, the final part of Hering's nerve was exposed along with the origin of the superior cardiac branches. The sympathetic chain appeared interspersed with two small ganglia: the middle cervical one enveloping the inferior thyroid artery and the vertebral one lying on the vertebral artery. The middle cardiac nerve was seen arising from the middle cervical ganglion and its relationship with vagus and cervical plexus were highlighted.

At the thoracic level, the stellate ganglion gave the inferior cardiac nerve, which contributed to the cardiac plexus. The latter could be divided in a superficial plexus, located below the aortic arch and in front of the right pulmonary artery, and a deep plexus, at the tracheal bifurcation.

The anterior and posterior esophageal plexuses, resulting from anastomoses of the vagus nerves, were also isolated.

In the abdomen, the celiac and superior mesenteric ganglia seemed to adhere to their respective vessels with a felt-like appearance. Relationship with the vagus, greater and lesser splanchnic nerves and the adrenal glands were also highlighted.

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Sertoli cells counteract muscle atrophy *in vitro* and in preclinical models of cancer cachexia

Sara Chiappalupi[1,2,#], Laura Salvadori[2,3,#], Iva Arato[1], Francesca Mancuso[1], Mario Calvitti[1], Francesca Riuzzi[1,2], Riccardo Calafiore[1], Giovanni Luca[1] and Guglielmo Sorci[1,2]

[1] Department of Medicine and Surgery, University of Perugia, Perugia 06132, Italy
[2] Interuniversity Institute of Myology (IIM), Perugia 06132, Italy
[3] Department of Translational Medicine, University of Piemonte Orientale, Novara 28100, Italy
[#] Contributed equally

Duchenne muscular dystrophy (DMD) is a lethal X-linked disease due to mutations in the dystrophin gene resulting in chronic muscle inflammation and degeneration [1]. Despite an intense effort in finding a cure, the proposed therapies have shown substantially unsatisfying results so far, due to limitation in survival and homing of the engrafted cells, treatment of respiratory muscles, application limited to specific *DMD* gene mutations, need of pharmacological immunosuppression, and multiple side effects [2]. We set up a preclinical protocol to treat DMD based on the peculiar immunological properties of Sertoli cells (SeC) [3]. We purified SeC from prepubertal piglets' testes and encapsulated them into clinical-grade alginate microcapsules (MC-SeC). Intraperitoneal (i.p.) injection of MC-SeC in dystrophic mice translated into dramatic reduction of infiltrating inflammatory cells, fibrosis, and necrosis in muscles, and in SeC-derived heregulin β1-mediated induction of the dystrophin paralogue, utrophin at the sarcolemma, thus resulting in recovery of muscle performance [4]. Here we show that SeC protect C2C12 myotubes against atrophy induced by different stimuli (i.e., TNF$\alpha\pm$IFNγ, dexamethasone or starvation). Independently from the stimulus applied, SeC protected myotubes against reduction of diameters and loss of myosin heavy chain (MyHC), and restrained atrogenes (*Fbxo32* and *Trim63*) expression in a dose-dependent manner. These effects were confirmed in *in vivo* models of cancer-induced cachexia. Indeed, WT mice subcutaneously injected with (pro-cachectic) Lewis Lung Carcinoma or B16 melanoma cells in the presence of i.p.-injected MC-SeC showed reduced i) loss of body weight, ii) shift towards lower myofiber cross-sectional areas, iii) MyHC degradation, and iv) atrogenes expression, in comparison with tumor-bearing mice injected with empty microcapsules. Together with reported results [5], our data show that i.p. injection of MC-SeC as a treatment to DMD is endowed with several advantages: it can easily reach all body muscles, it is equivalent to a combinatorial approach, it does not require any pharmacological immunosuppression, and it represents a universal approach being efficacious irrespective of the *DMD* mutation.

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Functional ablation of the receptor for advanced glycation end-products (RAGE) in LLC cells translates into reduced tumorigenic and cachectic potential

Sara Chiappalupi[1,2,#], Giulia Gentili[1,2,#], Laura Salvadori[2,3], Cristina Marchetti[1], Martina Paiella[3], Francesca Riuzzi[1,2,$] and Guglielmo Sorci[1,2,$]

[1] Department of Medicine and Surgery, University of Perugia, Perugia 06132, Italy
[2] Interuniversity Institute of Myology (IIM), Perugia 06132, Italy
[3] Department of Translational Medicine, University of Piemonte Orientale, Novara 28100, Italy
[#] Contributed equally [$] Shared senior authorship

Cachexia is a highly debilitating multifactorial syndrome affecting more than 50% of patients with advanced cancer, especially lung and upper-gastrointestinal cancer, and accounting for about 20% of cancer-associated deaths. Skeletal muscle atrophy is the major clinical feature of cachexia leading to pronounced weight loss, poor quality of life, reduced response to chemotherapy, and poor prognosis and outcome [1,2]. Nevertheless, cachexia remains a poorly understood process for which a complete pharmacological cure is not available. We reported that genetic ablation of the multiligand receptor RAGE (receptor for advanced glycation end-products) in mice translated into delayed loss of muscle mass and strength, reduced tumor progression, and increased survival, suggesting a major role of RAGE in the cachectic syndrome [3]. To elucidate the role of RAGE in the tumor environment promoting cachexia, we generated LLC (Lewis lung carcinoma) clones stably transfected with pcDNA3/RAGEΔcyto (RAGE antagonist), pcDNA3/RAGE (full-length RAGE, to increase RAGE expression) or empty vector. We found that functional ablation of RAGE in LLC (LLC/RAGEΔcyto) cells translated into morphological changes and increased apoptosis extent, which was accompanied by reduced phosphorylation (activation) levels of ERK1/2. LLC/RAGEΔcyto cells also exhibited reduced migration in *in vitro* assay and almost inability to form colonies in soft agar. When injected subcutaneously in WT and *Ager*-/- (RAGE-KO) mice, LLC/RAGEΔcyto cells formed smaller masses than LLC/RAGE and LLC/empty-vector cells, with the smallest masses found in *Ager*-/- mice. Interestingly, *tibialis anterior* muscles of mice injected with LLC/RAGEΔcyto showed reduced expression of the atrogene, *Fbxo32*, which was found at levels comparable to those of untreated controls in *Ager*-/- mice. In accordance, and contrary to medium conditioned by LLC/RAGE or LLC/empty-vector, LLC/RAGEΔcyto-conditioned medium was unable to induce C2C12 myotube atrophy *in vitro*.

Altogether, our data confirm reported findings about the role of RAGE expressed by the host in cachexia conditions [3], and reveal an important role of RAGE expressed by LLC cells in sustaining the tumor properties of these latter and the muscle atrophy induced by the presence of LLC tumor masses, suggesting that the inhibition of RAGE might represent a potential approach to counteract loss of muscle mass and strength in cancer patients.

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Chitosan-based breast cancer cell cultures for in vitro evaluation of anticancer treatments

Bianca Bazzolo[1], Elisabetta Sieni[2], Annj Zamuner[3], Monica Dettin[3], Rosa Di Liddo[1], Maria Teresa Conconi[1]

[1] University of Padova, Department of Pharmaceutical and Pharmacological Sciences, 35131 Padova, Italy
[2] Department of Theoretical and Applied Sciences, University of Insubria, via Dunant, 3, 21100 Varese, Italy
[3] University of Padova, Department of Industrial Engineering, via Marzolo, 9, 35131 Padova, Italy

In drug development, two-dimensional (2D) cell cultures are widely used to choose the most effective drug candidates that are then tested in animal models. Nevertheless, these cultures, almost completely lacking extracellular matrix (ECM), may lead to unreliable results because they do not reflect the tissue architecture. Thus, several three-dimensional (3D) in vitro models, such as spheroids and hydrogel-based cultures, have been developed to resemble the tumour microenvironment and reduce the use of animals in testing according to the 3R rule (Reduction, Replacement, Refinement).

Herein, a 3D in vitro model of breast cancer has been obtained by seeding HCC1954 cells on lyophilized scaffolds composed of chitosan that derives from partial deacetylation of chitin and presents a structural similarity to the glycosaminoglycans (GAG) of ECM. Cultures were characterized at various time points (1, 3, and 7 days from seedings): cell morphology, cell growth, stemness, and matrix deposition were evaluated by means of phase contrast microscopy, cell viability, histochemical staining, and mRNA expression analysis. Then after, the responses of breast cancer cells to anticancer drugs were evaluated. All results were compared to that obtained on 2D cell cultures.

Our data have shown that chitosan hydrogels allowed the formation of 3D cultures where breast cancer cells organized themselves to form not only monolayer but also spheroids and produce ECM molecules, such as collagen1A1 and laminin B1. In 3D cultures cancer stem cell population was enhanced, as demonstrated by increases in the expression of stemness markers, such as Nanog, SOX2, OCT4, and in sphere-forming ability. Furthermore, the N-cadherin/E-cadherin ratio was significantly higher than that detected in 2D cultures. Finally, chitosan-based cultures were less sensitive to doxorubicin than adherent cell cultures. Collectively, results suggest that chitosan-based breast cancer cell cultures may represent a promising model for in vitro evaluation of anticancer treatments.

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Manuka essence associated with Vitamin C and E positively influences the vitality and viability of fibroblasts

Dolaji Henin[1], Luiz Fiorin[1,2], Mariachiara Perrotta[1], Elena Canciani[1], Marilisa Toma[1], Riccardo Sirello[1], Gabriela Carrara[1,2], Claudia Dellavia[1]

[1] Thin Section Laboratory, Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano (UNIMI)- Milan, Italy
[2] Department of Diagnosis and Surgery- Division of Periodontics, São Paulo State University "Júlio de Mesquita Filho"- Araçatuba, Brazil, UNESP.

Background and aim: Wound healing in compromised subjects is still a critical issue in oral surgeries. Studies have confirmed the role of the Hyaluronic Acid (HA) and amino acids in promoting the healing of the oral mucosa, favoring neoangiogenesis and reducing the inflammatory process [1]. Also, Vitamins C and E showed antioxidant and collagen stimulating effect [1, 2]. However, the addiction of substances with antibacterial and antifungal properties may help the biological response to the tissue injury. Manuka essential oil is known for its antimicrobial and analgesic features. Aim of this in vitro study was to test the behavior of gingival fibroblasts towards a new topical device developed at improving the oral wound healing.

Materials and methods: A vehicle gel based on HA, Glycine, Leucine and Proline was enriched with Manuka essence 3%, Vitamin C 1.4% and Vitamin E 0.14%. The new compound was diluted to 5% with culture medium (HAM F12) to create a stock solution. Primary gingival fibroblasts were obtained and cultured at 37 °C in an incubator at 5% of CO2 until the end of the experiment. Fibroblasts were brought to a confluence of 70-80% before application of the gel. On the fourth day, the medium was replaced with a medium at the different concentrations of the gel (5%, 2.5%, 1%). The Alamar Blue test was performed at 24h and 48h to evaluate the toxicity of the gel and the cellular metabolism.

Results: At 24h the survival rate of the gingival fibroblasts was: 52.45% with 5% gel, 76.6% with 2.5% gel and 76.6% with 1% gel. Compared to the vehicle gel, the new compound at all concentrations presented an increase in vitality between 24 and 48h as follows: 110.75% (5% gel); 124.55% (2.5% gel); 127.55 (1% gel).

Conclusions: Manuka and vitamins enriched gel demonstrated to favor the viability and vitality of gingival fibroblasts, particularly when applied at 1% concentration.

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TRPV1 as a potential target for improving skincare

Gaia Favero[1,2], Giano` Marzia[1], Caterina Franco[1], Claudio Lonati[1,3], Elisa Borsani[1,2,3], Daniela Pinto[4], Fabio Rinaldi[4]

[1] Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[2] Interdipartimental University Center of Research "Adaption and Regeneration of Tissues and Organs (ARTO)", University of Brescia, Brescia, Italy
[3] Italian Society for the Study of Orofacial Pain (Società Italiana Studio Dolore Orofacciale – SISDO), Brescia, Italy
[4] Human Microbiome Advanced Project Institute, Milan, Italy

The skin helps to regulate the temperature, performs a secretory function, protects against external toxic chemicals and harmful microorganisms, as well as the sun's rays. Intrinsic skin aging involves chronological damage caused by slow tissue degeneration; whereas skin photoaging is primarily the result of ultraviolet (UV) exposure [1,2]. However, cutaneous aging physio-pathological processes have not been fully clarified. The transient receptor potential vanilloid 1 (TRPV1) is a nonselective cation channel linked to heat, pH and pain [3]. To date, the TRPV1 signalling involvement in skin have not been elucidated clearly. In this study, our research group investigated the putative epidermal TRPV1 signaling pathway related to aging. We observed in human face skin biopsy (lateral ocular angle) that epidermal responses to numerous environmental and physio-pathological stimuli may be mediated by activation of TRPV1 signaling pathway, so displacing the notion that keratinocytes function strictly in a barrier role for the body.

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Preliminary study at ultrastructural level on prostate specific antigen (PSA) in salivary glands

Michela Isola[1], Lai Ylenia[1], Roberta Noli[1], Francesco Loy[1], Raffella Isola[1]

[1] Department of Biomedical Sciences, Section of Cytomorphology, University of Cagliari, Italy

Prostate-specific antigen (PSA), a biomarker used for the detection of prostate cancer diagnosis, has been found in serum and in saliva. The PSA reactivity was also observed by indirect immunoperoxidase staining in parotid and submandibular glands in the apical portion of the cytoplasm in ductal cells (1). Aim of this study was to verify the presence of PSA in human major salivary glands by transmission electron microscopy.

Samples of salivary glands were treated to detect at ultrastructural level, the PSA by immunogold staining technique (2,3).

We observed PSA immunoreactivity in both parotid and submandibular glands mainly localized in the cytoplasmic compartment of secretory cells. Few granules showed a negligible PSA reactivity. Moreover, both the intercalated and striated ductal cells exhibited a similar labelling to PSA in the cytoplasm among mitochondria.

Our findings represent a further evidence that PSA exerts a role in major salivary glands. Nevertheless, the specific role and the traffic of PSA release in oral cavity remain to be elucidated

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Osteocalcin localization in human parotid glands

Michela Isola[1], Lai Ylenia[1], Roberta Noli[1], Raffella Isola[1], Francesco Loy[1]

[1] Department of Biomedical Sciences, Section of Cytomorphology, University of Cagliari, Italy

Osteoblasts synthesize osteocalcin, one of the most important non-collagenous proteins in bone. Circulating osteocalcin acts locally in different organs and tissues (1).

Osteocalcin has been detected in saliva, (2), but its source is still questioned.

The aim of this study was to investigate ultrastructural localization of salivary osteocalcin in human parotid glands.

Samples of parotid glands were fixed, embedded in Epon Resin, treated to localize osteocalcin by immunogold staining technique and observed by transmission electron microscopy (3).

We observed osteocalcin immunoreactivity localized in the secretory serous cells principally associated to the granules.

Our data provided new morphological evidences of the osteocalcin extra-osteoblastic localization, fundamental prerequisite for a better understanding of the roles of this protein in other tissues and, in particular, in human secretory salivary cells.

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Aging-related aquaporin modulation

Marzia Gianò[1], Alessandra Stacchiotti[2,3], Elisa Borsani[1,4,5], Rita Rezzani[1,4,5], Gaia Favero[1,4]

[1]Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[2]Department Biomedical Sciences for Health, University of Milan, Milan, Italy
[3] Laboratory of Applied Human Morphology, IRCCS San Donato Polyclinic, Milan, Italy
[4] Interdipartimental University Center of Research "Adaption and Regeneration of Tissues and Organs (ARTO)", University of Brescia, Brescia, Italy
[5] Italian Society for the Study of Orofacial Pain (Societa` Italiana Studio Dolore Orofacciale – SISDO), Brescia, Italy

Aging is a complex and progressive process involving every organ in the body and it is the result of coordinated biology events that can span decades. The maintenance of tissue homeostasis is highly dependent on the distribution of water throughout the body, pH balance and electrolyte levels [1]. Aquaporins (AQPs), protein channels allowing transport of water, glycerol and other small neutral solutes across cell membranes [2,3], are emerging as important players in metabolic and energy homeostasis with potential important physiopathological implications. With aging, fluid loss occurs in all tissues and this loss may be associated with change in density and/or functions of AQPs. The aim of this study was to investigate AQPs modulation at heart level of senescence-accelerated prone mice (SAMP8) respect relative controls, senescence-accelerated resistant mice (SAMR1). Notably, SAMP8 heart showed, together with morphological alteration, increased expression of AQPs respect SAMPR1 heart. In conclusion, AQPs may have significant roles for the development of age- related hypertension and the tendency of cardiac ischemia, underlining their potential therapeutic implication at cardiovascular level.

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99m Tc-labeled keratin gold-nanoparticles in a nephron-like microfluidic chip for photo-thermal therapy applications. An ultrastructural point of view

Selenia Miglietta[1], Viviana Frantellizzi[2] Francesca Petronella[3] , Paolo Rosa[4], Vanessa Verrina[4], Vincenzo Petrozza[4], Giuseppe Familiari[1,] Antonella Calogero[4] , Luciano De Sio[4].

[1] Department of Anatomical, Histological, Forensic and Orthopedic Sciences, Sapienza University of Rome, Rome, Italy
[2] Department of Radiological Sciences, Oncology and Anatomical Pathology, Sapienza University of Rome, Italy
[3] Institute of Crystallography CNR-IC, National Research Council of Italy, Monterotondo, Italy
[4] Department of Medico-Surgical Sciences and Biotechnologies - Research Center for Biophotonics, Sapienza University of Rome, Latina, Italy

Biocompatible gold nanoparticles (AuNPs) are of particular interest for photo-thermal therapy (PTT) of cancer treatment because of their ability to efficiently convert light into heating. For this reason, efficient targeting and monitoring of AuNPs into the selected tissues is of paramount importance. This study reports on a new generation of 99m Tc-labeled AuNPs coated with keratin (Ker-AuNPs) and their spatial localization investigated by nuclear imaging techniques and by transmission electron microscopy. The effective radiolabeling of Ker-AuNPs with 99mTc was is achieved by using the chelating agent Diethylenetriaminepentaacetic (DTPA), resulting in the 99mTc-DTPA-Ker-AuNPs nanoconjugate. The 99m Tc-DTPA-Ker-AuNPs displayed an excellent photothermal properties and a good biocompatibility with healthy human embryonic kidney (HEK293T) cells, used to localize and study the stability of 99m Tc-DTPA-Ker-AuNPs. Ultrastructural TEM analysis allowed us to understand HEK293T morphology, the interaction with 99m Tc-DTPA-Ker-AuNPs, and their uptake mechanisms. Cells shows an abundance of mitochondria, where their number, size, and morphology are not altered. Cytoplasmic organelles appear preserved as well, thus, there are no noticeable ultrastructural changes. Our results indicate that treatments using 99m Tc-DTPA-Ker-AuNPs do not increase cytotoxicity and genotoxicity. A Lab-On-a-Chip (LoC) approach is used to localize and study the stability of 99m Tc-DTPA-Ker-AuNPs under dynamic conditions; to this end, the nano-conjugates were are injected into a PDMS microfluidic chip mimicking the renal filtering unit, the nephron, and monitored via radio-imaging and thermo-optical experiments. These detailed studies establish that DTPA-assisted 99m Tc-labeled Ker-AuNPs exhibit potential application as eexcellent candidates as biocompatible and non-invasive radiolabeled nanotherapeutic for PTT-based applications.

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Bone Morphogenic Proteins and their antagonists in the lower airways of stable COPD patients

F Pieretti[1], A Di Stefano[2], I Gnemmi[2], U Rosani[3], A Leonardi[4], P Brun[1]

[1] Department of Molecular Medicine, Histology Unit, University of Padova, Padova, Italy
[2] Divisione di Pneumologia e Laboratorio di Citoimmunopatologia dell'Apparato Cardio Respiratorio, Istituti Clinici Scientifici Maugeri, IRCCS - Veruno (NO), Italy
[3] Department of Biology, University of Padova, Padova, Italy
[4] Department of Neuroscience, Ophthalmology Unit, University of Padova, Padova, Italy

Background: Bone morphogenic proteins (BMPs) and their antagonists are involved in development and homeostasis in various organs.

Aim: To determine the protein expression of BMPs and their antagonists in stable COPD.

Methods: The expression and localization of BMPs and some relevant antagonists was measured in bronchial biopsies (bb) of patients with stable COPD with mild/moderate, severe/very severe disease, control smokers (CS) and control non-smokers (CNS), and in lung parenchyma of mild/moderate COPD, CS and CNS using immunohistochemistry and transcriptome analysis.

Results: In large airways (bb) the BMP4 antagonists CRIM1 and Chordin were significantly increased in bronchial epithelium and in the lamina propria of COPD. BMPER was slightly increased in the bronchial epithelium of all smokers, with or without COPD, compared to control non-smokers. BMP4 expression was decreased in the bronchial epithelium of severe-very severe and mild-moderate COPD compared to control non-smokers. Chordin levels (cells/mm^2) in bb significantly inversely correlated with Forced Expiratory Volume in the 1st second (FEV1%) predicted values in patients with COPD. In vitro stimulation of 16HBE epithelial cells with RANTES and IL-8 (100ng/ml) induced a significant decrease in BMP4 over 0-24h. In the peripheral airways. Transcriptome data were similar in all groups studied. Transcriptome data revealed poor regulation for most of these mRNA molecules between the tested groups and low expression levels, except for NBL1 and BMPR2.

Conclusion: These data show an imbalance of BMPs proteins and their antagonists in the bronchial mucosa of stable COPD.

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Accessory fissures and lobes of the lung: a better knowledge from dissection classes

Francesco Potenza[1], Anna Venzi[1], Alessandra Pacini[1], Jacopo Junio Valerio Branca[1], Cristiana Veltro[1], Ferdinando Paternostro[1]

[1] Dept Experimental and Clinical Medicine, Human Anatomy Section, University of Firenze, Lgo Brambilla 3, 50134, Firenze, Italy

The lung is the pivotal respiratory organ of the human body and a defective pulmonary development might rise to variability in term fissures and lobes, as previously reported [1]. Additional lung segmentation has a functional and evolutionary role since it can limit circumscribe a region thus limiting the spread of infection or other pathologies [2]. Sometimes, diagnostic imaging such as radiography or computed tomography can be wrongly interpreted or missing detailed [3].

In the present report, we describe lung fissure and lobe variability observed during routine undergraduate dissection classes of the thorax, from adult cadavers.

The lung weight and dimension were ranging in a physiological window but additional fissure and lobe was present.

The term "gold-standard" in human anatomy seems to be an approximation since during different kind of investigation, the anatomical variations are not so uncommon.

A deep awareness of anatomical variations is imperative both for physician and clinicians in order to elude difficulties during surgical interventions but also for better interpreting the diagnostic imaging.

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Identificazione di marcatori molecolari e metabolici responsabili dell'invecchiamento precoce in soggetti sopravvissuti ad un cancro infantile

Silvia Ravera[1], Marina Podestà[2], Tiziana Vigliarolo[2], Silvia Bruno[1], Fabio Morandi[2], Danilo Marimpietri[2], Federica Sabatini[2], Monica Dagnino[2], Andrea Petretto[3], Martina Bartolucci[3], Monica Muraca[4], Eleonora Biasin[5], Riccardo Haupt[4], Marco Zecca[6], Franca Fagioli[5], Daniela Cilloni[7], Francesco Frassoni[1,7,8]

[1] Dipartimento di Medicina Sperimentale – Sezione di Anatomia Umana, Università degli Studi di Genova, Genova
[2] Laboratorio Cellule Staminali e Centro di Terapia Cellulare, IRCCS Ospedale Pediatrico Giannina Gaslini, Genova
[3] Core Facilities-Clinical Proteomics and Metabolomics, IRCCS Ospedale Pediatrico Giannina Gaslini, Genova
[4] Unità di Epidemiologia e Biostatistica e Clinica DOPO, IRCCS Ospedale Pediatrico Giannina Gaslini, Genova
[5] Dipartimento di Onco-ematologia Pediatrica, Ospedale Infantile Regina Margherita, Università di Torino, Torino
[6] Ematologia Oncologica Pediatrica, Fondazione IRCCS Policlinico San Matteo, Pavia, Italia
[7] Dipartimento di Scienze Cliniche e Biologiche, Facoltà di Medicina e Chirurgia, Università di Torino, Torino
[8] Dipartimento di Matematica (DIMA), Università degli Studi di Genova, Genova

Sebbene il tasso di sopravvivenza dei pazienti affetti da cancro infantile sia migliorato notevolmente negli ultimi quarant'anni, i pazienti sopravvissuti ad un cancro infantile (Childhood Cancer Survivors - CCS) sono maggiormente soggetti a sviluppare complicazioni cliniche associate alla chemio/radioterapia coerenti con un invecchiamento precoce In particolare, i CCS manifestano decenni prima del previsto sintomi di fragilità tra cui disturbi cardiovascolari, debolezza muscolare e declino cognitivo [2]. I CCS sono anche più predisposti a sviluppare un secondo tumore primario rispetto alla popolazione sana di pari età. Tuttavia, ad oggi, non si conoscono ancora le basi cellulari/molecolari che portano a sviluppare l'invecchiamento precoce.

Considerando che tra le alterazioni coinvolte nel processo di invecchiamento, la funzionalità, la biogenesi e la dinamica mitocondriale giocano un ruolo fondamentale [3], utilizzando cellule mononucleate (MNCs) isolate da sangue periferico dei CCS, sono stati valutati l'efficienza della fosforilazione ossidativa (OxPhos), l'equilibrio tra la produzione di stress ossidativo e le difese antiossidanti cellulari e marcatori della biogenesi e dinamica mitocondriale. Le analisi sono state eseguite su 196 campioni provenienti da CCS di età compresa tra 5 e 20 anni, e i risultati sono stati confrontati con quelli ottenuti su MNCs di 154 soggetti sani di età compresa tra 5 e 106 anni.

I dati mostrano che l'efficienza OxPhos nelle MNCs isolate dai CCS risulta 3 volte inferiore rispetto ai campioni sani corrispondenti per età, ma è simile a quella osservata nei soggetti con un'età uguale o superiore ai 60 anni [4]. L'alterazione del metabolismo energetico mitocondriale determina un aumento della glicolisi anaerobica e del danno ossidativo rispetto ai campioni sani corrispondenti per età, come mostrato dall'aumento di 4 volte dell'attività della lattico-deidrogenasi e del livello di malondialdeide [4]. Applicando un modello matematico di previsione dell'età basato sul metabolismo del glucosio [5], i CCS presentano un'età biologica significativamente aumentata di decenni rispetto all'età anagrafica. Inoltre, analisi proteomiche mostrano come le MNCs dei CCS siano caratterizzate da una over-espressione di marcatori legati all'invecchiamento, ma da una bassa espressione delle difese ossidanti rispetto ai alle MNCs isolate da soggetti sani coetanei [4]. Infine, le MNCs dei CCS, ma non dei soggetti di pari età e degli over 60, mostrano un'espressione genica e proteica di 5 volte inferiore di CLUH, PGC1-α e SIRT6, tre marcatori legati alla biogenesi e alla dinamica mitocondriale [4], suggerendo che i

meccanismi che sottendono all'invecchiamento precoce dei CCS sono differenti rispetto a quelli coinvolti nell'invecchiamento fisiologico.

In conclusione, questo studio ha identificato alcune alterazioni molecolari e metaboliche che potrebbero contribuire alla fisiopatologia dell'invecchiamento precoce nei CCS, aiutando ad individuare potenziali bersagli terapeutici volti a ripristinare la funzionalità e la dinamica mitocondriale, rallentando le patologie associate all'invecchiamento.

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Effect of probiotics on wound healing in a 3D bioprinted human mucosa model

Filippo Renò[1], Sarah Gino[1], Yerra Preetham[1], Marco Invernizzi[1], Maurizio Sabbatini[2], Mario Migliario[1]

[1] Innovative Research Laboratory for Wound Healing, Health Sciences Department, Università del Piemonte Orientale, Novara , Italy
[2] Sciences and Innovative Technology Department, Università del Piemonte Orientale, Alessandria, Italy

Probiotics are nonpathogenic live microorganisms that, when administered in foods or as dietary supplements, could confer benefits to the host's health protecting it from pathogenic infections and modulating the immune response. Moreover probiotics promote the healing of wounds and intestinal and skin ulcers. They act through different mechanisms, such as direct killing or the competitive displacement of pathogenic bacteria, reinforcement of the epithelial barrier, induction of fibroblastic activity and epithelial cell migration (1). Predominant in vitro systems used to study probiotics effects on wound healing include epithelial cells two-dimensional (2D) monolayers lacking physiological conditions such as cell-matrix interactions. To overcome the limitations related to current in vitro systems, an in vitro 3D bioprinted model of mucosa has been developed to mimic the structural and functional characteristics of native tissue. Cellink printer, a bioprinter widely used in the field of tissue engineering and a newly synthetized bioink (2) have been used to create a 3D model of human mucosa to be wounded. The *in vitro* tissue obtained (a cylinder of 1cm^2 of surface by 0.5 cm of height) consisted of a basal layer of human dermal fibroblasts (HF) and an apical epithelial part consisting of immortalized epithelial cells of the oral mucosa (hTERTOME). Inside the 3D model, a cylindrical perforation was created through the entire upper epithelial layer and half of the lower connective layer. Some probiotics strains (Lb. acidophilus, Lb. plantarum, Lb.rhamnosus, Lb. reuteri, Lb. johnsonii, B. infantis, B. longum) were added inside the "wound" at different concentrations to analyse their pro-healing ability.

After 7 and 14 days the release of cytokines related to wound healing (FGF7, TGF beta1 and TNF alpha was quantified by ELISA assay and, at day 14, 3D models were fixed, embedded in paraffin and sections were obtained for histochemical analysis of general in vitro tissue organization and cells counting. Within the three-dimensional protein matrix, which simulates the organization of the ECM in a mucosa, the direct interaction between some strains of probiotics and fibroblasts and keratinocytes significantly stimulated the release of pro-healing factors and induced cell proliferation compared to untreated "wounded" samples.

It is therefore possible to imagine innovative and safe therapies with the topical use of probiotics for the treatment of wounds / ulcers in patients suffering from systemic diseases (e.g. diabetes) capable of altering the normal wound healing processes or in patients in whose normal reparative processes are physiologically slowed down (elderly).

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Frozen Section Analysis and Real- Time Magnetic Resonance Imaging of Surgical Specimen Oriented on 3D Printed Tongue Model to Assess Surgical Margins in Oral Tongue Carcinoma: Preliminary Results

Flavio L. Ronzoni[1], Caterina Giannitto[2], Giuseppe Mercante[1,3], Luca Disconzi[1,3], Riccardo Boroni[4], Elena Casiraghi[5], Federica Canzano[3], Michele Cerasuolo[3], Francesca Gaino[3], Armando De Virgilio[1,3], Barbara Fiamengo[6], Fabio Ferreli[1,3], Andrea Alessandro Esposito[7], Paolo Oliva[4], Luigi Terracciano[1,6], Giuseppe Spriano[1,3] and Luca Balzarini[2]

[1] Department of Biomedical Sciences, Humanitas University, Milan, Italy
[2] Department of Diagnostic Radiology, Humanitas Clinical and Research Center Istituti di Ricovero e Cura a Carattere Scientifico (IRCCS), Milan, Italy
[3] Otorhinolaryngology Unit, Humanitas Clinical and Research Centre Istituti di Ricovero e Cura a Carattere Scientifico (IRCCS), Milan, Italy
[4] Department of Supply Chain, Humanitas Clinical and Research Center Istituti di Ricovero e Cura a Carattere Scientifico (IRCCS), Rozzano, Milan, Italy
[5] Department of Computer Science (DI), Universita degli Studi di Milano, Milan, Italy
[6] Department of Pathology, Humanitas Clinical and Research Center Istituti di Ricovero e Cura a Carattere Scientifico (IRCCS), Milan, Italy
[7] Department of Radiology, Azienda Socio Sanitaria Territoriale di Bergamo Ovest, Treviglio, Italy

Background: A surgical margin is the apparently healthy tissue around a tumor which has been removed. In oral cavity carcinoma, a negative margin is considered ≥ 5 mm, a close margin between 1 and 5 mm, and a positive margin ≤ 1 mm. Currently, the intraoperative surgical margin status is based on the visual inspection and tissue palpation by the surgeon and intraoperative histopathological assessment of the resection margins by frozen section analysis (FSA). FSA technique is limited and susceptible to sampling errors [1,2]. Definitive information on the deep resection margins requires postoperative histopathological analysis [3].

Methods: We described a novel approach for the assessment of intraoperative surgical margins by examining a surgical specimen oriented through a 3D-printed specific patient tongue with real-time Magnetic Resonance Imaging (MRI) [4]. We reported the preliminary results of a case series of 10 patients, prospectively enrolled, with oral tongue carcinoma who underwent surgery between February 2020 and April 2021. Two radiologists with 5 and 10 years of experience, respectively, in Head and Neck radiology in consensus evaluated specimen MRI and measured the distance between the tumor and the specimen surface. We performed intraoperative bedside FSA. To compare the performance of bedside FSA and MRI in predicting definitive margin status we computed the weighted sensitivity (SE), specificity (SP), accuracy (ACC), area under the ROC curve (AUC), F1-score, Positive Predictive Value (PPV), and Negative Predictive Value (NPV). To express the concordance between FSA and *ex vivo* MRI we reported the jaccard index.

Results: Intraoperative bedside FSA showed SE of 90%, SP of 100%, F1 of 95%, ACC of 0.9%, PPV of 100%, NPV (not a number), and jaccard of 90%, and *ex vivo* MRI showed SE of 100%, SP of 100%, F1 of 100%, ACC of 100%, PPV of 100%, NPV of 100%, and jaccard of 100%. These results needed to be validated in a larger sample size of 21- 44 patients.

Conclusion: The presented method allows a more accurate evaluation of surgical margin status, and the first clinical experiences underline the high potential of integrating FSA with *ex vivo* MRI of the fresh surgical specimen.

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Testing the angiogenic potential of bioactive glasses onto the chick chorioallantoic membrane (CAM): a simple, versatile and recently re-proposed with ethical meaning tool for bone tissue engineering applications

Virginia Stanzani[1], Marta Checchi[1], Devis Bellucci[2], Francesco Cavani[1], Marzia Ferretti[1], Valeria Cannillo[2] and Carla Palumbo[1]

[1] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Via del Pozzo 71, 41124 Modena, Italy
[2] Department of Engineering "Enzo Ferrari", University of Modena and Reggio Emilia, Via P Vivarelli 10, 41125 Modena, Italy

Since their discovery, bioactive glasses have paved the way to new scenarios in bone repair and regeneration, thanks to their osteoinductive and osteoconductive abilities, angiogenic potential and anti-bacterial activity. The 45S5 Bioglass® (45S5), in particular, stood as the "gold standard" of the bioactive glasses family for its capacity to form a chemical bond with bone while stimulating osteoprogenitor cells at the genetic level. Although these outstanding properties, 45S5 has the tendency to crystallize during the thermal treatments that are necessary to many processes, such as the production of porous scaffolds for regenerative medicine. The crystallization of the glass often leads to a low biological property and implant instability once placed into the body. To overcome the 45S5 drawbacks, the authors created two novel bioglass compositions containing both strontium and magnesium while enhancing the crystallization temperature. In the last years, clinical trials showed that strontium and magnesium could favor bone compressive strength and cell adhesion while reducing the risk of vertebral fractures in women with osteoporosis. The two compositions, here named BGMS_10 and BGMS_new, have already proven to display osteogenic abilities, supporting bone cell adhesion, colonization and differentiation *in vitro*. Since vascularization is a mandatory requisite to trigger and support bone tissue repair and regeneration, in this work, for the first time, the bioactive glasses were tested for their angiogenic potential. Specifically, the two novel bioglasses and the gold standard were tested for their ability to support vascularization and improve angiogenesis onto the chick chorioallantoic membrane (CAM), a natural bioreactor that could rapidly predict the angiogenic potential of the materials grafted[1]. The CAM assay is a simple, rapid and easy-to-read method, which has the ability to overcome both the limitations of *in vitro* tests and the ethical concerns related to animal experimentation[2]. Following the 3Rs rule, it also represents an economical solution in terms of times and costs, reducing the use of animals, replacing their maintenance costs and refining their distress. In conclusion, all the bioglasses showed an evident angiogenic response over the CAM, visible as different newborn vessels disposed around the graft. This demonstrates the ability of 45S5, BGMS_10 and BGMS_new to trigger vascularization also in a more complex system than *in vitro* 2D cell cultures.

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Evaluation of Proliferation and Morphological features of Human Periodontal Ligament Fibroblasts in Contact with Different Bovine Bone Grafts Treated with Low-Temperature Deproteinisation Protocol

Diana Torge[1], Sara Bernardi[1,2], Serena Bianchi[1], Guido Macchiarelli[1]

[1] Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy
[2] Center of Microscopy, University of L'Aquila, 67100 L'Aquila, Italy

Deproteinised bovine bone mineral (DBBM), obtained through different thermic and chemical processes, are commonly used in periodontal regenerative procedures with different outcomes [1]. The use of lower temperatures during the production of the DBBM is a current option to reduce the possible particle crystallization [2]. The aim of this study is to evaluate the bio-morphological behavior of periodontal fibroblasts in contact with different DBBM particles treated with a low-temperature protocol and without exposure to sodium hydroxide (NaOH). Morphological evaluation was performed using light, confocal laser, and scanning electron microscopy, while proliferation XTT assays were performed at 24 h (T1), 72 h (T2), and 7 days (T3). Morphological analysis suggested that the presence of the materials promotes significant cellular changes in the morphology of the periodontal fibroblasts into a polygonal shape, induces surface reactions with membrane thickening and increases actin expression. Specifically, the morphological changes were detectable by T1, with a progressive increase in the considered morphological characteristics at T2 and T3 follow-ups. The proliferation assay showed a statistically significant difference between the different experimental materials and the negative control in T2 and T3 follow-ups. The post hoc analysis revealed no differences between the materials. These results suggest that the grafts obtained with the low-temperature extraction protocols, not submitted to NaOH solution, showed positive morphological reactions without differences in particles size. In these perspective, available alternative deproteinisation protocols, which are less invasive to produce xenografts, might improve osteoconductive properties in regenerative procedures.

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Morphological alterations of the lung in the Sickle Cell Trait

Marcello Trucas[1], Sabrina Burattini[4], Michela Battistelli[4], Michela Simbula[3], Maria Serafina Ristaldi[3], Marina Quartu[1], Maria Pina Serra[1], Andrea Perra[2], Pietro Gobbi[4]

[1] Department of Biomedical Sciences, Section of Cytomorphology, University of Cagliari, Italy
[2] Department of Biomedical Sciences, Unit of Oncology and Molecular Pathology, University of Cagliari, Italy
[3] National Research Council of Italy (CNR) - IRGB, Cagliari, Italy
[4] Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy

Sickle Cell Trait (SCT), besides the increasing evidence of erythrocyte stiffness and the long-term onset of chronic kidney disease in the trait carriers[1], is still considered a benign condition. So far, there are still little data regarding the ultrastructural modifications responsible for a possible multi-organ damage in the SCT[2,3]. To extend our previous studies on the liver and spleen, we investigated the microanatomical alterations of the lung by conventional light microscopy and transmission electron microscope (TEM) in "healthy" heterozygous subjects.

To this aim, we used 13 humanized Townes mice (a chimera expressing falcemic human hemoglobin - HbS): 3 homozygotes (Sickle Cell Disease-SCD), 5 heterozygotes (SCT), and 5 healthy controls; all specimens were analyzed by conventional histological staining, whereas 2 controls and 2 Sickle Cell Trait mice underwent TEM analysis. After surgical removal, part of the tissue samples was paraffin-embedded to perform a light microscopy histopathological analysis. Another part of tissue was rapidly immersion-fixed in glutaraldehyde, reduced into 1x1x3 mm pieces, postfixed in OsO_4, dehydrated, and embedded into epoxy resin. Ultrathin sections of 50-80 nm underwent staining with UranyLess and lead citrate for TEM analysis.

The histopathological analysis of SCD lungs showed the classic signs of ischemia. By contrast, in the SCT lungs, low-grade inflammation and minimal capillary congestion were observed. In the semithin sections, the basement membrane of the pulmonary alveoli was often thickened and gave the parenchyma a denser and more irregular appearance compared to controls. In SCT mice, the TEM analysis revealed irregular morphology of pulmonary capillaries, their basal lamina showed a thickness three times that of control mice. Moreover, the presence of extremely irregularly shaped erythrocytes was observed. The endothelium also presented numerous extroflexions towards the vascular lumen.

The present findings confirm the presence of chronic vascular damage that could affect long-term organ function even in heterozygous mice. While, according to our previous observations, the endothelial alterations are virtually ubiquitous in the liver and spleen, the lung shows the most significant parenchymal alterations. Collectively, these findings contribute novel data to better characterize the morpho-pathological modifications in the SCT mouse model and suggest translational usefulness for in-depth investigation of the subclinical multi-organ involvement present in patients heterozygous for HbS.

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Morphological and functional assessment of NCX2 potential role in axonal damage related to Oxaliplatin

Paola Alberti[1,2], Elisa Ballarini[1,2], Alessio Malacrida[1,2], Eleonora Pozzi[1,2], Virginia Rodriguez-Menendez[1,2], Laura Monza[1,2], Alessia Chiorazzi[1,2], Annalisa Canta[1,2], Gabriella Nicolini[1,2], Arianna Scuteri[1,2], Paola Marmiroli, Guido Cavaletti[1,2]

[1] University of Milano-Bicocca, Monza, Italy
[2] NeuroMI (Milan Center for Neuroscience), Milan, Italy

Oxaliplatin (OHP) induced peripheral neurotoxicity (OIPN), a potentially persistent sequela of chemotherapy, is characterised by a chronic and acute neurotoxicity syndrome; the latter is due to transient axonal hyperexcitability. We have already demonstrated (Alberti et al., 2020) that modulating acute OIPN, chronic OIPN is prevented. Acute OIPN is related to unbalance on sodium voltage-operated channels; this dysfunction might lead to a toxic increase of intra-neuronal calcium via the Sodium-calcium exchanger 2 (NCX2), thus potentially causing the chronic axonal damage. We explored the role NCX2 might play in OIPN pathogenesis in an *in vivo* and *in vitro* setting.

In the *in vitro* experiments, embryonic rat Dorsal Root Ganglia (DRG) organotypic cultures treated with different concentrations of SEA0400 (SEA), a NCX inhibitor, were used to assess NCX2 role in OIPN development. In the *in vivo* experiment, OHP treated mice (7 mg/Kg, iv, once a week per 8 weeks; 1qw8ws) were compared with a vehicle-treated group (n=8 each). Neurophysiological and behavioural testing were performed to characterise both acute and chronic OIPN. Extensive morphological analyses were performed to verify axonal damage (intraepidermal nerve fiber density [IENFD] and caudal nerve morphometry). Morphological analysis (immunohistochemistry and immunofluorescence) were performed on DRG as well as western blot (WB) analysis - on DRG pool - to detect changes in NCX2 levels/expression.

In the *in vitro* setting, the inhibition of NCX2 was able to significantly reduce the neurotoxic effect of oxaliplatin on DRG neurite elongation (from 40.8 to 58.4%). In the *in vivo* part, neurophysiology and behavioural tests showed acute and chronic OIPN pattern had ensued. Neuropathology confirmed an axonal, sensory, polyneuropathy had ensued in OHP group; NCX2 WB analysis showed a significant alteration in OHP group ($p<0.001$) which was matched by results obtained via immunohistochemistry and immunofluorescence.

Therefore, NCX2 might be a pivotal element in the development of acute and chronic OIPN, shedding light on a potential novel neuroprotectant strategy.

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Morphological and functional study of the caudal nerve in a rat model of peripheral neuropathy

Paola Alberti[1,2], Eleonora Pozzi[1,2], Laura Monza[1,2], Alessia Chiorazzi[1,2], Annalisa Canta[1,2], Elisa Ballarini[1,2], Virginia Rodriguez-Menendez[1,2], Mario Bossi[1,2], Luca Crippa[1,2], Paola Marmiroli, Guido Cavaletti[1,2]

[1] University of Milano-Bicocca, Monza, Italy
[2] NeuroMI (Milan Center for Neuroscience), Milan, Italy

Animal models of peripheral neuropathy (PN) allow a detailed morphological testing to be matched with neurophysiological recordings. The rat caudal nerve is an easy site to be evaluated and it is highly relevant in case of length dependent processes. Different information can be detected if testing different sites of the nerve; main published protocols rely on one or, maximum, two different sites to perform analyses (Monza et al, 2021). We aimed at refining our experimental approach increasing the set of measures we routinely perform both with histopathology and nerve conduction study (NCS). A model of moderate-severe PN, due to paclitaxel (PTX) chronic administration, was used as a proof-of-concept setting.

A control (vehicle [VEH] treated, n=8) and a PTX treated group (PTX 10 mg/Kg, 1qw4ws, n=8) were tested. Caudal nerve sensory nerve action potential (SAP) was recorded in multiple sites for the whole length of the tail from proximal to distal. The whole caudal nerve was harvested at the end of treatment and, after fixation, the same segments studied with NCS underwent morphological examination. The whole tail was also collected to describe normal changes of the nerve itself for the whole length of the tail.

At the end of treatment, NCS showed the typical pattern of a moderate-severe PN ensued in PTX group with clear length-dependent features (no recordable traces as soon as 5 cm form the base of the tail). A matching pattern in axonal damage was clearly demonstrated by histopathological examination at optical and electron microscopy.

We obtained a refinement of our experimental procedures to detect PN in rat models. Therefore, careful planning should be implemented to select the appropriate sites to be tested, adapting for multiple recordings/specimen sampling to grasp the complexity of observed phenomena.

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Health benefits of outdoor water sports in chronic disease: a systematic review

Lucia Cugusi[1], Martina Meloni[1], Marco Bergamin[2], Stefano Gobbo[2], Andrea Di Blasio[3], Corrado Conca[1], Pier Paolo Bassareo[4], Alessandra Piras[5], Andrea Montella[1], Maria Alessandra Sotgiu[1], Pasquale Bandiera[1]

[1] Department of Biomedical Sciences, University of Sassari, Sassari, Italy
[2] Sport and Exercise Medicine Division, Department of Medicine, University of Padova, Padova, Italy
[3] Endocrine Section, Department of Medicine and Sciences of Aging, G d'Annunzio University of Chieti-Pescara, Chieti, Italy
[4] Department of Cardiology, Mater Misericordiae University Hospital Crumlin, University College of Dublin, Dublin, Ireland
[5] Department of Cardiology, Azienda Ospedaliero Universitaria (AOU), Cagliari, Italy

Background. Although outdoor water sport activities are gaining increasing attention for their therapeutic potential in the social and care management of populations with chronic diseases, these practices are currently underutilised. Moreover, the available body of literature on the topic has not been critically and systematically assessed yet.

Aims. (1) To appraise the health effects of outdoor water sport activities for chronic disease populations; (2) to identify potential gaps and avenues of development for this emerging field.

Methods. A systematic review was carried out searching PubMed (including MEDLINE), Physiotherapy Evidence Database (PEDro) and Scopus from inception to December 2021. Only outdoor water sport interventions specifically designed for therapeutic purposes for individuals with chronic disease were included. The quality score of each study was calculated with the Tool for the assEssment of Study qualiTy and reporting in Exercise (TESTEX) tool.

Results. Fifteen studies (five RCTs, seven non-RCTs and three CTs with healthy subjects as controls) met the inclusion criteria and were assessed. Among the studies selected, two focused on canoa kayak, one on stand-up paddle, two on surfing, two on sailing activity, and eight on dragon boat padding. The median TESTEX score for study quality and reporting was 6/15, i.e ., "very low" (range 5–8). Meta-analyses were not performed because none of the five RCTs retrieved shared data on at least one similar outcome. Based on the qualitative analysis, the few individual studies that could be included reported generally positive results, ranging from improvements in antioxidant action and cardiovascular function for dragon boating, to beneficial effects on balance, postural control, and flexibility for on-water paddle board activities. Overall, outdoor water sport interventions were associated to higher rates of adherence than conventional trainings.

Conclusions. Very low to low quality evidence from a limited set of pilot studies seems to suggest beneficial effects of outdoor water sports for chronic disease populations. However, such preliminary findings need to be replicated through large, high-quality RCTs to be conducted in target populations. Avenues of development and translational perspectives for this specific research field are proposed and discussed.

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Persistent paradoxical effects on striatal and limbic a-synuclein and tyrosine hydroxylase following methamphetamine withdrawal

Francesca Biagioni[1], Cinzia Fabrizi[2], Elena Pompili[2], Rosangela Ferese[1], Carla Letizia Busceti[1], Stefano Puglisi-Allegra[1], Francesco Fornai[1,3]

[1] IRCCS Neuromed, Pozzilli, Italy
[2] Dep of Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza University of Rome, Rome, Italy
[3] Dep Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Methamphetamine (METH) produces a variety of epigenetic effects in the brain, which are seminal to establish long-lasting alterations in neuronal activity. A number of studies were carried out aimed at rough assessment of the amount of either histone acetylation and methylation or direct DNA methylation, without a selective analysis of specific genes. In the present study we wish to assess whether METH-induced epigenetic alterations may specifically engage the expression of a-synuclein, which is a key protein in neurodegeneration and synaptic plasticity. In this way, a potential long-term alteration of brain circuitries may produce a variation in the threshold for neurotoxicity, sensitization, addiction and neurodegeneration. Thus, the occurrence of long-term changes in the expression of the protein were analyzed in parallel with persistent changes in a specific marker of integrity of meso-striatal/meso-limbic pathway, which is the expression of tyrosine hydroxylase (TH) both in the mesencephalon and within dorsal striatum. The integrity of dopamine (DA) projection was assessed at the level of the olfactory tubercle, the *nucleus accumbens* and *fundus striati*.

Prolonged exposure to small doses of METH, produces nigro-striatal toxicity, when assessed at short time intervals following prolonged exposure. However, at prolonged time intervals a paradoxical increase progressively occurred in TH immunostaining within limbic regions. Such an increase exceeds at large the amount of TH expressed in controls. This occurs concomitantly with an overexpression of the primary transcript as well as the protein alpha synuclein within the same brain regions and dorsal striatum. This increase is persistent at prolonged time interval of METH withdrawal.

The increase in the primary a-synuclein transcript is due to hypomethylation of specific CPG islands placed in the SNCA gene promoter which ranged roughly ten-fold of controls, it was steady, and it persisted at least 21 days following METH withdrawal. Thus, such an apparent synucleinopathy induced by METH indeed was associated with increased mesolimbic DA innervation, which equally surpasses several folds the amount which was measured in controls and persists at least for three weeks. The increase in SNCA is not associated with an increase of SNCA copy number. Nonetheless, the amount of the native protein, which is detected by ultrastructural stoichiometry, exceeds the increase reported following genetic SNCA multiplications (ten-fold of controls).

These findings are discussed in the light of METH-induced phenotype changes which accompany toxicity, sensitization, addiction and neurodegeneration.

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Morphological and immunohistochemical changes in the enteric nervous system of normal and inflamed intestine

Arianna Casini[1], Rosa Vaccaro[1], Stefano Leone[1], Paolo Onori[1], Romina Mancinelli[1]

[1] Department of Anatomical, Histological, Forensic Medicine and Orthopedic Sciences, Sapienza University of Rome

The autonomic nervous system consists of three divisions defined as sympathetic, parasympathetic, and enteric. The neural crest cell-derived enteric nervous system (ENS) is a complex neural network embedded in the wall of the gastrointestinal tract that regulates the intestinal reflex behaviors and represents an intrinsic innervation acting independently respect to the central nervous system (CNS) input.

The ENS has two ganglionated plexuses, the myenteric and the submucosal plexuses, in which almost all intrinsic nervous cells are located (1). The submucosal plexus is prominent only in the small and large intestines. The myenteric plexus extends the full length of the digestive tract, between the outer longitudinal and circular muscle layers, from the esophagus to the rectum. The myenteric plexus is composed of neurons surrounded by a barrier consisting of proteins and enteric glial cells: the encapsulated ganglia connected by interganglionic segments (2).

Our aim is to investigate and characterize the changes between normal and inflamed intestine through: (i) the morphology of the myenteric plexus using various histological techniques (Hematoxylin and Eosin, Masson's Trichrome and Silver Impregnation), and (ii) immunohistochemical expression of the main enteric neurotransmitters and glial markers, to characterize respectively neuron (ChAT, alfa-Syn, Vip, TH), glia (GFAP) and microglia (CD11b). Surgical specimens of small and large intestine were collected from patients with Crohn's disease and from control patients.

In this preliminary study the myenteric ganglia revealed significant differences in shape, size, neurons population, enteroglia distribution, in the microglia and in the capsule surrounding the ganglia. Recent study in the myenteric plexus suggests that the ENS and CNS use similar mechanisms of neuroimmune interaction, although exposed to different environmental signals, it seems likely that both tissues produce equivalent soluble factors (3). Modified features of myenteric ganglia we found in Crohn disease needs further exploration, but they will probably shrink the current idiopathic category of gastrointestinal disease starting to include them in the disorders in common with the CNS and the ENS, the so-called brain–gut diseases.

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Role of PACAP-ADNP axis in Glioblastoma multiforme

Agata Grazia D'Amico[1], Grazia Maugeri[2], Benedetta Magrì[2], Salvatore Saccone[3], Concetta Federico[3], Velia D'Agata[2]

[1]Department of Drug Sciences, University of Catania, Italy
[2] Department of Biomedical and Biotechnological Sciences, Section of Anatomy, Histology and Movement Sciences, University of Catania, Italy
[3]Department of Biological, Geological and Environmental Sciences, Section of Animal Biology, University of Catania, Italy

Glioblastoma multiforme (GBM) is a lethal form of brain cancer, characterized poor prognosis due to high rate of cells migration. The uncontrolled cell proliferation produces hypoxic niches in tumor mass showing induction of hypoxia inducible factors (HIFs). The signaling cascades triggered by HIFs are responsible of neoangiogenesis. This event is responsible tumormalignant progression and recurrence [1].

Recently, it has been demonstrated that the pituitary adenylate cyclase-activating polypeptide (PACAP) is involved in GBM malignancy by interfering with cells invasiveness towards surrounding tissue [2]. This neuropeptide is widely expressed in central nervous system where exerts many biological effects directly by binding to its related receptors (PAC1-R, VPAC1-R and VPAC2-R), or indirectly by the induction of the activity-dependent neuroprotective protein (ADNP).

Considering the main role exerted by hypoxic microenvironment in tumor malignancy, we here investigated the PACAP ability to regulate this process. By using confocal microscopy, we have detected higher expression of PACAP, PAC1R and ANDP in hypoxic areas as compared to non-hypoxic areas of GBM frozen sections.

This result suggested a direct relation between peptides upregulation and the hypoxic microenvironment. To understand the biological impact of PACAP and ADNP overexpression in hypoxic niches of GBM, we performed an *in vitro* study by using human U87MG glioblastoma cells exposed to deferoxamine (DFX), a hypoxic mimetic agent.

Results demonstrated that PACAP-ADNP axis modulated the hypoxic-angiogenic pathway in GBM cells by reducing VEGF expression and its extracellular secretion. This data was confirmed by reduced formation of vessel-like structures obtained culturing H5V endothelial cells in conditioned medium derived from U87MG cells exposed to DFX and treated with peptides. Moreover, PACAP-ADNP axis also affects the invasiveness of GBM cells cultured under hypoxia as demonstrated through wound healing assay.

Although additional investigations are warranted to determine the role of PACAP-ADNP axis in GBM malignancy, the data provide new insight on its involvement in glioblastoma progression.

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Role of Pannexin-1 channels during SARS-CoV-2 infection: focusing on neuroinflammation

Daniela D'Amico[1,2], Rosario Barone[1], Valentina Di Felice[1], Eliseo A. Eugenin[2]

[1] Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo, Italy
[2] Department of Neuroscience, Cell Biology, and Anatomy, University of Texas Medical Branch, USA

The SARS-CoV-2 virus infection has rapidly spread around the world causing variable symptoms in the population ranging from asymptomatic, albeit contagious, to fever, difficulty breathing and even death, especially in older people with other conditions. Trying to investigate infectious pathogenesis and chronic inflammation, several host proteins have been identified to be used by the virus to enter and replicate in different cell types; first of all the angiotensin-converting enzyme 2 (ACE2) receptor. However, the inflammatory processes triggered by SARS-CoV-2 virus is still under investigation. It has already shown that the interaction of the spikeS-CoV-2 protein with the ACE2 receptor is able to achieve the transient opening of the Panx-1 channels with the consequent release of factors into the extracellular space inflammatory agents such as ATP, PGE2 and IL-1β, and induce the activation of purinergic receptors [1]. Our hypothesis is that "SARS-CoV-2 virus infection induces the release of ATP through the opening of Panx-1 channels with consequent activation of purinergic receptors, contributing to neuroinflammation"; therefore, blocking Panx- 1 channels should be considered a therapeutic goal. Thus, we propose to investigate *in vitro* the molecular mechanisms triggered by the opening of Panx-1 channels, determining whether the blocking of the latter or of purinergic receptors can reduce neuronal apoptosis and inflammation associated with infection.

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Braf activation and Pten deletion in peripheral neural stem cells give rise to Schwannoma and peripheral nerve tumors

Susanna Dolci[1], Ambra Colopi[1], Eugenia Guida[1], Serena Fuda[1]

[1] Cattedra di Anatomia, Facoltà di Medicina e Chirurgia, Università di Roma Tor Vergata

Gli schwannomi sono tumori della guaina nervosa derivati dalle cellule di Schwann che compaiono sporadicamente o in associazione con sindromi genetiche come la neurofibromatosi di tipo 1 o 2 (NF1/NF2). Studi recenti suggeriscono che le neurofibromatosi (NFs) insorgano da una mutazione genetica nei geni della famiglia NF nei precursori delle cellule di Schwann (SCP). Abbiamo recentemente sviluppato un modello murino in cui l'attivazione della mutazione BRafV600E e la delezione di Pten, guidate dal deleter Sox2-CreERT2 inducibile dal tamoxifene, provoca la formazione di oligodendrogliomi e schwannomi gangliari. Poiché è noto che la nestina è espressa nelle cellule precursori di Schwann, abbiamo utilizzato topi transgenici Nestin-Cre-ERT2 per ottenere la mutazione BRafV600E e la delezione di Pten specificamente nelle SCPs. Trenta giorni dopo l'iniezione di tamoxifene, i topi hanno mostrato segni neurologici di malattia e sono stati sottoposti a eutanasia. Simile ai topi mutanti inducibili da Sox2, l'analisi istologica del cervello ha rivelato la presenza di lesioni nodulari bilaterali identificate come schwannomi del nervo vestibolo-cocleare che erano immunoreattive per Egr2 e S100. Inoltre, il midollo spinale sezionato da topi iniettati con tamoxifene ha mostrato anche schwannomi del ganglio della radice dorsale (DRG), neurofibromi plessiformi associati a tumore maligno della guaina del nervo periferico (MPNST). I nostri risultati suggeriscono che l'attivazione di Braf e la delezione di Pten nei precursori delle cellule di Schwann pluripotenti adulte possono essere responsabili dello schwannoma e della formazione di MPNST.

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Duodenal alpha-Synuclein pathology and enteric gliosis in advanced Parkinson's Disease patients

Aron Emmi[1,2,7], Michele Sandre[2,7], Francesco Paolo Russo [3,7], Giulia Tombesi[4], Federica Garrì[2], Marta Campagnolo[2,7], Miryam Carecchio[2,7], Roberta Biundo[2,5,7], Gaya Spolverato[3,7], Veronica Macchi[1,7], Edoardo Savarino[3,7], Fabio Farinati[3,7], Piero Parchi[8,9], Andrea Porzionato[1,7], Luigi Bubacco[4,7], Raffaele De Caro[1,7], Gabor G Kovacs[10,11,12], Angelo Antonini[2,7]

[1] Institute of Human Anatomy, Department of Neuroscience, University of Padova, Padova, Italy
[2] Parkinson and Movement Disorders Unit, Centre for Rare Neurological Diseases (ERN-RND), Department of Neuroscience, University of Padova, Padova, Italy
[3] Department of Surgery, Oncology and Gastroenterology, Padova University Hospital, Padova, Italy
[4] Department of Biology, University of Padova, Padova, Italy
[5] Department of General Psychology, University of Padova, Padova, Italy
[6] Department of Pathobiology and Laboratory Medicine, University of Toronto, Toronto, Ontario M5S 1A8, Canada
[7] Center for Neurodegenerative Disease Research (CESNE), University of Padova, Padova, Italy
[8] IRCCS, Istituto delle Scienze Neurologiche di Bologna (ISNB), Bologna, Italy
[9] Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy
[10] Tanz Centre for Research in Neurodegenerative Disease, University of Toronto, Toronto, Ontario, Canada
[11] Department of Laboratory Medicine and Pathobiology and Department of Medicine, University of Toronto, Toronto Ontario, Canada
[12] Laboratory Medicine Program & Krembil Brain Institute, University Health Network, Toronto, Ontario, Canada

Objectives: In Parkinson's Disease (PD), the role of the gut-brain axis has been greatly highlighted by recent developments in both clinical and preclinical research. Considering the involvement of the enteric nervous system (ENS) in the prodromal stages of PD and its relationship with gut motility, the detection of αSyn aggregation and its deposition in gut tissues appears to be of great relevance. In the present study we aim to investigate the histopathological changes in the enteric nervous system by characterizing both αSyn aggregates and enteric glial responses in duodenum biopsies of advanced PD patients with extensive clinical and demographical documentation.

Materials: Eighteen patients with advanced PD who required initiation of Levodopa Carbidopa Intestinal Gel (LCIG) infusion and 18 control subjects comparable for age- and sex- undergoing screening diagnostic endoscopy were included in the study. Four 3 mm3 duodenal-wall biopsies were sampled in a topographically unrelated district to PEG-J placement for histopathological and immunohistochemical analyses.

Methods: Biopsies were fixed, paraffin-embedded, and microtome sectioned. Immunoperoxidase and immunofluorescent staining was performed for aggregated αSyn (Clone 5G4), Glial Fibrillary Acidic Protein (GFAP) and Beta-III Tubulin. Sections were evaluated by experienced morphologists and underwent morphometrical analyses for the quantification of immunoreactivites.

Results: Duodenal samples collected from PD patients were characterized by marked immunoreactivity for aggregated αSyn (18/18; 100%), while absent (4/18) or barely detectable (14/18) immunoreactivity was found in controls. Semi-automatic morphometrical quantification for aggregated αSyn revealed statistically significant higher immunoreactive tissue area in PD patients compared to controls (****$p<0.0001$). Thread-like aggregated αSyn reactivites were detected exclusively in PD patients, and colocalized with pan-neuronal maker Beta-III Tubulin, indicating aggregated αSyn deposits in duodenal nerve fibers. Morphometrical analyses revealed both increased EGC density and increased cell size when compared to controls (***$p=0.0002$), suggesting for local reactive gliosis.

Discussion: In the present study, we documented marked immunoreactivity for aggregated αSyn and morphological changes in EGC suggestive of reactive gliosis in the duodenum of

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advanced PD patients. These findings expand our knowledge on the involvement of the enteric nervous system in PD and suggest the duodenum as possible target for early disease detection.

Conclusions: In conclusion, our data suggest that duodenal biopsy may represent a safe, feasible and useful tool for characterizing PD pathology in the GI tract and discerning patients from controls. Future studies will be required to confirm these findings in a prodromal or early PD phase.

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The thrombin/PAR1 axis as regulator of Schwann cell functions in health and disease

Cinzia Fabrizi[1], Benedetta Mantua[1], Elena De Santis[1], Claudia Giampietri[1], Francesca Somma[1], Valerio de Franchis[1], Elena Pompili[1]

[1]Department of Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza University, Rome, Italy

Thrombin, the key effector protease of the coagulation cascade, mediates hemostasis, thrombosis, and inflammatory responses to vascular injury predominantly acting through its main receptor, protease-activated receptor 1 (PAR1). PAR1 is a member of a family of four G-protein-coupled receptors which are activated by proteolytic cleavage of their N-terminal extracellular domains. The expression and role of PAR1 in peripheral nervous system is still poorly investigated, although high PAR1 expression was found in the dorsal root ganglia and in the non-compacted Schwann cell myelin microvilli at the nodes of Ranvier.

Our previous results indicate that rat Schwann cell plasticity can be widely modulated by thrombin acting through PAR1 (Pompili et al., Mol and Cell Neurosci 2017; Pompili et al., Eur J Histochem 2020). Here we extend those previous data showing that thrombin regulates proliferation and survival of human Schwann cells increasing the expression of factors, such as matrix metalloprotease 2 (MMP2) and macrophage migration inhibitory factor (MIF), which are key in modulating nerve regeneration.

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An immunohistochemical study on the neurotensiergic system in the human cerebellar cortex

Paolo Flace[1,2], Demetrio Milardi[3], Alberto Cacciola[3], Vincenzo Coviello[2], Giuseppe Anastasi[3], Antonella Bizzoca[4], Diana Galletta[5], Paolo Livrea[6], Andrea Marzullo[7] and Gianfranco Gennarini[4]

[1] Medical School, University of Bari 'Aldo Moro', Bari, Italy
[2] Hospital Structures of Universo Salute Opera Don Uva, Bisceglie, Italy
[3] Department of Biomedical, Dental Sciences and Morphological and Functional Images, University of Messina, Messina, Italy
[4] Department of Basic Medical Sciences, Neurosciences and Sensory Organs, Medical School, University of Bari 'Aldo Moro', Bari, Italy
[5] Unit of Psychiatry and Psychology, Federico II University Hospital, Naples, Italy
[6] University of Bari 'Aldo Moro', Bari, Italy
[7] Section of Pathology, Department of Emergency and Organ Transplantation, University of Bari 'Aldo Moro', Bari, Italy

Neurotensin (NT) is a neuropeptide widely distributed in the mammals central and peripheral nervous systems. NT is mainly involved in neurotransmission/neuromodulation mechanisms of the dopaminergic system [(1)]. Several studies suggest a role of NT in dopamine-related diseases such as Parkinson's disease and schizophrenia [(2,3)]. Currently, in the cerebellum no data are available on the presence and distribution of neurotensinergic neurons, and in it, only few neurotensinergic extrinsic fibers, and of the neurotensin receptor subtypes NTR_2 and NTR_3 has been detected. Therefore, the goal of this immunohistochemical study, was to evaluate in the human cerebellar cortex, the presence and the distribution of an intrinsic neuronal neurotensinergic system. The goal of this immunohistochemical study, was to evaluate in the human cerebellar cortex the presence of an intrinsic neuronal neurotensiergic system.

The study was carried out on fragments of postmortem human cerebellar cortex 36-48h after death. Each fragment were fixed in an aldehyde and picric acid solution, embedded in paraffin, cut into 5 μm sections, and subjected to light microscopy immunohistochemical procedures using rabbit and goat polyclonal antibodies respectively against NT and NTR_1. For positive controls were used fragments of rat intestine subjected to the same experimental procedure. In the cerebellar cortex, NT and NTR_1 immunoreactivity were observed in the molecular layer, in subpopulations of stellate and basket neurons; in the Purkinje neuron layer, in a subpopulation of Purkinje neurons; in the granular layer, in a subpopulation of granules and Golgi neurons and some non-traditional large neurons (candelabrum, synarmotic, perivascular neuron) [(4)]. NTR_1 immunoreactivity was also observed in form of fine 'puncta' (putative axon terminals) in the neuropil and in close relationship to the wall of microvessels. The present study evidenced in the human cerebellar cortex the existence of a neurotensinergic neuronal system, may involved in neurotransmission/neuromodulation mechanisms of intrinsic circuits, in the microvascular innervation, and in cerebellar cortico-nuclear projective circuits. Moreover, we plan to carry out further studies to evaluate the co-expression level of the corticocerebellar neurotensiergic system with the dopaminergic cerebellar system and its possible role in neuronal circuits related to dopaminergic diseases, such as Parkinson's disease, schizophrenia and autism spectrum disorders.

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Acetylated α-Tubulin and Parkinson's disease: a study in *post-mortem* human brain

Federica Giampietro[1], Berkcan Isilgan[1], Gloria Gagliardi[1], Alessandra M Calogero[1], Giorgio Giaccone[2], Manuela Bramerio[3], Samanta Mazzetti[1,4], Gianni Pezzoli[4,5], Graziella Cappelletti[1,6]

[1] Department of Biosciences, Universita` degli Studi di Milano, Milan, Italy,
[2] Unit of Neuropathology and Neurology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
[3] S C Divisione Oncologia Falck and S C Divisione Anatomia Patologica, Ospedale Niguarda Ca' Granda, Milan, Italy
[4] Fondazione Grigioni per il Morbo di Parkinson, Milan, Italy
[5] Parkinson Institute, ASST "GPini-CTO," Milan, Italy
[6] Center of Excellence on Neurodegenerative Diseases, Universita` degli Studi di Milano, Milan, Italy

Microtubules defects are emerging as a contributing player in neurodegenerative process that leads to Parkinson's disease (PD) [1,2]. Tubulin post-translational modifications influence microtubule functions and, among them, α-tubulin acetylation is mainly associated to long-lived microtubules. To date, α-tubulin acetylation is strongly investigated because of its unbalance in different cellular and animal PD models [3,4,5]. Moreover, recent data obtained in *post-mortem* human brain show that acetylated α-tubulin is decreased in PD [6].

We aimed to deeply investigate microtubule alterations in PD human brains studying acetylated α-tubulin localization in dopaminergic neurons and glial cells of *substantia nigra* and acetylated α-tubulin distribution in different areas involved in PD pathology using immunohistochemistry approach and confocal microscopy. Additionally, acetylated α-tubulin interplay with total α-Synuclein and α-Synuclein oligomers was analysed in Lewy body morphogenesis, combining Proximity Ligation Assay (PLA) with immunohistochemistry, visualised with high resolution microscopy and 3D reconstruction by Arivis.

Our results showed that acetylated α-tubulin strongly accumulates in dopaminergic neuronal cell bodies of PD patients compared to controls, while no differences are found in glial cells. Interestingly, this acetylated α-tubulin redistribution occurs in all the regions involved in PD pathology. Dorsal motor nucleus of vagus, *locus coeruleus* and *substantia nigra* show a significant accumulation in neuronal cell bodies while acetylated α-tubulin decreases in the bundles of fibers of putamen.

Focusing on the interplay between acetylated α-tubulin and total α-Synuclein, we found that neurons strongly positive for acetylated α-tubulin rarely contain Lewy bodies. Starting from

this, we wondered if acetylated α-tubulin plays a role in the early phases of Lewy body morphogenesis, from the formation of early aggregates to *pale body* assembly. Using PLA, we detected α-Synuclein oligomers with acetylated α-tubulin staining in different stages of *pale body* maturation. The data we obtained allow us to link intraneuronal enrichment of acetylated α-tubulin to α-Synuclein aggregation.

In conclusion, this is the first study that points out a redistribution of acetylated α-tubulin in PD human brains and its interplay with α-Synuclein during Lewy bodies morphogenesis. Although its role in neurodegeneration is still controversial, we suggest that acetylated α- tubulin could play an active role in PD.

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Role of the sigma-1 receptor on mitophagy in amyotrophic lateral sclerosis

Benedetta Magrì[1], Grazia Maugeri[1], Agata Grazia D'Amico[2], Velia D'Agata[1]

[1] Department of Biomedical and Biotechnological Sciences, Section of Anatomy, Histology and Movement Sciences, University of Catania, Italy
[2]Department of Drug Sciences, University of Catania, Italy

Amyotrophic Lateral Sclerosis (ALS) is a multifactorial neurodegenerative disease characterized by progressive degeneration of upper and lower motor neurons. Of the numerous form of inherited ALS, approximately 20% are caused by mutations in the gene Cu/Zn superoxide dismutase (SOD1) leading to its gain of function [1]. Several molecular mechanisms have been proposed to explain SOD1-mediated toxicity including defects of mitochondrial calcium homeostasis, endoplasmic reticulum (ER) stress, deficits in autophagy and mitophagy processes [2]. Some studies have also highlighted that Sig-1R gene mutations are involved in a juvenile form of ALS [3]. Sigma-1 receptor (Sig-1R) is localized to the specific microdomains of the ER called mitochondrial-associated membranes (MAM) where regulates calcium flux from the ER to mitochondria. Several studies have reported that a loss of function of Sig-1R reduces ER–mitochondria cross talk by inducing MAM disruption and mitochondrial alteration. In the present study, we investigated the molecular mechanisms involved in neuroprotective role of Sig-1R in ALS. In particular, we evaluated the effect of a Sig-1R agonist, PRE-084, as well as a Sig-1R antagonist, BD1047, on autophagy and /or mitophagy process in a model in vitro of ALS. Cell viability of neuroblastoma-spinal cord-(NSC-34) wild type (WT) cells and NSC-34 stably bearing a human Cu/Zn superoxide dismutase1 G93A mutation (G93A) was evaluated after treatment with PRE-084 or BD1047 through MTT analysis. As demonstrated by western blot and immunofluorescence analysis, Sig 1 R activation reduced cell death by modulating the parkin/Pink1 system, a molecular marker of mitophagy as well as LCII and p62 expression, two molecular marker of mitophagy. Further studies are needed to better characterize the role of Sig-1R ALS pathogenesis.

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Plasmatic changes for VGF-derived peptides in severe psychiatric disorders

Barbara Noli[1], Elias Manca[1], Mirko Manchia[3,4], Claudia Pisanu[2], Raffaella Ardau[5], Carlo Arzedi[3,4], Alberto Bocchetta[2,5], Donatella Congiu[2], Eleonora Cossu[3,4], Mario Garzilli[3,4], Anna Meloni[2], Maria Antonietta Montis[3,4], Andrea Mura[3,4], Pasquale Paribello[3,4], Federica Pinna[3,4], Giovanni Severino[2], Maria Del Zompo[2,5], Caterina Chillotti[5], Bernardo Carpiniello[3,4], Cristina Cocco[1], Alessio Squassina[2]

[1] Department of Biomedical Sciences, NEF-Laboratory, University of Cagliari, 09042, Monserrato, Cagliari, Italy
[2] Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, 09042, Monserrato, Cagliari, Italy
[3] Unit of Psychiatry, Department of Public Health, Clinical and Molecular Medicine, University of Cagliari, 09042, Monserrato,Cagliari, Italy
[4] Unit of Clinical Psychiatry, University Hospital Agency of Cagliari, Cagliari, Italy
[5] Unit of Clinical Pharmacology, University Hospital Agency of Cagliari, Cagliari, Italy

Accurate classification of mental disorders is very complex due to overlapping symptoms and limited knowledge of the neurobiology underneath. As such, to find specific diagnostic biomarkers becomes very important. In this field, VGF and VGF-derived peptides are potential novel biomarkers, as they have been often found dysregulated through multi-omic studies in major psychiatric diseases. The neurosecretory protein VGF (non-acronymic) belongs to the granin family. The *vgf* gene encodes a precursor protein (proVGF) that undergoes proteolytic processing by prohormone convertases and other proteases in the regulated secretory pathway to produce several active VGF-derived peptides. In the present study, we aimed to investigate plasma changes for the VGF-derived peptides namely AQEE, NAPP, TLQP, C-terminal in severe psychiatric disorders. Fasting blood samples from patients with a diagnosis of major depressive disorder (MDD, n=37), bipolar disorder (BD, n=40) or schizophrenia (SZ, n=41) and 36 non-psychiatric controls (NPC) were collected. Plasma levels of VGF peptides, high-sensitivity C-Reactive Protein (hsCRP) and tumor necrosis factor-α (TNFα) were measured by Enzyme linked immunosorbent assays (ELISA). Generalized linear models with correction for multiple testing were used for statistical analysis while Pearson's correlation was used to measure statistical relationships among VGF-peptides, clinical general information (e.g. gender, age of patients) and inflammatory markers (hsCRP and TNFα). Plasma AQEE levels were significantly lower in BD and SZ patients (decrease of ~50% and ~35% respectively, $p<0.05$) compared with NPC, while TLQP and NAPP-peptides were reduced in BD patients only (decrease of ~30% in both assays, $p<0.05$) compared with NPC. It should be noted that higher levels of AQEE, TLQP and NAPP peptide (increase of ~40%, ~48% and ~50%, respectively, $p<0.05$) were observed in younger DMM patients (less than 40 years old) than in NPC and older DMM patients (whose plasma levels are similar to NPC). No difference was found for C-terminal VGF peptide in all samples analyzed. Levels of hsCRP were higher in SZ compared to NPC (increased of ~42%, adjusted $p=0.027$), while patients with treatment-resistant MDD had higher levels of TNFα compared to NPC ($p=0.028$) and to non-treatment resistant ($p=0.039$). An inverse correlation between plasma VGF levels (AQEE, TLQP and NAPP peptides) and age of DMM patients was observed (r_p = -0.509, -0.591, -0.589 respectively, $p<0.005$). No correlation was found between plasma VGF levels and hsCRP, while a negative correlation was only found between AQEE and TNFα (r_p= -0.428; $p=0.005$). Our study shows a distinct dysregulation of VGF and increased inflammation in patients with severe mental disorders, and supports the hypothesis that VGF and VGF peptides would be potential psychiatric disease biomarkers.

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Liposome-based nanoparticles modulate Macrophage and regulatory T cell and effector phenotypes in Multiple Sclerosis patients

Alessandra Pistilli[1], Francesco Ria[2], Maria Tredicine[2], Noemi Poerio[3], Matteo Lucchini[4], Chiara Camponeschi[2], Massimiliano Mirabella[4], Anna Maria Stabile[1], Desirée Bartolini[1], Maurizio Fraziano[3], Mario Rende[1], Gabriele Di Sante[1]

1 Department of Surgery and Medicine, Institute of Human, Clinical and Forensic Anatomy, Piazza L Severi 1, 06125 Perugia, Italy
2 Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy
3 Department of Biology, University of Rome "TorVergata", Via della Ricerca Scientifica 1 - 00173 Rome, Italy
4 Department of Neuroscience, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy

Current available treatments of Multiple Sclerosis (MS) aim to non-specifically reduce or eliminate symptoms and neuroinflammation, but can drive severe side effects and have a limited efficacy in slowing the progression of the disease[1,2].

Here, we evaluated in vitro the potential of a new class of microvesicles –liposomes of four different types[3,4]: single-sided, constituted by a double layer of phosphatidylserine (PSCho/PS), double-faced, with an outer layer of phosphatidylserine and an inner layer of phosphatidic acid (PSCho/PA), either alone or in the presence of the myelin basic protein (MBP) peptide (residues 85–99) (PSCho/PS -MBP and PSCho/PA-MBP).

Results showed that PSCho/PS are equally and efficiently internalized by pro- and anti-inflammatory monocytes (M1 and M2 respectively), while PSCho/PA were internalized better by M2 than M1[4,5]. PSCho/PS liposomes were able to inhibit the secretion of innate pro-inflammatory cytokine IL-1β. PSCho/PA liposomes were unable to dampen pro-inflammatory T cells and to promote immune-regulatory phenotype (Treg), while PSCho/PS liposomes expanded Tregs, reducing Th1 and Th17 cells. PSCho/PS liposomes were more effective in decreasing Th1 (but not Th17) cells in MS patients with a disease duration > 3 months. On the other hand, down-modulation of Th17 cells was instead evident in MS patients with active, Gadolinium enhancing lesions at the RMN and in MS patients with a high basal expression of M1-associated markers in the monocytes. Ability of PSCho/PS liposomes to up-regulate Treg cells was more pronounced in MS patients with high basal expression of M2 markers. The same findings were observed in the modulation of MBP-driven Th1/Th17/Treg responses. Together these data indicate that monocyte/macrophage may play an important regulatory role during MS course. Early MS associate to a hard-wired pro-Th1 phenotype of M1 that is lost later during disease course.

Acute inflammatory events, on the other hand, reflect a temporary decrease of M2 phenotype that however is amenable to restauration by treatment with PSCho/PS liposomes and suggest a role for PSCho/PS and PSCho/PS -MBP as new therapeutic strategies to dampen the pro-inflammatory immune responses and to promote its regulatory branch.

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Conjugated Linoleic Acid isomers reduce inflammatory response in murine microglia cells

Clara Porcedda[1], Franca Piras[1], Elisabetta Murru[1], Claudia Manca[1], Gianfranca Carta[1], Sebastiano Banni[1], Valeria Sogos[1]

[1] Department of Biomedical Sciences, University of Cagliari

Conjugated Linoleic Acid (CLA) refers to a family of a stereo and positional isomers of linoleic acid, largely contained in ruminant meat and dairy products. CLA has a variety of biological effects associated to diabetes, obesity and cancer; moreover, in central nervous system it plays an important role in immune and inflammatory responses as modulator of lipid metabolism and eicosanoid formation.

Microglia are the resident immune cells of the brain and play an important role in maintaining tissue homeostasis. However, when there is a neuronal injury or other insult, activated microglia can synthesize and secrete a great variety of soluble factors, including cytokines, chemokines, complement factors, fatty acid metabolites, proteolytic enzymes and free radicals. Sustained or chronic activation of microglia can lead to irreversible CNS damage and is often associated to neurodegenerative disorders. We previously demonstrated that CLA isomers easily pass the blood brain barrier in rats and in humans and downregulate inflammatory markers in human cultured astrocytes.

The objective of this study was to investigate the anti-inflammatory effects of the two main CLA isomers c9,t11 and t10,c12 by the evaluation of cytokine expression and secretion in BV-2 murine microglia cells.

Cells were treated with 25 μM of c9,t11 or t10,c12 CLA isomers or Oleic Acid as control for 48 hours. We found that CLA isomers were able to modulate the expression of inflammatory molecules either in basal condition or after an inflammatory stimulus by lipopolysaccharide (LPS).

We verified that both CLA isomers were incorporated and metabolized into BV-2 cells; consequently, we noticed a decrease in mRNA expression of IL-1β, IL-6, iNOS and RANTES compared to the fatty acid control Oleic Acid. Western blot showed a diminution in IL-1β and iNOS expression after CLA isomers treatment. Furthermore, we observed a reduction of IL-6 and RANTES secretion, as assessed by ELISA.

This study extends our understanding of fatty acids relevance in neuro-inflammation and suggests that CLA may ameliorate some of the inflammatory features through its nutritional role; the availability of more clinical data should clarify the implications in human health.

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Extracellular vesicles modulate microglia activation and function

Chiara Porro[1], Maria Ester La Torre[1], Maria Antonietta Panaro[2], Melania Ruggiero[2], Antonia Cianciulli[2], Dario Domenico Lofrumento[3], Rita Polito[1], Laura Antonucci [1], Giovanni Messina[1]

[1] Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy
[2] Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy
[3] Department of Biological and Environmental Sciences and Technologies, Section of Human Anatomy, University of Salento, Lecce, Italy

Microglia are the resident immune cells in the brain that are involved in immune surveillance and inflammatory responses in the central nervous system. Microglia activation is a mechanism of homeostatic regulation in the brain. Activated microglia can be triggered by exogenous or endogenous stimuli, including inflammation, injury and pathogens. When the immune homeostasis is disturbed, microglia can be phenotypically transformed into two types of polarization: the classically activated M1 (pro-inflammatory) and alternatively activated M2 (anti-inflammatory) phenotypes. Extracellular vesicles (EVs) represent a heterogeneous group of cell-derived membranous structures released by all cell types, including brain cells. Microglia release EVs both in normal conditions than after addition of proinflammatory stimuli, the content of these two types of EVs is different.

There are three types of EVs, classified on the basis of their origin and size, exosomes, microvesicles, and apoptotic bodies which originate from the endosomal system, shed from the plasma membrane, or produced by cells undergoing apoptosis respectively. They are present in biological fluids and are involved in multiple physiological and pathological processes. Extracellular vesicles are now considered as an additional mechanism for intercellular communication, allowing cells to exchange proteins, lipids and genetic material.

In our study we have analyzed the effects of the EVs obtained from microglia with different stimuli, on microglial cell activation. Upon activation, microglia also undergo dramatic morphologic changes, from resting ramified shape into activated amoeboid morphology. These changes are concomitant with up-regulation of several transcription factors and release of soluble factors, such as proinflammatory cytokines. Lipolysaccharides (LPS) is a potent pro-inflammatory stimulus largely utilized for induce neuroinflammation. In this study we have used LPS for stimulate BV2 microglia cells in order to induce microglia activation and EVs release; the EVs obtained with LPS stimulation, called EVs-LPS, are then utilized as stimuli on microglia BV2 resting cells for to investigate their ability to induce microglia polarization towards microglia inflammatory state. We have analyzed the change of morphology of BV2 cells, proliferation and migration of the microglia after EVs-LPS stimulation, and we have also investigated the expression and the release of pro-inflammatory and anti-inflammatory cytokines. The encouraging results of this study have demonstrated that EVs-LPS are able to induce microglia activation in the same way then the LPS alone, instead the EVs obtained from control cells are incapable to induce microglia polarization towards pro-inflammatory state. Microglial activation has been considered as a result of neuronal damage, however, recently it becomes to recognize as a possible cause of the damage in various neurodegenerative diseases. This study has confirmed the important role of EVs in communication and has demonstrated that EVs produced in inflammatory environment are able to exacerbate the inflammatory process inducing microglia activation and this may influence all the brain cells.

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Neuroprotective effects of vitamin C: new insight about the GSK3β signaling pathway modulation

Melania Ruggiero[1], Francesca M. Filannino[1], Antonia Cianciulli[1], Rosa Calvello[1], Chiara Porro[2], Dario D. Lofrumento[3], Giuseppe Nicolardi[3], Francesco De Nuccio[3], Maria Antonietta Panaro[1]

[1] Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, I- 70125 Bari, Italy
[2] Department of Clinical and Experimental Medicine, University of Foggia, I-71100 Foggia, Italy
[3] Department of Biological and Environmental Sciences and Technologies, Section of Human Anatomy, University of Salento, I-73100 Lecce, Italy

Neuroinflammation is an inflammatory response through which the brain immune cells eliminate pathogens, cell debris or misfolded proteins. As a defence mechanism, it is finalized to the preservation of the brain, however the prolonged inflammatory status leads to the onset of neurodegenerative disorders [1].

Oxidative stress is an imbalance between free radicals and antioxidants in favour of pro-oxidants. Given that both neuroinflammation and oxidative stress are implicated in the pathogenesis of neurodegenerative disorders, antioxidants and anti-inflammatory compounds may be useful for counteracting them. Vitamin C (Vit C) is known to have anti-inflammatory and antioxidant properties and, although its neuroprotective effects have been elucidated, the underlying molecular mechanisms remain unclear [2].

Glycogen synthase kinase 3β (GSK3β) is a serine/threonine kinase which acts as a potent driver of inflammation, rendering GSK3β inhibitors a promising target of anti-inflammatory research [3]. In this study, we have investigated the role of GSK3β inactivation in the context of Vit C neuroprotective effects by using a well-known 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced preclinical animal model of Parkinson's disease (PD) and LPS-treated BV2 cells as alternative model system for activated primary microglia cultures.

We found that Vit C regulates the inflammatory response up-regulating the expression of the anti-inflammatory cytokine IL-4 and down-regulating the pro-inflammatory cytokine IL-6 both in in vitro and in vivo models. In addition, the levels of superoxide dismutase 1 (SOD1) antioxidant enzyme were increased by Vit C in both models. In response to Vit C, GSK3β protein was inactivated and p-p38 underwent an increase compared to control, suggesting that p-p38 may play a role in inactivating GSK3β. Moreover, Vit C is able to increase the cytosolic levels of β-catenin and consequently its nuclear translocation, giving a possible explanation to the anti-inflammatory and antioxidant functions of Vit C. Collectively, these results demonstrate that Vit C exhibits substantial neuroprotective effects through the modulation of GSK3β pathway, attenuating pro-inflammatory and up-regulating anti-inflammatory processes.

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Mitochondrial transfer: the key for Mesenchymal Stem Cell-induced neuroprotection?

Olga Tarasiuk[1], Elisa Ballarini[1], Elisabetta Donzelli[1], Virginia Rodriguez-Menendez[1], Angelica Squarzoni[1], Mariarosaria Miloso[1], Guido Cavaletti[1], Arianna Scuteri[1]

[1] Experimental Neurology Unit and Milan Center for Neuroscience, School of Medicine and Surgery, Milano-Bicocca University, Italy

Mesenchymal stem cells (MSCs) are adult stem cells residing in many tissues with unique properties: they are able to modulate the immune system and overall to provide a strong support to neuronal survival. During the last decade, many studies have investigated the mechanisms of this neuroprotective effect, with the identification of multiple mechanisms, potentially involved: from the release of trophic molecules such as growth factors, cytokines, antioxidants, to the secretion of vesicles containing molecules important for tissue repair.

In our model, we analyzed the positive effect of MSCs on sensory neurons derived from Sprague Dawley rat embryo and, by using Immunofluorescence and in vivo imaging analysis, we identified the transfer of mitochondria from MSCs to neurons, which could take place by different modalities, such as extracellular vesicles, gap junctions and tunneling nanotubes. The block of these interactions could abrogate the neuroprotective effect of MSCs, thus confirming their importance for MSC-dependent support of neuronal survival.

Such a knowledge could add a piece to the puzzle of MSC neuroprotective potential, which could be exploited to maximize their positive effect in the perspective of a clinical use.

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High-fat diet impairs mouse median eminence: a study by transmission and scanning electron microscopy coupled with Raman spectroscopy

Ilenia Severi[1], Georgia Colleluori[1], Marco Fosca[2], Angelica Di Vincenzo[1], Julietta V. Rau[2], Antonio Giordano[1]

[1]Dipartimento di Medicina Sperimentale e Clinica, Universita` Politecnica delle Marche, Ancona
[2] Istituto di Struttura della Materia, ISM-CNR, Roma

Hypothalamic dysfunction is an initial event following diet-induced obesity, primarily involving areas regulating energy balance such as arcuate nucleus (Arc) and median eminence (ME). To gain further insights into the early hypothalamic diet-induced alterations, adult CD1 mice fed an high-fat diet (HFD) for 6 weeks were studied and compared with normo-fed controls. Transmission and scanning electron microscopy and histological staining were employed for morphological studies of the ME, while Raman spectroscopy was used for the biochemical analyses of the Arc-ME complex. In HFD mice, ME β2-tanycytes, glial cells dedicated to blood-liquor crosstalk, exhibited remarkable ultrastructural anomalies, including altered alignment, reduced junctions, degenerating organelles and higher content of lipid droplets, lysosomes and autophagosomes. Degenerating tanycytes also displayed an electron transparent cytoplasm, filled with numerous vesicles, and dilated extracellular spaces extending up to the subependymal layer. Consistently, Raman spectroscopy analysis of the Arc-ME complex revealed higher glycogen, collagen and lipid bands in HFD mice compared with controls, and higher band corresponding to the cyanide group in the former compared to the last. Collectively, these data show that ME β2-tanycytes exhibit early structural and chemical alterations due to HFD and reveal for the first time hypothalamic cyanide presence following high dietary lipids consumption, a novel aspect with potential implications in the field of obesity.

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Tumor Microenvironment and Microvascular Density in Human Glioblastoma

Roberto Tamma[1], Tiziana Annese[1 2], Domenico Ribatti[1]

[1] Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari Medical School, Bari, Italy
[2] Department of Medicine and Surgery, Libera Università del Mediterraneo (LUM) Giuseppe Degennaro University, Bari, Italy

Glioblastoma (GBM) is one of the most common and aggressive primary brain tumors in adults. It is characterized by rapid proliferation, diffuse invasion, necrosis, high angiogenesis and resistance to treatment. The tumor microenvironment (TME) is a complex environment surrounding the cancer cells that includes cellular and extracellular components and a vascular network which are involved in the tumorigenesis and in the determination of therapeutic efficacy. Recent data indicate that GBM cells can manipulate the microenvironment surrounding themselves developing a niche sustaining tumor growth and angiogenesis. This process involves immune cells, astrocytes, glial cells, neurons, extracellular matrix, and vascular cells. In this retrospective study we analyzed serial histological sections derived from bioptic specimens of 30 adult patients diagnosed for IDH1 wild type GBM. By immunohistochemistry we determined the percentages of total and M2-positive macrophages, CD4-positive and CD8-positive lymphocytes and CD34 positive microvessels in both the center of tumor and in the healthy surrounding areas. Moreover, we measured the percentage of cells expressing bcl6 and p53 in order to determine any possible correlations with TME. Morphometric analysis showed a significant increase of total and M2 type macrophages, of CD4 and CD8 lymphocytes and of CD34-positive microvessels in the tumoral area respect to the healthy zone. Previously, we demonstrated that Bcl-6 translocation induced an overexpression of Bcl-6 at messenger and protein levels in GBM, data correlated with the expression of p53. In this work we confirmed the increased percentage of p53 and BCL6 positive cells in the tumor and the latter resulted positively correlated with the CD34-positive microvessels. Overall, these data confirm the important role played by microenvironment components, including macrophages, lymphocytes, microvascular density and epigenetic events, including BCL6 translocation, p53 expression, in GBM progression. Finally, these results could contribute to the development of targeted new therapies useful in countering GBM progression.

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Anatomical localization of ACE2R and TMPRSS2 expression patterns in the Human Brainstem

Aleksandar Tushevski[1], Aron Emmi[1,2,3], Silvia Riccetti[4], Michele Sandre[2,3], Angelo Antonini[2,3], Luisa Barzon[4], Veronica Macchi[1,3], Andrea Porzionato[1,3], Raffaele De Caro[1,3]

[1] Institute of Human Anatomy, Department of Neuroscience, University of Padova, Padova, Italy
[2] Parkinson and Movement Disorders Unit, Centre for Rare Neurological Diseases (ERN-RND), Department of Neuroscience, University of Padova, Padova, Italy
[3] Center for Neurodegenerative Disease Research (CESNE), University of Padova, Padova, Italy
[4] Department of Molecular Medicine, University of Padova, Padova, Italy

Background: Angiotensin Converting Enzyme 2 Receptor (ACE2R) represents the major cell entry receptor for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus causing Coronavirus Disease 2019 (COVID-19). Despite being a respiratory virus, SARS-CoV-2 may directly access the central nervous system and infect neurons and glial cells alike. However, even though numerous studies have assessed SARS-CoV-2 neurotropism, very little information is available concerning the distribution of ACE2 Receptors in the human brain, with particular regard to its topographical expression in the brainstem.

Aim: Here, we assess the topography and expression pattern of ACE2 Receptor and TMPRSS2 in the human brainstem, in order to identify possible entry sites more susceptible to SARS-CoV-2 infection.

Materials and methods: The brains of 24 COVID-19 patients and 18 matched controls underwent immunoperoxidase and immunofluorescent staining to determine ACE2R and TMPRSS2 topography and expression within standardized sections of the medulla, pons and midbrain. Semi-quantitative morphometrical assessment was performed to compare protein expression within the structure.

Results and discussion: ACE2R and TMPRSS2 appear to be expressed in neuronal and oligodendroglial cells of the brainstem, particularly at the level of the medullary and midbrain tegmentum. These sites coincide with the most frequent sites of reported SARS-CoV-2 tropism.

Conclusions: This study helps to define anatomically susceptible regions to SARS-CoV-2 infection in the brainstem, advancing knowledge on the neuropathological underpinnings of neurological manifestations in COIVD-19.

Cellule staminali, istogenesi e differenziamento

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Dual role of endolysosomal two-pore channel two (tpc2) in malignant traits of melanoma cells

Samantha Barbonari[1], Antonella D'Amore[1], Fioretta Palombi[1], Silvia Sideri[1], Nicolò Morina[2], Anna Riccioli[1], Diletta Di Mitri[2] and Antonio Filippini[1]

[1] Unit of Histology and Medical Embryology, Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Sapienza University of Rome, 00161 Rome, Italy
[2] Humanitas Clinical and Research Center IRCCS, Rozzano (MI), Italy

The ion channel TPC2 (Two-pore channel 2), localized on the membranes of acidic organelles such as endo-lysosomes and melanosomes, is known to play a role in relevant pathologies, e.g. tumour metastasis and viral infection. Our aim is to explore whether TPC2 plays a different role in primary versus metastatic malignant melanoma (MM), as suggested by its differential expression in human MM clinical samples. We have explored which hallmarks of the malignant phenotype are TPC2-regulated traits, using an in vitro model represented by B16F0 (primary) and B16F10 (metastatic sub-line) MM cells, well known murine models for melanoma studies. In these cells, we have comparatively tested how the genetic or pharmacological inhibition of TPC2 impacts on either malignant phenotype. Our data show that in the less aggressive (B16F0) cells the inhibition of TPC2 determines anti-oncogenic responses, i.e. the downregulation of autophagy, the inhibition of migration and impairment of vasculogenic mimicry, while in the highly aggressive (B16F10) cells it results in enhancement of malignant traits. The different functional role of TPC2 in the two MM samples indicates that differences in aggressiveness, even within the same tumor cell type, must be considered when attributing a functional role to a specific gene. In a parallel set of experiments, aimed to explore the impact of TPC2 expression in MM cells on the tumor microenvironment, we are also testing the response of MM secretome on the polarization of macrophages, a relevant factor in tumor development. Our data on the secretome of the B16F10 cells indicate that the role of TPC2 strongly influences macrophage polarization resulting in a pro-tumoral modulation of the tumour microenvironment.

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Reactive oxygen species regulation of CD271, a potential cancer stem cell marker associated with chemoresistance and metastatic capacity of melanoma

Francesca Beretti[1], Martina Gatti[1], Manuela Zavatti[1], Tullia Maraldi[1]

[1] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena 41125, Italy

Cutaneous melanoma remains the deadliest form of skin cancer with a rapidly increasing global incidence. Oncogenic BRAF mutations, such as the common V600E substitution present in 30–50% of melanomas. activate the downstream effector mitogen-activated protein kinase (MEK/ERK) to drive melanoma tumour growth. Despite improvements in clinical outcome since the introduction of selective BRAF and MEK inhibitors (BRAFi/MEKi) and immune checkpoint inhibitors, development of resistance to these drugs remains a major problem, with little improvement in overall patient survival.

Although the level of CD271 in tumors is likely unstable, increased expression of this stem-cell marker, and its ligand NGF, was observed in chemo-resistant melanoma cells featuring increased migration. Concordantly, the selective inhibitor of oncogenic BRAFV600E/K vemurafenib mediated resistance *via* increased expression of CD271, indicating that CD271 may potentially give tumour cells a survival advantage during metastasis. It has recently been shown that BRAF signaling results in transcriptional upregulation of the oxidase Nox4, which promotes ROS generation. After acquisition of resistance increased ROS levels were found.

Here we examined how Nox4 derived ROS in BRAF mutated melanoma cells regulate their drug sensitivity and metastatic potential, focusing on ROS modulation of cellular signalling by Nox4 inhibition *in vitro*. In this study we demonstrated that DPI, a Nox inhibitor, reduced the resistance to vemurafenib of a melanoma cell line, SK-MEL-28, and of a primary culture derived from a BRAFV600E mutated biopsy. The signalling pathways of ERK and Akt were involved in the DPI role on the drop of epithelial-mesenchymal transition (EMT) and migration capacity. Indeed, DPI treatment affected the expression of CD271, leading to a decrease in expression of Vimentin and ZEB1, markers of EMT process which undoubtedly promotes an invasive phenotype in melanoma. More importantly, scratch test demonstrated the efficacy of the Nox4 inhibitor in blocking migration, supporting its use to counteract drug resistance and, thus, cell invasion and metastasis of BRAF mutated melanoma.

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Mucopolisaccaridosi 1H (MPS-1H): studio del rimodellamento osseo

Giacomina Brunetti[1], Albina Tummolo[2], Laura Piacente[3], Antonio Marzollo[4,5], Alessandra Biffi[4,6], Alberto Burlina[7], Maria Felicia Faienza[3].

[1] Dipartimento di Bioscienze,Biotecnologie e Biofarmaceutica, Università di Bari, Bari, Italia
[2] Dipartimento dei disordini metabolici, Genetica clinica e Diabetologia, Ospedale pediatrico Giovanni XXIII, Azienda Ospedaliero-Universitaria Consorziale, Bari,
[3] Dipartimento di Scienze Biomediche e Oncologia Umana, Sezione di Pediatria, Università di Bari, Italia
[4] Divisione di Ematologia pediatrica, Oncologia e Cellule staminali, Università di Padova, Padova, Italia
[5] Fondazione Città della Speranza, Istituto di Ricerca Pediatrica, Padua, Italy
[6] Dipartimento di Salute della donna e del bambino, Università di Padova, Padova, Italia
[7] Dipartimento di Pediatria, Divisione di Malattie metaboliche ereditarie, Università di Padova, Padova, Italia

La mucopolisaccaridosi-1H (sindrome di Hurler, MPS-1H) è la forma più grave di un disturbo da accumulo lisosomiale (LSD) causato da varianti del gene IDUA, codificante per l'alfa-L-iduronidasi (IDUA). MPS-1H è anche caratterizzata da numerosi difetti scheletrici dovuti all'accumulo di glicosaminoglicani (GAG) parzialmente degradati nei lisosomi delle cellule del tessuto connettivo. L'efficacia del trapianto di cellule staminali ematopoietiche (HSCT) e della terapia enzimatica sostitutiva (ERT) sulle manifestazioni scheletriche di MPS1H sono ancora considerate non soddisfacenti.

Pertanto, abbiamo studiato il caso di una giovane ragazza, che ha mostrato cambiamenti significativi nei marcatori di rimodellamento osseo e potenziale di osteoclastogenesi in vitro dopo HSCT combinato con ERT. Ha ricevuto ERT e ha subito due HSCT. Le alterazioni scheletriche della diagnosi, hanno mostrato una tendenza al miglioramento sia della mobilità che del pattern radiologico dopo HSCT. Abbiamo misurato i livelli più alti di attivatore del recettore del fattore nucleare kappa-B ligando (RANKL) e del rapporto RANKL/osteoprotegerina (OPG) alla diagnosi e durante ERT, coerentemente con l'osteoclastogenesi spontanea osservata. Al contrario, dopo il successo dell'HSCT con ERT in corso, sono stati osservati i livelli più alti di osteocalcina e tutti i marker di formazione e riassorbimento osseo sono migliorati.

In conclusione, la terapia combinata di ERT e HSCT è stata efficace nella riduzione dell'attività degli osteoclasti e nell'aumento dell'attività degli osteoblasti, questi cambiamenti supportano il fenotipo osseo riscontrato nel bambino, l'attività di IDUA e i livelli di GAG.

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Endocannabinoid system functions in spermatozoa: potential role of the CB1 receptor during epididymal maturation

Raffaella D'Auria[1], Marianna Marino[1], Elena Mele[2], Grazia Maria Giovanna Pastorino[3], Francesca Felicia Operto[3], Rosaria Meccariello[2], Andrea Viggiano[1], Antonietta Santoro[1]

[1] Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno, Italy
[2] Department of Movement Sciences and Wellbeing, Parthenope University of Naples, Italy
[3] Child and Adolescent Neuropsychiatry Unit, Medical School University of Salerno, Italy

The "endocannabinoid system," (ECS) comprising the cannabinoid receptors type 1 (CB1R) and type 2 (CB2R), their endogenous ligands including N-arachidonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), and the proteins that regulate endocannabinoid biosynthesis and degradation, is a lipid cell signalling system involved in the production of sex steroids and high-quality gametes, fertilization and embryo implantation [1]. However, the mechanisms through which this regulation occurs are still poorly elucidated. In order to fill the gap knowledge about the functional role of ECS in sperm differentiation and fertilization, we studied the effects of ECS stimulation via CB1R by administering the selective CB1R agonist AEA ± its antagonist SR141716 to male rats at puberty (n=4 /group, PND45) for three weeks. Sperm survival and motility was determined and CB1R localization and distribution was investigated in spermatozoa from epididymis caput and cauda. Briefly, spermatozoa have been obtained from freshly collected epididymal caput and cauda. Some aliquots of spermatozoa were fixed in 2% PFA for immunofluorescence (IF) analysis. Results showed that CB1R stimulation reduces sperm motility without affecting cell survival. Moreover, in the epididymis caput of untreated rats, CB1R follows the contour of the acrosomic vesicle and marks the region docking sperm tail to the nucleus. Instead, in mature spermatozoa from the cauda, CB1R distributes mainly in the dorsal acrosomal membrane being completely absent in the posterior region of the head connecting the sperm tail to the head. In AEA treated rats, CB1R expression decreases in the hook and is only slightly detectable in the sperm head, shifting almost completely to the tail, in both epididymis caput and cauda. These results suggest a role for the CB1R in controlling spermatozoa maturation and acquisition of competence critical for fertilization. On this hand, since the Kisspeptin system is known to be crucial for male reproduction by regulating the upstream activity of GnRH secreting neurons and the downstream modifications related to spermatozoa formation and maturation, we also investigate Kiss1 receptor (Kiss1R) localization in the same sperm samples. Notably, the treatment with AEA induced changing in Kiss1R distribution that appears to be localized in sperm tail whereas in the control group the immunoreactivity was limited to the perforatorium, and posteriorly overlaps with CB1R expression in the region marking the passage from to sperm head to the tail. The selective inhibition of the CB1R by SR141716 reverted all the functional and morphological-induced effects suggesting that ECS, via CB1R can influence spermatozoa epididymal maturation also modulating Kiss1R.

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The effect of nitrogen-containing bisphosphonates, alendronate and zoledronate, on PDLSCs-induced angiogenesis

Jessica Bria[1], Anna Di Vito[1], Emanuela Chiarella[1], Francesco Baudi[1], Alessandro Antonelli[2], Marta Greco[2], Amerigo Giudice[2], Tullio Barni[1]

[1] Department of Experimental and Clinical Medicine, University Magna Græcia of Catanzaro, 88100 Catanzaro, Italy
[2] Department of Health Sciences, University Magna Græcia of Catanzaro, 88100 Catanzaro, Italy

Alendronate (ALN) and zoledronate (ZOL) are two potent amino-bisphosphonate (N-BPs) widely used for cancer indications and osteoporosis [1]. At the cellular level, N-BPs mainly exert their effects on osteoclasts, however, their impact on osteoblasts, endothelial and mesenchymal cells has also been described. At the molecular level, N-BPs inhibit the action of farnesyl pyrophosphate synthase in the mevalonate pathway, thereby blocking the prenylation of small GTPases that are major regulators of intracellular trafficking [2].

When N-BPs bind to bone, they localize preferentially at sites of resorption, where osteoblasts and endothelial cells are exposed to a much lower concentration *in vivo* than the osteoclasts. Although the association of N-BPs and osteonecrosis of the jaw (MRONJ) has been widely described, pathophysiology of the MRONJ has not been elucidated. We and others reported dose-dependent effects of ALN and ZOL on periodontal ligament stem cells (PDLSCs), which play a key role in periodontal tissue remodeling [3,4]. Recently, more attention has been directed to the role of N-BPs-induced vascular alteration in the initiation and progression of the disease.

With this aim, we evaluated the release of VEGF and MCP-1, two important angiogenic growth factor and chemokine, respectively, in the supernatant of PDLSCs exposed to increasing doses of ALN (2 μM, 5 μM, 10 μM,) and ZOL (1 μM, 1,5 μM, 2 μM) for 24 h, 48 h, and 120 h. Then, we investigated if those modulations have any role in impairing PDLSCs-induced angiogenesis, *via* tube formation assay.

By microarray analysis, we recorded increased VEGF and MCP-1 levels in the medium of PDLSCs after exposure to 1 mM ZOL compared to unexposed cells. Conversely, a reduction of VEGF and MCP-1 release was reported after exposure to 1,5 mM and to a lesser extent to 2 mM ZOL at all times considered, compared to cells exposed to 1 mM ZOL. Similarly, an increase in both VEGF and MCP-1 was reported after exposure to 2 mM ALN at all time points tested. Also in this case, exposure to the higher ALN concentrations (5 and 10 mM) accounted for a reduction in VEGF and MCP-1 production compared to cells exposed to 2 mM ALN. Tube-formation capacity of HUVECs were then tested in the presence of supernatant from PDLSCs +/- N-BPs. Quantitative analysis was performed by ImageJ software, and we found that both ALN and ZOL were able to modulate the number of meshes, nodes, and total segment length in a dose-dependent manner, compared with conditioned medium without drug.

Although additional studies are needed, our study suggest new insights on modulatory action of N-BPs, ALN and ZOL, in PDLSCs-induced angiogenesis.

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Extracellular Vesicles Derived from Human Gingival Mesenchymal Stem Cells: a Protective Role in Cardiomyocytes Acute Hypoxia

Della Rocca Y.[1], Fonticoli L.[1], Marconi G.D.[2], Pizzicannella J.[3], Trubiani O.[1], Diomede F.[1]

[1] Department of Innovative Technologies in Medicine & Dentistry, University "G d'Annunzio" Chieti-Pescara, Via dei Vestini, 31, 66100 Chieti, Italy
[2] Department of Medical, Oral and Biotechnological Sciences, University "G d'Annunzio" Chieti-Pescara, Via dei Vestini, 31, 66100 Chieti, Italy
[3] Cardiology Intensive Care Unit, "Ss Annunziata" Hospital, ASL 02 Lanciano-Vasto-Chieti, 66100 Chieti, Italy

Hypoxia has an impact on pathological conditions of different tissues and especially on the heart where it can have different consequences depending on the duration of exposure to the hypoxic state [1].

Acute hypoxic exposure can result in reversible acclimatization in heart tissue, maintaining a good systemic oxygen supply, while chronic hypoxic exposure leads to tissue damage exacerbating hypoxia-induced cardiac dysfunction.

Extracellular vesicles (EVs) are small membrane vesicles, of the order of nanometers, secreted by different cell types. EVs are mediators of intercellular communication in both physiological and pathological conditions [2]. EVs produced by oral-cavity-derived Mesenchymal Stem Cells (MSCs), including human gingival mesenchymal stem cells, have pro-angiogenic and anti-inflammatory effects. For this reason, the EVs can be identify as a new therapeutic potential for tissue regeneration.

The aim of the present work was to evaluate the effect of treatment with EVs produced by human gingival mesenchymal stem cells (hGMSCs) on an in vitro model of HL-1 cardiomyocytes cultured under acute hypoxia state (0,2% hypoxia) followed by normoxia conditions. The HIF-α, p300 and NFkB expression was downregulate with EVs treatment as well as Nrf2 and VEGF expression was increased with EVs treatment. EVs could represent an innovative platform to prevent the hypoxic damages and to tissue regeneration.

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Road to metastasis: alternative mRNA translation regulates Triple-Negative Breast Cancer bioenergetics

Paola Falletta[1], Marco Gaviraghi[1,2], Alessia Loffreda[1], Martina Crippa[1], Vincenzo Salemme[3], Jagat Chauhan[4], Colin Goding[4], Paola Defilippi[3] and Carlo Tacchetti[1]

[1] Università Vita-Salute San Raffaele, Milano, IT
[2] Fondazione Human Technopole, Milano, IT
[3] DBMSS, Università di Torino, IT
[4] Ludwig Institute for Cancer Research, University of Oxford, UK

Metastasis is the leading cause of carcinoma–related morbidity and mortality. Identification of effective therapeutic strategies for the metastatic disease is a crucial unmet need.

Numerous microenvironmental inputs have been implicated in driving phenotypic transition towards metastasis in cancer, and many converge on the transcriptional and translational reprogramming driven by the Integrated Stress Response (ISR). The ISR is a conserved network of signaling pathways evolved to adapt to a dynamic milieu and to maintain homeostasis by integrating the sensing of a variety of stresses which converge to the phosphorylation of the rate limiting eIF2α (eukaryotic translation Initiation Factor 2α) and result in alternative mRNA translation.

In cancer cells, ISR can be triggered by microenvironmental cues as well as by oncogene activation, and its role in the metastatic process in TNBC is still emerging. We focus on Triple-negative breast cancer (TNBC), which accounts for the 20% of diagnosed breast cancers, carries the highest mortality risk and its metastatic nature often leads to early recurrence.

Our analysis of TNBC cells translatome reveals that activation of the ISR profoundly alters mitochondrial protein synthesis. Indeed, a wide subset of mRNAs of mitochondrial protein, both coded by nuclear and mitochondrial DNA, is more efficiently translated upon ISR activation. Here we show that the ISR regulates mitochondria phenotype and bioenergetics, it drives the mitochondrial production of Reactive Oxygen Species (ROS), and enhances the migratory and invasive potential of TNBC cells.

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Potential role of PLCβ2 in DOX-induced cardiotoxicity

Antonietta Fazio[1], Alessandra Cappellini[1], Sara Mongiorgi[1], Camilla Evangelisti[1], Alessandra Ghigo[2], Michele Russo[2-3], Lucio Cocco[1], Lucia Manzoli[1] and Stefano Ratti[1]

[1] Cellular Signalling Laboratory, Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, 40126 Bologna, Italy
[2] Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino, Italy
[3] Department of Translational Medical Sciences, Federico II University, Naples, Italy

Anthracycline therapy is a cornerstone in the treatment of several cancers. Nevertheless, anthracycline therapy is limited by cumulative dose-dependent cardiotoxicity which imply a decrease of long-term survival of patients. Doxorubicin (DOX) is one of the most potent anthracycline agents used for the treatment of solid tumors and certain types of leukemia[1]. Though, its clinical use is limited because of severe cardiotoxic side effects ranging from alterations in morpho-functional myocardial dysfunction to dilated cardiomyopathy and heart failure resulting in cardiac transplantation or death. Unfortunately, no guidelines have been identified for screening and diagnostic monitoring of these patients and, due to the incomplete understanding of molecular mechanisms, effective therapeutic strategies are still lacking. Accordingly, the investigation of cellular mechanisms underlying anthracycline cardiotoxicity is crucial for the prevention of cardiac side effects.

It has been reported that PLCβ expression and activity is influenced in cardiomyocytes under pathological conditions, such as cardiac hypertrophy and chamber dilatation[3]. In a screen for signal transduction genes in human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes, phospholipase C beta 2 (PLCβ2) upregulation was identified after DOX treatment[2]. For this reason, the present study aims to elucidate the potential function of PLCβ2 in DOX-induced cardiotoxicity *in vitro*. H9C2 cells were exposed to DOX (1μM) and incubated up to 24h. The treatment confirmed a significative upregulation of PLCβ2 expression at both protein and mRNA levels correlated to the increase of cell surface area, altered mitochondrial function and increase of reactive oxygen production (ROS). Furthermore, the investigation of downstream transduction pathways confirmed the impairment of cellular mechanisms due to drug-induced cardiotoxicity, including phosphatidylinositol-3-kinase (PI3K)/Akt/ mammalian target of rapamycin (mTOR), mitogen-activated protein kinase (MAPK) and diacylglycerol kinase (DGK) signalling.

Based on these findings, future experiments will be focused on the modulation of PLCβ2 expression, in order to evaluate whether PLCβ2 may be a potential target in DOX-induction cardiotoxicity.

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Neurotoxicity of plastic pollutants: effects of endocrine-disruptors on developing human neuronal cells

Giulia Gaggi[1,2], Andrea Di Credico[1,2], Pierpaolo Di Martino[1], Valentina Marcozzi[1], Barbara Ghinassi[1,2] and Angela Di Baldassarre[1,2]

[1] Department of Medicine and Aging Sciences, "G D'Annunzio" University of Chieti- Pescara, 66100 Chieti, Italy
[2] Reprogramming and Cell Differentiation Lab, Center of Advanced Studies and Technology, University "G d' Annunzio" Chieti-Pescara, Italy

Health concern is rising about the immediate and long-term consequences of human exposure to plastic pollutants that contaminate the environment with chemicals such as Bisphenols (BPs) and perfluoroalkyles (PFs) known to be endocrine disruptor (EDs). EDs can impact the endocrine system and subsequently impair human development and health. It has been reported that EDs can cross the blood-placenta barrier, accumulating in the amniotic fluid, umbilical cord and fetal plasma. Despite the recent advances in the ED research, little is still known about their effects on human developing neurons. Aim of this study was to elucidate the effects of diverse EDs (BPA, BPS, PFOS and PFOA) on a mixed population of human iPSC-derived neurons (glutamatergic, GABAergic and dopaminergic) and astrocytes of the central nervous system. The neuronal population was treated with the EDs (alone or in combination) at doses that are considered typical of human exposures (0.1uM) for 24h and then the mitochondrial health and the electrical activity were analysed.

Data demonstrated that all the EDs were detrimental for mitochondria activity changing the mitochondrial membrane potential; the myotoxicity was particularly dramatic when different EDs were administered in combination (cocktail effect). Moreover, alterations of the neuronal electrical activity and connectivity were registered in first 24h following the treatment: indeed, we detected a modification of the mean firing rate, of the burst frequency and of the network activity. In conclusion our data demonstrated that exposure to EDs leached from plastic, especially in combination, can affect developing neurons by altering the mitochondrial function and the electrophysiological features, thus suggesting that they could be also involved in the development of neurological disorders.

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Ultrastructural evaluation of mouse oocytes after exposure to the fungicide Mancozeb

Marta Gatti[1], Maria Grazia Palmerini[2], Mariacarla De Rubeis[1], Ilaria Antenisca Mascitti[2], Stefania Annarita Nottola[1] and Guido Macchiarelli[2]

[1]Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Sapienza University, Rome, Italy
[2]Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

Mancozeb is a fungicide, a member of the Dithiocarbamates group, used in the management of fungal diseases of plants, and classified as an endocrine disruptor [1]. Although its low mammalian toxicity, high or repeated exposure to Mancozeb may interfere with reproductive biology. Recent studies on the effect of Mancozeb on female fertility evidenced morphological changes associated with an altered p53 expression, in mice granulosa cells and hampered embryo development [2,3]. Data from *in vitro* experiments have revealed alterations in the meiotic spindle structure of mice oocytes and granulosa cells morphology [4,5]. However, there are no ultrastructural studies performed by Light Microscopy (LM) and Transmission Electron Microscopy (TEM), on mammalian oocytes exposed to Mancozeb. This preliminary study aims to evaluate the ultrastructure of M-II mouse mature oocytes obtained by puncturing antral follicles of PMSG-treated prepubertal CD1 female mice and cultured *in vitro* in DMEM+5%FBS+pen/step without (control) or with increasing concentrations of Mancozeb (from 0.001 to 1 μg/ml). After collection, oocytes were fixed in 2.5% glutaraldehyde/PBS and subjected to the standard preparative for LM and TEM [5]. At low concentrations, ultrastructural data showed intact zona pellucida, thin perivitelline space, few and irregular microvilli distributed on the oolemma, normal mitochondria dispersed in the cytoplasm, and a few cortical granules visible. At higher concentrations, the zona pellucida appeared intact with an indistinguishable perivitelline space, an irregular or reduced distribution of microvilli, and a decreased presence/distribution of the organelles. In particular, differently from the previous group, the ooplasm showed the presence of hooded mitochondria, characterized by a reduced functional surface, with no visible cortical granules. In conclusion, Mancozeb induced dose-dependent toxicity on the mouse oocyte ultrastructure, with alterations in the oolemma, a reduction of the microvilli, and a decreased density of intracellular organelles. These morphological changes could adversely affect developmental competence and reproductive potential, as observed in cases of infertility associated with pesticide exposure.

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Bone marrow stromal cells support acute myeloid leukemia stem cell enhancing antioxidant defenses

Silvia Masciarelli[1], Francesca Liccardo[1], Martyna Śniegocka[1], Viviana Romano[1], Tiziana Ottone[2,3], Serena Travaglini[2,3], Selenia Miglietta[4], Stefania Annarita Nottola[4], Giuseppe Familiari[4], Vincenzo Petrozza[5], Luca Tamagnone[6,7], Maria Teresa Voso[2,3], Francesco Fazi[1]

[1] Department of Anatomical, Histological, Forensic & Orthopaedic Sciences, Section of Histology and Medical Embryology, Sapienza University of Rome, Rome, Italy
[2] Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy
[3] Santa Lucia Foundation, Neuro-Onco-Hematology Laboratory, Rome, Italy
[4] Department of Anatomical, Histological, Forensic & Orthopaedic Sciences, Section of Human Anatomy, Sapienza University of Rome, Rome, Italy
[5] Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy
[6] Department of Life Science and Public Health, Histology and Embryology Unit, Catholic University of the Sacred Hearth, Rome, Italy
[7] Fondazione Policlinico Universitario A Gemelli IRCCS, Roma, Italy

Acute myeloid leukemia (AML) is a heterogeneous group of diseases due to chromosomal abnormalities and gene mutations that impair myeloid progenitor differentiation. However, increasing evidences highlight the relevance of bone marrow (BM) niche remodeling during leukemia onset and progression. BM microenvironment of patients suffering from leukemia undergo a complex process of specific adaptations which exert important effects on the hematopoietic stem cell compartment, create a favorable habitat for leukemic stem cell (LSCs) and increase drug resistance. Functional changes in stromal components in the bone marrow must be considered as one of the aspects of leukemia biogenesis, because they create an aberrant microenvironment which supports survival and expansion of leukemic cells[1,2].

We recently designed a strategy to arrest AML cells based on a combination of a differentiative agent (retinoic acid -R-), an inducer of proteotoxic stress (the proteasome inhibitor Bortezomib -B-) and an inducer of oxidative stress (arsenic trioxide -A-)[3]. We found that, although the combination RBA is very efficient in inducing proliferation arrest, differentiation and cell death of AML stem and progenitor cells, the same treatment, in a co-culture system with BM stromal cells becomes ineffective. Thus we set up to identify the mechanisms underlying the protective effects of stromal cells on AML cells. Our data show that stromal cells strongly activate their oxidative stress response, thus reducing the presence of reactive oxygen species in AML cells. Furthermore, we observed that stromal cells undergo substantial morphological changes, in particular by increasing the number of focal adhesions. We also demonstrate that direct contact between stromal and AML cells is necessary to ensure the protective effects. Of note, on the basis of the observations obtained in the *in vitro* co-culture system, we are setting up a approach to overcome resistance to therapy, provided by the bone marrow niche, in an orthotopic murine model of human AML.

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Expression of connexins in human minor salivary glands

Stefania Moscato[1], Alessandra Falleni[1], Enza Polizzi[1], Giovanni Fulvio[1], Manuela Cabiati[2], Silvia Del Ry[2], Chiara Baldini[1] and Letizia Mattii[1].

[1] Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy
[2] Biochemistry and Molecular Biology Laboratory, Institute of Clinical Physiology, CNR, Pisa, Italy

Introduction: Connexins (Cxs) are transmembrane proteins involved in the formation of connexons/hemichannels. Two connexons of adjacent cells can dock one each other forming an intercellular channel at gap junction (GJ), by which ions and small metabolites can pass between cells. Single hemichannels participate in the exchange of small metabolites between cells and the extracellular space [1].

There are at least 21 isoforms of Cxs expressed in humans and they are localized in almost every tissue. More than one Cx can be expressed in each tissue and connexons can be made by a single Cx or by different types of Cxs.

GJ are involved in various physiological functions including secretion in glandular tissue [2-5]. Cx26, Cx32 and Cx43 are the mainly expressed Cxs in glands, but no data are available about their expression in human salivary glands.

Aim: The aim of our study is to investigate the presence and the localization of these Cxs in human minor salivary glands.

Methods: human minor salivary gland biopsies (hMSGBs) were obtained as part of the routine diagnostic procedures when primary Sjögren's Syndrome (pSS) was suspected. Samples without pSS were selected for the study. Immunofluorescence and immunoelectron microscopy were employed to evaluated Cx26, Cx32 and Cx43 protein expression while RT-PCR was used to detect their mRNA expression.

Results and Conclusions: Cx expression was found at both protein and mRNA level in all hMSGBs analysed. Cxs were observed at level of the duct and acinar cell membranes, as well as in myoepithelial cells. The localization of the three Cx types was very similar, suggesting a colocalization of these Cxs in the same connexons. Further studies are necessary to confirm this hypothesis. However, these preliminary results are important because demonstrate for the first time the presence of Cxs in human salivary glands. Moreover, in the few analysed pSS samples we observed an alteration of Cx expression indicating that these proteins may be involved in salivary gland dysfunctions.

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Microvascular alterations in idiopathic forms of Chronic Intestinal Pseudo- Obstruction: a morphometric and molecular analysis

Irene Neri[1], Elisa Boschetti[1], Leonardo Caporali[2], Roberto D'Angelo[3], Rita Rinaldi[3], Valerio Carelli[1,2], Roberto De Giorgio[4], Stefano Ratti[1], Lucio I. M. Cocco[1]and Lucia Manzoli[1]

[1] Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy
[2]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
[3]IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy
[4]Department of Translational Medicine, University of Ferrara, Ferrara, Italy

Introduction: Chronic intestinal pseudo-obstruction (CIPO) is a rare syndrome characterized by severe gastrointestinal (GI) dysmotility that mimics a GI obstruction in the absence of any mechanical cause CIPO is due to muscular-neuronal-glial-interstitial cells of Cajal abnormalities[1] and can be idiopathic or secondary to a genetic cause Recently we documented that changes in the microvasculature supplying the gut contribute to GI abnormalities in a genetically driven form of CIPO[2]

Aim: The object of this study was to investigate if jejunal microvasculature alterations may occur and contribute to the disease also in idiopathic forms of CIPO.

Methods: Full thickness jejunal biopsies were collected from n=23 CIPO and n=10 controls. Formalin fixed-paraffin embedded tissue sections were 1) stained with orcein to identify, measure and count blood vessels, that were subdivided in >300μm (large), 300-101μm (medium), 100-51μm (small) and <50μm (very small), 2) stained with Sirius Red/Fast Green- collagen-assay to measure longitudinal muscle layer thickness and to spectrophotometrically determine fibrosis. Frozen tissue was used to assess by western blot Thymidine Phosphorylase (TP) protein expression, as this enzyme is involved in physio-pathological angiogenesis and was found absent in the previously documented form of CIPO secondary to a genetic disorder[2]; and the expression of HIF-1α as marker of hypoxia.

Results: CIPO patients showed a lower vascular area with more vessels/mm^2 submucosa vs. controls. Vessels <50 μm increased at the expense of medium and large vessels. As compared to controls, CIPO showed, a higher fibrosis index, hypoxia, a decreased thickness of the longitudinal muscle layer and a decreased number of myenteric neurons. Finally, TP enzyme expression was found decreased in the jejunum of CIPO patients.

Conclusion: Our results indicate that the jejunum of CIPO patients is characterized by quantitative and qualitative vascular changes, muscular and neuronal atrophy, fibrosis and hypoxia. Thus, this study allows us to hypothesize that abnormal GI vascularization might be a possible mechanism underlying gut dysfunction in CIPO.

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Hepatic stem/progenitor cell activation and ductular-canalicular junctions in primary biliary cholangitis

Diletta Overi[1], Antonio Franchitto[1], Vincenzo Cardinale[2], Marco Carbone[3], Domenico Alvaro[4], Pietro Invernizzi[3], Guido Carpino[5]

[1] Sapienza Università di Roma, Dipartimento di Scienze Anatomiche, Istologiche, Medico-Legali e dell'Apparato Locomotore, Roma, Italia
[2] Sapienza Università di Roma, Dipartimento di Scienze e biotecnologie medico-chirurgiche, Latina, Italia
[3] Università degli Studi di Milano-Bicocca, Dipartimento di Medicina e Chirurgia, Monza, Italia
[4] Sapienza Università di Roma, Dipartimento di Medicina Traslazionale e di Precisione, Roma, Italia
[5] Università di Roma "Foro Italico", Dipartimento di Scienze Motorie, Umane e della Salute, , Roma, Italia

Hepatic stem/progenitor cells (HpSCs) are facultative bipotential stem cells, located in the canals of Hering and surrounded by a specialized niche [1]. Primary biliary cholangitis (PBC) is a chronic cholangiopathy characterized by immuno-mediated injury of interlobular bile ducts leading to intrahepatic cholestasis and progressive liver fibrosis [2]. The aim of the present study was to investigate the pathogenetic relevance of HpSC activation in PBC.

Liver biopsies were collected from PBC patients (N=87). Clinical-serological parameters were obtained at diagnosis. Histological staging was performed on all slides according to multiple scoring systems and criteria for PBC. HpSC niche was studied by immunohistochemistry and immunofluorescence. Liver samples were obtained from Mdr2-/- mice. Samples were processed for histology, immunohistochemistry, and immunofluorescence.

HpSC activation in PBC patients correlated with the disease stage and with liver fibrosis, but not with disease activity; an extensive HpSC activation correlated with patients' estimated survival, independently from other histological parameters, including disease stage. In PBC patients, HpSC activation was correlated with fibrosis and fibrogenetic cell activation. HpSC phenotype showed low or negligible expression of mature cholangiocyte markers; instead, HpSCs formed reactive bile ductules which were associated with the establishment of junctions with bile canalicular system. Consistently, in a mouse model of intrahepatic cholestasis, HpSC activation was associated with fibrosis and disarrangement of ductular-canalicular junctions.

In conclusion, extensive HpSC activation outlines a severe histologic phenotype associated to progressive fibrogenesis and linked to ductular-canalicular junction establishment. HpSC activation represents a histological hallmark which identifies PBC patients with a worse clinical outcome.

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Involvement of TAF, TDF and INSTIs on adipocyte differentiation

Angelica Perna[1], Carmine Sellitto[2], Eleonora Hay[2], Paolo De Blasiis[2], Vincenzo Esposito[3], Alfonso Baldi[4], Angela Lucariello[5], Antonio De Luca[2], Germano Guerra[1]

[1] Department of Medicine and Health Sciences "Vincenzo Tiberio", University of Molise, Campobasso, Italy
[2] Department of Mental and Physical Health and Preventive Medicine, Section of Human Anatomy, University of Campania "Luigi Vanvitelli", Naples, Italy
[3] Infectious Diseases and Gender Medicine Unit "D Cotugno" Hospital – AO dei Colli, Naples Italy
[4] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Università degli Studi della Campania "L Vanvitelli", Caserta, Italy
[5] Department of Sport Sciences and Wellness, University of Naples "Parthenope", Naples, Italy

The drugs available today for the treatment of HIV patients successfully block the replication of the virus to such an extent that its presence in the blood is undetectable and life expectancy very close to that of a healthy person. Widely used is the integrase strand transfer inhibitor (INSTI) class of antiretroviral drugs, characterised by a good tolerability profile and a relatively high genetic barrier to HIV drug resistance. However, an increase in patient weight is observed with treatment, compared to those on other regimens. In combination with INSTIs, reverse transcriptase inhibitor nucleotide analogues (NRTIs) are often used, which may or may not amplify this contraindication; their effect on the adipogenesis pathway is not well understood.

In this study, using the 3T3-L1 cell line as an in vitro model of adipogenesis, we evaluated the effects of the new NRTI, tenofovir alafenamide fumarate (TAF), and the previously used tenofovir disoproxil fumarate (TDF), both in single and in combination with the four INSTIs, raltegravir (RAL), elvitegravir (EVG), dolutegravir (DTG) and bictegravir (BIC) on adipose differentiation. Using the western blotting technique, the expression levels of PPARγ and C/EBPα, markers of adipogenesis, and the levels of intracellular lipid droplet deposition were assessed by means of Red Oil staining. RAL, EVG, DTG and BIC, as in the control curve, are able to induce differentiation into adipocytes, and in particular RAL and ELV appear to be more inductive.

Single TAF, on the other hand, would appear to inhibit slightly differentiation. This inhibiting effect of TAF would also seem to persist in combinations with INSTIs, where a reduction in markers is observed, particularly in combinations with DTG and BIC. TDF, used alone, also showed an inhibiting activity on adipogenesis, and a reduction in the metabolic activity of the cells.

TDF, on the other hand, in combination with the other INSTI molecules, showed an increase in adipocyte differentiation, particularly in combination with DTG and RAL.

A morphological change in the cells was also observed at different treatment points. Therefore, assuming that the cells could take on a fibroblastic phenotype, through the technique of immunohistochemistry, we evaluated the expression of TR7, a specific marker, detecting low expression in control cells, as in those treated with TAF, TDF and RAL; in BIC-treated cells, however, a fair level was observed, while ELV- and DTG-treated cells showed high expression of TR7. The interaction between INSTI and TAF leads to an antagonistic effect on adipocyte differentiation, whereas the interaction between INSTI and TDF appears to induce increased differentiation, perhaps even anticipating it, particularly in combinations with DTG and RAL.

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Patient-derived colorectal cancer multicellular tumor spheroids and their mouse xenograft show different exosome secretion

Michela Relucenti[1], Orlando Donfrancesco[1], Federica Francescangeli[2], Maria Laura De Angelis[2], Xiaobo Li[3], Zeuner Ann[2], Giuseppe Familiari[1]

[1] Department of Anatomical, Histological, Forensic Medicine and Orthopedic Sciences, Section of Human Anatomy, Sapienza University of Rome, Rome, Italy
[2] Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy
[3] School of Public Health, Capital Medical University, Beijing, PR China

Background and aim: Colorectal Cancer (CRC) represents 10% of the global cancer incidence CRC is the third leading cause of cancer-related deaths in both genders worldwide. CRC survival rate is related to the time of diagnosis, when the patients show the symptoms of CRC, such as rectal bleeding, anemia, or abdominal pain, they are in advanced CRC stages when the cancer is aggressive, malignant, and metastatic.

To reduce mortality in CRC the development of personalized therapies to treat patients in the more advanced stages of the CRC is required. To achieve this goal a simultaneous development of up-to-date in vitro and in vivo preclinical models, that express the patient-specific cancer lineage and genetic diversity has to be carried on. In the last years are emerging new preclinical models of cancer, such as the three-dimensional patient-derived tumor organoids (PDTOs) and the multicellular tumor spheroids (MTS), and the role of exosomes as specific biomarkers in CRC prediction and screening is growing in importance. In our study we focused on the exosome secretion pattern in patient-derived MTS and in mouse xenografts, to find possible differences between "in vivo" and "ex vivo" models.

Methods: MTS originated from CRC of a 63 year old man were then transplanted in immunodeficient mice. Both MTS and xenograft biopsies were analyzed by scanning and transmission electron microscopy. Exosome and multivesicular body size and secretion patterns were compared and statistically analyzed.

Results: Exosome secretion pattern in MTS and xenograft is different, in MTS exosome secretion increases as spheroid complexity does and is generally less intense than what is observed in xenograft. The size of exosomes is about 70 nm in both samples as well as for multivesicular bodies (about 240 nm).

Conclusions: MTS can be considered a useful 3D preclinical model, they reproduce several aspects of CRC biology. MTS-derived xenografts appear as a further improvement of the cancer behavior model, they show a well-sustained exosome production/secretion, which could be possibly due to the influence of factors present in the microenvironment of the in vivo model.

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Sorcin promotes cell proliferation, migration and invasion in cancer by regulating the EGF-dependent EGFR signaling pathways

Claudia Tito[1], Ilaria Genovese[2], Flavia Giamogante[3], Selenia Miglietta[4], Loredana Cristiano[5], Silvia Masciarelli[1], Stefania Annarita Nottola[4], Giuseppe Familiari[4], Vincenzo Petrozza[6], Mattia Lauriola[7], Luca Tamagnone[8], Tito Calì[3], Gianni Colotti[2], Francesco Fazi[1]

[1]Department of Anatomical, Histological, Forensic & Orthopaedic Sciences, Section of Histology and Medical Embryology, Sapienza University of Rome, Rome, Italy
[2]Institute of Molecular Biology and Pathology, Italian National Research Council, IBPM-CNR, Rome, Italy
[3]Department of Biomedical Sciences, University of Padova, Padova, Italy
[4]Department of Anatomical, Histological, Forensic & Orthopaedic Sciences, Section of Human Anatomy, Sapienza University of Rome, Rome, Italy
[5]Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy
[6]Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy
[7]Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy
[8]Department of Life Science and Public Health, Histology and Embryology Unit, Catholic University of the Sacred Hearth, Rome, Italy

The epidermal growth factor receptor (EGFR) is one of the main tumor drivers, and is an important therapeutic target for many cancers[1]. Calcium is important in EGFR internalization and in EGFR signaling pathways[2]. Sorcin is one of the most important calcium sensor proteins, overexpressed in many tumors, that promotes cell proliferation, migration, invasion, epithelial-to-mesenchymal transition, malignant progression and resistance to chemotherapeutic drugs[3]. The present work elucidates an important mechanism that links calcium homeostasis to EGFR signaling in cancer. Sorcin and EGFR overexpression are significantly correlated in cancer patients. Sorcin directly binds EGFR in a calcium-dependent fashion and regulates calcium (dys)homeostasis linked to EGF-dependent EGFR signaling. Sorcin controls EGFR signaling, increases its recycling, activates the PI3K/AKT signaling cascade, and controls the RAS/ERK cascade, participating in the regulation of cellular proliferation, migration and invasion. Sorcin expression leads to increased cell migration, invasion and EMT, via PI3K/AKT signaling; Sorcin silencing reverses these cancer features, synergistically with EGFR inhibitors.

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Can secretome by human mesenchymal stromal cells mitigate age-related ovarian dysfunctions?

Maria Assunta Ucci[1], Serena Marcozzi[1], Giulia Salvatore[1], Antonio Libonati[2], Cosimo Tudisco[2], Vincenzo Campanella[2], Francesca G. Klinger[3], Massimo De Felici[1] and Antonella Camaioni[1]

[1] Department of Biomedicine and Prevention - University of Rome Tor Vergata, Rome, Italy
[2] Department of Clinical Sciences and Translational Medicine - University of Rome Tor Vergata, Rome, Italy
[3] Saint Camillus International, University of Health Sciences, Rome, Italy

The continued lengthening of female life expectancy has resulted in a marked increase in the number of years beyond menopause and women today can expect to live a third of their life in a potential hormone-deficient state. Natural ovarian aging is characterized by a gradual decrease in the quantity and quality of oocytes of the primordial follicular reserve, which leads to the loss of fertility [1]. It has also been proposed that inflammatory processes and fibrosis contribute to ovarian aging [1,2]. Hormone replacement therapy has been used to treat menopause side effects, but it might increase the risk of cancer insurgence or recurrence in survivors. Therefore, it is urgent to identify alternative and effective intervention to counteract ovarian aging. Recently, our [3] and other groups [4] have proven that transplantation of mesenchymal stromal cells (MSCs) is a promising strategy for the treatment of premature ovarian failure and female infertility. Growing evidence indicates that MSCs are able to restore tissue or organ functions mainly by secreting a variety of compounds named "secretome". Studies of stem cell-derived secretome have identified various growth factors, cytokines, chemokines as well as microvesicles and exosomes in the MSC conditioned medium (MSC-CM) [5].

The main purpose of our study is to determine whether CM from two different sources, human dental pulp (DPSCs) and adipose tissue (ASCs), normally discarded material, has the ability to recover age-related ovarian dysfunctions in a mouse model.

DPSCs and ASCs obtained and grown in culture following standard protocols, were first characterized by analysing their proliferation rate, the capacity to differentiate toward osteogenic, chondrogenic and adipogenic lineages and the expression of typical MSC markers by flow cytometer. To produce MSC-CM, cells were allowed to reach 80% confluency, and the medium was replaced by serum-free medium. After 3 days of culture, the CM was harvested and centrifuged to remove cell debris, filtered and stored at -80 °C. Our protocol consisted in intravenous administration of 5mL/Kg of CM or saline, in 8-months-old 129/Sv female every other day for three times. One month after treatment, some females from each group were sacrificed to perform morphological and molecular analyses while the others were monitored to follow their reproductive output. Count of follicle classes in histological sections of the ovaries did not reveal significant differences within the experimental groups, while preliminary results on the pregnancy rate showed an increase in both groups compared to controls (CTRL = 8,33%±5,27; DPSC-CM = 25,00%±9,13; ASC-CM = 60,67%±10,27). Moreover, the analysis of oestrous cycle evaluated by vaginal smears showed that both treatments improved cyclicity by significantly decreasing the duration of the diestrus of ~35%.

These data suggest that MSC-CM injection might mitigate the ovarian aging mainly by improving the quality of oocytes related to their developmental competence and, possibly, the functional status of the reproductive system.

In line with [2], we found higher levels of transcripts of the pro-inflammatory genes *TNFα*, *IL6* and *IL1β* in the ovaries of old (8-months) in comparison to young (2-months) females and the presence of unique multi-nucleated macrophage giant cells in the aged ovaries. Experiments are in progress to evaluate whether the CM effects reported above can be attributed to a reduced inflammatory status at both local (ovary) and systemic levels.

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Resident self-tissue of pro-inflammatory cytokines rather than their systemic levels correlates with development of myelofibrosis in *Gata1*^{low} mice

Maria Zingariello[1], Paola Verachi[2], Francesca Gobbo[2,3], Fabrizio Martelli[4], Mario Falchi[5], Maria Mazzarini[2], Mauro Valeri[6], Giuseppe Sarli[2], Christian Marinaccio[7], Johanna Melo-Cardenas[8], John D. Crispino[8] and Anna Rita Migliaccio[1,9]

[1] Department of Medicine, Campus Biomedico, Rome, Italy
[2] Department of Biomedical and Neuromotorial Sciences, Alma Mater University, Bologna, Italy
[3] Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy
[4] National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy
[5] National Center HIV/AIDS Research, Istituto Superiore di Sanità, Rome, Italy
[6] Center for animal experimentation and well-being, Istituto Superiore di Sanità, Rome, Italy
[7] Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
[8] Department of Hematology, St Jude Children's Research Hospital, Memphis, TN, USA
[9] Altius Institute for Biomedical Sciences, Seattle, WA, USA

Primary myelofibrosis (PMF) is a Philadelphia-chromosome negative myeloproliferative neoplasm with adverse prognosis and is associated with bone marrow fibrosis and extramedullary hematopoiesis. The disease is sustained by driver mutations in the thrombopoietin (TPO) axis. Serum levels of inflammatory cytokines (such as a lipocalin-2, LCN2, IL-8 and TGF-β1, are currently investigated as prognosis markers in myelofibrosis. To clarify the relevance of systemic cytokine levels with respect to microenvironment bioavailability in disease progression, we performed an extensive analysis of the cytokine profile of Gata1^{low} mice, a strain that harbor a hypomorphic mutation that induce megakaryocyte abnormalities similar to those found in PMF patients and develop with age a phenotype that recapitulate PMF. Mice were evaluated before and after disease onset and data were compared to those observed in age matched wild-type littermates. Cytokine content of the serum was evaluated by Luminex-bead-assay and ELISA while cytokine levels in the bone marrow (BM) were detected by immunohistochemistry. We also determined the localization of GFP-tagged hematopoietic stem cells (HSC) within the bone marrow architecture with respect to those of relevant stromal cells by confocal microscopy. Differences in serum levels of 32 inflammatory-cytokines, including LCN2, CXCL1, the murine equivalent of human IL-8, and TGF-β1 between pre-fibrotic and fibrotic *Gata1*^{low} mice and their wild-type littermates were modest. By contrast, BM from fibrotic *Gata1*^{low} mice contained higher levels of LCN-2, CXCL1, and TGF-β1 than wild-type BM. The increased bioavailability of proinflammatory cytokines was mostly due to increased expressed by the malignant megakaryocytes. Accordingly, in Gata1^{low} mice, HSC are localized in the femur diaphysis in areas surrounded by micro-vessels, neo-bones, and megakaryocytes, while wild-type HSC are localized in the femur epiphysis around adipocytes. In conclusions, BM bioavailability of inflammatory cytokines, rather than blood levels, possibly by reshaping the HSC-supporting niches, correlates with myelofibrosis in *Gata1*^{low} mice.

Stili di vita e prevenzione: scienze del movimento, della nutrizione e del benessere

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A functional approach for fitness to prevent injuries in daily life: a preliminary investigation

Gianluca Azzali[1], Gabriella Cusella[1], Gabriele Ceccarelli[1]

[1] Department of Public Health, Experimental Medicine and Forensic, Human Anatomy Unit, University of Pavia, Via Forlanini 8, 27100 Pavia, Italy

Too often fitness for adults is based on exercises from bodybuilding, so the real goals of physical preparation, defined from ACSM as performing ADLs with stamina and preventing injuries (1), are lost. Unfortunately, the literature is poor in studies about using a strength and coordination integrated approach for this argument. Therefore, as an exploratory case study, we recruited 3 active adults to evaluate parameters of flexibility of upper (Shoulder Mobility Test of Gray Cook's FMS) and lower limbs (Sit and Reach Test), musculoskeletal pain (modified Nordic Musculoskeletal Questionnaire) and strength (3RM Hexagonal Bar Deadlift Test) at the beginning, at half and at the end of an 18 weeks program, consisting of 2 sessions of neuromotor strength and coordination and 2 of endurance. We recorded meaning improvements among initial test and final test in Sit and Reach test of 0,06 ± 0,01 m, which represents 122,5%. Difference between right and left side on Shoulder Mobility Test recorded a meaning improvement of 0,01±0,02 m, 30,7%. We reported an improvement in 3RM test between initial and final tests of 58,3%. Results of modified NMQ reported a reduction of pain to the 26% of initial meaning score. Two participants suffered respectively a Chronic Neck Pain and a Tendinosis of Achilles Tendon, inserting specific exercises from literature, has been recorded a reduction of Chronic Neck Pain from 7/10 to 1/10 and about tendinosis from 6/10 to 2/10.

Hence, we demonstrated the effectiveness of the proposed programming in relation to the improvement of the parameters of mobility, perceived pain and strength. In addition, we have also achieved the secondary objective, which is to propose a sequence of rapid, low-cost and easily repeatable evaluation tests to assess the health of patients and also determine their critical issues and risks of injury and work on them.

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Does exist a microbiota healthy "organ"?

Battistelli Michela[1], Sisti Davide[1], Amatori Stefano[1] , Pazienza Valerio[2], Citterio Barbara[1], Salucci Sara[3], Trucas Marcello[4], Sestili Piero[1], Piccini Fabio [5], Rocchi Marco BL[1]

[1] Department of Biomolecular Sciences, University of Urbino Carlo Bo, 61029 Urbino, Italy
[2] Division of Gastroenterology, IRCCS "Casa Sollievo della Sofferenza" Hospital, 71013 San Giovanni Rotondo, Italy
[3] Cellular Signalling Laboratory, Department of Biomedical and NeuroMotor Sciences (DIBINEM), University of Bologna, 40126 Bologna, Italy
[4] Department of Biomedical Sciences, Section of Cytomorphology, University of Cagliari, Italy
[5] Progetto Microbioma Italiano – IMP

Introduction: Several factors, from genetic background, age, lifestyle, dietary habits to local environments, contribute to microbiota heterogeneity in humans. A major challenge in microbiome research is understanding the variability of the "healthy microbiome". (1) Recent studies underlined the emerging differences in microbiota composition even in population cohorts with a similar genetic and cultural background. Scientists are focusing their attention on how to manipulate microbiota to improve health status; however, the scientific community also agrees on studying the factors constituting the normal ranges of these features in healthy populations as a first step. Previously, some papers have been published about microbiota composition of Italians in specific diseases, or as a changing ecosystem, just a few data were reported about microbiota of healthy population.

This study aimed to define the reference intervals of the gut microbiota of a healthy Italian people sample with relatively homogeneous physiological features. (2)

Sample of 148 healthy control subjects of seventeen Italian regions, with a gender ratio of 69 male / 79 female and an age ranging from 23 to 57 years, were collected and analysed. A questionnaire has been administered to each participant to collect information regarding the demographic and anthropometric variables, dietary habits and physical activity.

The analysis performed using Elbow and Silhouette methods showed that the best clustering solution was the one with two clusters of subjects; both methods showed the same results. The two different clusters are clearly defined; Cluster 1 (C_1) assembles 108 subjects, while Cluster 2 (C_2) includes 40 subjects.

Our study may contribute to primarily identify the existence, within the healthy Italian population, of a commonly distributed, constantly present, main microbiological pattern, thus suggesting the presence of a short of a microbiological scaffold. Taken together, our data highlights the significance of studies on population-specific variations in human microbiota composition. Nevertheless, at the same time, it can be highlighted here how the variability studies must be able to take into account even minimal variations in the intestinal microbiological population, given that, at least in the healthy population, there is a significant and reproducible presence of well-defined groups of bacteria.

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Comparative effect of Atenolol and Nebivolol on reverse remodeling of intramural coronary arterioles in the spontaneously hypertensive rat model

Francesca Bonomini[1,2,3], Lorena Giugno[1], Claudio Lonati[1,3], Carolina De Ciuceis[4], Claudia Rossini[4], Claudia Agabiti Rosei[4], Damiano Rizzoni[4], Elisa Borsani[1,2,3]

[1] Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia, 25123 Brescia, Italy
[2] Interdipartimental University Center of Research "Adaption and Regeneration of Tissues and Organs-(ARTO)", University of Brescia, Brescia, Italy
[3] Italian Society for the Study of Orofacial Pain (Societa` Italiana Studio Dolore Orofacciale – SISDO), Brescia, Italy
[4] Department of Clinical and Experimental Sciences, Clinica Medica, University of Brescia, Brescia, Italy

Arterial hypertension is an established major risk factor for ischemic heart disease. Accordingly, studies in hypertensive patients with or without left ventricular hypertrophy have shown that coronary flow reserve is often reduced at angiography [1]. The latter findings can be explained, at least in part, by functional and structural abnormalities at the level of the microcirculation that are believed to be the substrate of coronary microvascular dysfunction [2]. The aim of this study was to assess the effect on coronary microvascular remodeling in Spontaneously Hypertensive Rat (SHR) model of two drugs used for the treatment of hypertension and coronary artery disease: the beta-blocker Atenolol and the selective third-generation β1- adrenergic antagonist Nebivolol. Unlike traditional beta-blocker, Nebivolol has been demonstrated to possess direct vasodilator and antioxidant properties which may improve endothelial dysfunction and reduce smooth muscle cell proliferation [3].

For this aim we used three groups of SHR rats: 1) Hypertensive group (SHR rats without treatment); 2) SHR plus Atenolol (50 mg/kg/day by oral gavaging for 8 weeks) 3) SHR plus Nebivolol (20 mg/kg/day by oral gavaging for 8 weeks). Morphological analysis and protein expression levels of vasoactive factors, oxidative stress and remodeling markers in coronary microcirculation were determined by immunohistochemical analysis of Endotelin-1 (ET-1), inducible nitric oxide synthase (iNOS) and transforming growth factor-b1 (TGF-b1).

We found that, in comparison to Atenolol treated rats, animals treated with Nebivolol showed significant improvement in morphology of the coronary microcirculation; moreover, Nebivolol showed greater activity in the modulation of protein involved in vasodilation and oxidative stress protection which may provide additional benefit beyond blood pressure reduction and beta-receptor blockade.

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Increased dietary choline alters the plasma metabolome and worsens atherosclerosis development without impacting the immune system

Marco Busnelli[1], Elsa Franchi[1], Alice Colombo[1], Stefano Manzini[1], Mariel Anel García-Rivera[2], Jennifer Kirwan[2], Xufei Zhang[3], Philippe Gérard[3], Eugenio Scanziani[4], Giulia Chiesa[1]

[1] Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milano, Italy
[2] Micalis Institute, INRAE, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
[3] Berlin Institute of Health, Core Facility Metabolomics, Berlin, Germany
[4] Mouse and Animal Pathology Laboratory (MAPLab), Fondazione UniMi, Milan, Italy

Gut microbiota can influence atherosclerosis development by metabolizing dietary choline: experimental and observational studies have highlighted a positive correlation between increased plasma choline-derived TMAO concentrations and adverse cardiovascular events. The study was aimed at investigating whether the levels of other metabolites besides TMAO were affected by dietary choline intake. In addition, it was evaluated for the first time whether choline/TMAO had an impact on immune system activation.

Female EKO mice were fed two low-fat, no cholesterol diets differing for a low (0.09%) or a high (1.2%) choline content for 16 weeks. Atherosclerosis development was quantified at the aortic sinus and targeted plasma metabolomics was performed. The activation status of the immune system was assessed by FACS analysis in blood, spleen, cardiac lymph node, mesenteric lymph node, and Peyer's patches. In addition, inflammatory infiltrates present in atherosclerotic lesions and in a large panel of organs including liver, kidney, lung, adrenal, duodenum, jejunum, ileum, and large intestine were evaluated by histology.

High-choline intake increased plasma TMAO concentration and worsened atherosclerosis development. Plasma choline concentration was not affected by diet. High-choline feeding was associated with lower plasma levels of the pro-atherogenic metabolite homocysteine and a concomitant increase of its by-products methionine and sarcosine. Hexoses and carnitine were also increased by high-choline diet.

Increased choline intake had no impact on the activation of the immune response, either innate or adaptive. All organs considered showed no alterations due to increased leukocyte infiltrate.

Taken together, our data indicate that a boost of dietary choline leads to an altered plasma metabolome associated to atherosclerosis worsening. Dietary choline content does not affect the immune-inflammatory status either in the vasculature or systemically.

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Body composition affects vestibular and proprioceptive balance components in young adults: a cross-sectional study

Andrea Ciorciari[1], Antonino Mulè[1], Letizia Galasso[1], Lucia Castelli[1], Eliana Roveda[1,2], Angela Montaruli[1,2]

[1] Department of Biomedical Sciences for Health, University of Milan, Milan, Italy
[2] IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

Balance is a complex motor skill, composed of three integrated components: visual, vestibular and proprioceptive. They cooperate in order to allow the subject to maintain a stable posture and to be able to respond promptly to destabilizing stimuli. The literature is still uncertain in establishing whether body composition, in terms of percentage of lean mass (% LM) and fat mass (% FM) can influence this capacity. Furthermore, studies that show an effective correlation between these two factors have never investigated whether all components of balance are affected or only some of them(1). The goal of this study was to assess whether body composition and balance, or at least some of its components, are related. 32 young adults (23 females, 9 males; age = 21.9 ± 3.6; BMI = 22.83 ± 2.86) were recruited. Following a body composition analysis performed by plicometry (7-folds method (2)), the participants underwent a test for the assessment of balance (m-CTSIB, Modified Clinical Test of Sensory Interaction in Balance (3)).

This test can discriminate the three components of this ability, through 4 different subtests: 1. eyes open on a rigid surface; 2. eyes closed on a rigid surface; 3. eyes open on a soft surface; 4. eyes closed on a soft surface. An oscillation index was then derived in each of the tests. Linear correlation analyzes were performed. As expected, statistical significance emerged in the correlation between the general oscillation index, deriving from the average of the 4 tests described above, and % FM and % LM ($p < 0.05$; $r = 0.54$). However, by examining which components were actually correlated, only the vestibular ($p < 0.05$; $r = 0.41$) and proprioceptive ($p < 0.05$; $r = 0.53$) were statistically significant, while in the visual component no correlation was highlighted. Although this study presents some limiting factors, such as the distribution in terms of sex of the sample and the analysis of the body composition carried out by plicometry, it allows to analyze more in depth the relationship between body composition and balance, highlighting the critical components to act on in order to prevent possible adverse events.

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Melatonin supplementation in Autistic BTBR T+Itpr3tf/J Mice: goblet cells and sodium glucose transporter as new targets in autism spectrum disorder

Caterina Franco[1], Riccardo Guarneri[1], Francesca Bonomini[1,2,3], Rita Rezzani[1,2,3]

[1]Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia, 25123 Brescia, Italy
[2] Interdipartimental University Center of Research "Adaption and Regeneration of Tissues and Organs-(ARTO)", University of Brescia, 25123 Brescia, Italy
[3] Italian Society of Orofacial Pain (SISDO), 25123 Brescia, Italy

Autism spectrum disorder (ASD) identifies a neurodevelopmental syndrome characterized by a complex etiology. It primarily affects the brain, but actually 5 to 80 % of the autistic patients suffer from gastrointestinal (GI) symptoms and the existence of a link between GI diseases and ASD has already been demonstrated. In this sense, fundamental are the morphological alterations that affect the GI tract of autistic patients and the imbalance between the physiological condition and the inflammatory/stressed one [1]. Moreover, also the goblet cell's (GC) content and the expression of sodium glucose transporter (Sglt-1 and -3) undergo changes in mouse model of autism [2]. Starting from this background, the aim of this work was to evaluate the role of melatonin's (MT) supplementation in the gut of BTBR mice. These mice are considered a good ASD-like model because they present behavioral and psychological alterations like those observed in patients with ASD. On the other side these mice showed also the typical autistic metabolic pattern, showing insulin resistance, diabetes- induced nephropathy and phenylketonuria [3].

Considering the important role of MT as antioxidant and considering the role that GC and Sglt-1 and -3 have in ASD pathogenesis, we want to understand whether restoring the oxidative balance in the autistic patient can also have an impact on the gut's morphology and functionality [4].

Gut morphology and GC's content has been evaluated by histological staining, inflammation processes have been expressed considering some markers, such as SOD and CAT and Sglt-1 and -3 expression has been studied by immunohistochemical and quantitative analyses.

Our data showed that: 1) GI tract from autistic mice presented an alteration in GC's content and in the number of inflammatory cells; 2) the morphological changes are linked to functional alterations, represented by the different Sglt's expression; 3) MT supplementation could work on the stressed background restoring an oxidative balance and improving the clinical GI manifestations.

Despite these starting results, further experimental researches are needed to better evaluate the molecular mechanisms involved in GI diseases in order to protect the gut morphology and function reducing the number and severity of comorbidities that autistic patients often present.

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Effectiveness of an algorithm-based rehabilitation program for practical management and therapy of complex regional pain syndrome type 1 of the hand

Ghelarducci G[1], Ryskalin L[1,2], Marinelli C[1], Annoscia P[3], Bertozzi N[4], Gesi M[1,2]

[1] Center for Rehabilitative Medicine "Sport and Anatomy", University of Pisa, Italy
[2] Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy
[3] Unit of Hand Surgery, AOUP, Pisa, Italy
[4] Breast Surgery Unit, Morgagni-Pierantoni Hospital, Ausl Romagna, Forlì, Italy

Background: Complex regional pain syndrome (CRPS) type 1 is a rare but disabling pain condition, usually involving distal extremities such as the wrist, hand, ankle, and foot due to either direct or indirect traumas [1,2]. CRPS type 1 is characterized by a complex set of symptoms where no correlation can be identified between the severity of the initial injury and the ensuing painful syndrome [3]. Over the years, numerous treatment strategies have been proposed for CRPS management, but therapies still remain controversial. Indeed, at present, there is no successful therapeutic intervention exists for this condition.

Questions/purposes: The aim of the present study is to propose and to assess the effectiveness of a rehabilitative treatment algorithm for CRPS, which is actually in use at the Center for Rehabilitative Medicine "Sport and Anatomy" of the University of Pisa.

Methods: We retrospectively reviewed all the patients that underwent physical rehabilitative treatment algorithm for hand CRPS between 2014 and 2021 at our Institution.

Results: All the parameters taken into consideration, namely Purdue Pegboard test (PPT), Disability of the Arm, Shoulder and Hand (DASH), Visual Analog Scale (VAS), as well hand oedema recorded with a hand volumeter, were significantly improved at the end of the rehabilitation protocol.

Conclusion: The results obtained in the present study demonstrated that early and skillful rehabilitation intervention is of paramount importance for CPRS type 1 management to achieve a stable and optimal functional recovery while preventing the onset of deformities. In addition, our rehabilitation protocol was able to achieve substantial improvement in pain and quality of life scores.

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Characterization of metabolic syndrome-related morpho-functional changes in the lung and the role of testosterone treatment in an animal model

Giulia Guarnieri[1], Sandra Filippi[2], Alessandro Pini[1], Paolo Comeglio[3], Ilaria Cellai[3], Mario Maggi[3], Linda Vignozzi[3], Annamaria Morelli[1]

[1] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
[2] Department of Neurosciences, Drug Research and Child Health (NEUROFARBA), University of Florence, Florence, Italy
[3] Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy

Metabolic syndrome (MetS) is a cluster of metabolic and cardiovascular risk factors strictly linked to unhealthy lifestyle, dietary habit and physical inactivity. In male patients, MetS symptoms are often complicated by the onset of hypogonadotropic hypogonadism (HH), with low testosterone (T) and gonadotropin levels. Recent studies have shown the association of lung dysfunction to MetS, but the underlying mechanism remains unclear.

Here, we used a well-established rabbit model of high fat diet (HFD)-induced MetS, which recapitulates human phenotype (including HH), to better understand the pathogenesis of lung dysfunction in MetS. In addition, based on previous studies demonstrating the beneficial effects of T treatment in counteracting some MetS symptoms in both pre-clinical [1] and clinical studies [2], we investigated the potential protective action of T on MetS-induced lung alterations. Rabbits fed a HFD for 12 weeks were treated with T during the last 6 weeks and were compared to both untreated HFD and regular diet (RD) rabbits.

The assessment of lung function was performed by pressure airway opening (PAO) measurements and evidenced that airway resistance to inflation was significantly increased in HFD rabbits compared to RD. Accordingly, morphological and immunohistochemical analyses showed the occurrence of tissue inflammation, as detected by immunostaining for the macrophagic marker RAM11, and the presence of fibrotic processes, particularly at peribronchiolar level in the lung of HFD animals in comparison to RD. Treatment with T significantly improved not only some metabolic parameters, but also the lung ventilation compared to the HFD group, while it showed a tendency to counteract pro-inflammatory macrophage activation and peribronchiolar fibrosis. In addition, gene expression analysis of the main inflammatory and fibrotic markers confirmed a positive effect of T treatment.

In conclusion, our results showed that the HFD-induced MetS model is valid and useful *in vivo* tool for the study of MetS-related lung dysfunctions, adding new insight into the comprehension of the underlying mechanisms responsible for the morpho-functional changes. Moreover, T treatment could be beneficial in patients with MetS, hypogonadism and pulmonary comorbidities.

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Dysregulation of microRNAs and tRNA-Derived ncRNAs in mesothelial and mesothelioma cell lines after asbestiform fiber exposure

Carla Loreto[1], Veronica Filetti[1], Claudia Lombardo[2], Elisabetta Pricoco[3], Sergio Castorina[2]

[1] Human Anatomy and Histology Unit, Department of Biomedical and Biotechnological Sciences, University of Catania, 95123 Catania
[2] Human Anatomy Section, Department "GF Ingrassia", University of Catania, 95123 Catania
[3] Policlinico -San Marco, Anatomia Patologica, 95123 Catania

Experimental evidence demonstrated that fluoro-edenite (FE) can develop chronic respiratory diseases and elicit carcinogenic effects. Environmental exposure to FE fibers is correlated with malignant pleural mesothelioma (MPM), an aggressive and rare malignant neoplasm of the pleural surface. An early diagnosis of MPM, and a comprehensive health monitoring of the patients exposed to FE fibers are two clinical issues that may be solved by the identification of specific biomarkers. We reported the microRNA (miRNA) and transfer RNA-derived non coding RNA (tRNA-derived ncRNA) transcriptome in human normal mesothelial and malignant mesothelioma cell lines exposed or not exposed to several concentration FE fibers. We analysed several pathways that are involved in the pathogenesis of malignant mesothelioma. Furthermore, an interactive mesothelioma-based network was derived by using NetME tool.

In untreated condition, the expression of miRNAs and tRNA-derived ncRNAs in tumor cells was significantly different with respect to non-tumor samples. Moreover, interesting and significant changes were found after the exposure of both cells lines to FE fibers. There was the involvement of pathways that have important functions in inflammatory processes and in angiogenesis. The network-based pathway analysis showed several signalling and metabolic pathways potentially involved in the pathogenesis of MPM. From papers analysed by NetME, it is clear that many miRNAs can positively or negatively influence various pathways involved in MPM.

For the first time, the analysis of tRNA-derived ncRNAs molecules in the context of mesothelioma has been made by using *in vitro* systems. Further studies will be designed to test and validate their diagnostic potential in high-risk individuals' liquid biopsies.

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Baropodometric evaluation of postural stability and plantar pressure parameters in healthy subjects

Angela Lucariello[1], Allegra Fullin[2], Paolo Caravaggi[3], Pietro Picerno[4], Daniele Di Tella[5], Antonio De Luca[2], and Paolo De Blasiis[2]

[1] University of Naples "Parthenope", Department of Sport Sciences and Wellness, Naples, Italy
[2] University of Campania "Luigi Vanvitelli", Department of Mental and Physical Health and Preventive Medicine, Section of Human Anatomy, Functional Anatomy Laboratory, Naples, Italy
[3] Movement Analysis Laboratory, IRCCS Istituto Ortopedico Rizzoli, 40136 Bologna, Italy
[4] SMART Engineering Solutions & Technologies (SMARTEST) Research Center, Università telematica "e Campus", 22060 Novedrate, Italy
[5] Department of Medicine and Health Sciences "Vincenzo Tiberio", University of Molise, Campobasso, Italy

Postural stability and plantar pressure parameters can be assessed by baropodometry; nevertheless, they are often affected by low repeatability. The aim of the study was to test the accuracy and repeatability of a novel resistive sensor pressure plate and to establish the most reliable baropodometric parameters.

Accuracy and repeatability of the FM12050 BTS Bioengineering plate measurements were assessed by using different weights in static conditions across three sessions. Subsequently, 20 healthy subjects were assessed by 30-s stabilometric analysis in bipedal standing with open eyes across four trials in two sessions, morning and afternoon.

Pressure plate repeatability in measuring the static weights was very high, and plate measurements were correlated to the scale measurements (Pearson's coefficient = 0.99). Percentage of load distribution between left and right foot and in rearfoot and forefoot regions showed the largest repeatability (coefficient of variation < 5%) across trials. Eventually, median and percentiles (25–75%) were reported for each parameter.

This study helped to assess the accuracy and repeatability of a novel pressure plate in static conditions and to define the most reliable parameters for the assessment of postural stability and foot morphology. The present healthy-subject stabilometric dataset may be used as reference data in the evaluation of pathological populations.

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"Sport and Anatomy": teaching, research and assistance at the University of Pisa

Natale G[1,2], Ryskalin L[1,3], Morucci G[1,3], Dell'Agli S[1,3], Coluccia N[3], Soldani P[1,3], Gesi M[1,3]

[1] Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy
[2] Museum of Human Anatomy "Filippo Civinini", University of Pisa, Italy
[3] Center for Rehabilitative Medicine "Sport and Anatomy", University of Pisa, Italy

Over the last decades, the university system has experienced huge growth, facing several challenges. Study programs, teaching methods and technologies, learning environment, quality assurance, programmed student numbers, and research results are key features for the prestige of the scientific community. Within the framework of this evolving educational system, academic structures and centers of excellence contributed to the development of these new purposes, while ameliorating the cooperation between universities and local communities. In this respect, athletic training and rehabilitation managed by specialists have assumed an increasingly relevant role.

Concerning the importance of students' learning and research in sports education, the present research aimed to describe the unique experience of "Sport and Anatomy", a brand that includes an academic organization of the University of Pisa, with the aim of becoming a reference point for sports management, while creating indispensable links between basic and specialist sciences through different masters and schools. Besides didactic activity, "Sport and Anatomy" also coordinates additional tasks, encompassing research activity, medical assistance, and rehabilitation.

As an incoming ambitious project, from December 2021, the centre of "Sport and Anatomy" has started the expanse of university structures to organize sports medicine, thus becoming the reference point for the School of Specialization in Sports Medicine of the University of Pisa. Within this frame, further professional figures will be also considered, encompassing neurologists, nutritionists, and cardiologists to expand their specific competences to many other pathologies, including those related to metabolic dysfunctions. Moreover, the centre has recently inaugurated an advanced unit of Biomechanics. This represents a great opportunity for integrated activities, in terms of teaching, research, assistance, and rehabilitation.

Therefore, within the context of sports sciences, this center plays a key role in supervising and accomplishing in an innovative way all the three missions of the university (i.e. teaching, research, and dissemination of knowledge), thus strongly fulfilling the aims of modern university targets.

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Ciliary neurotrophic factor and CNTF receptor α in prostate cancer

Giovanni Tossetta[1], Sonia Fantone[1], Martina Senzacqua[1], Roberta Mazzucchelli[2], Daniela Marzioni[1]

[1] Department of Experimental and Clinical Medicine, Università Politecnica delle Marche, 60126 Ancona, Italy
[2] Department of Biomedical Sciences and Public Health, Section of Pathological Anatomy, Università Politecnica delle Marche, School of Medicine, United Hospitals, Ancona, Italy

Ciliary neurotrophic factor (CNTF) is a pleiotropic cytokine belonging to Interleukin 6 (IL-6) cytokine family that signals through a receptor complex containing a specific subunit, CNTF receptor α (CNTFRα). CNTF receptor complex is a heterodimer including gp130 and CNTFRα proteins triggering the activation of multiple intracellular signaling pathways including AKT/PI3K, MAPK/ERK and Jak/STAT pathways. At present no data are available on the localization and function of CNTF and CNTFRα in prostate cancer. In this study we analysed CNTF and CNTFRα localization by immunohistochemistry and investigated the role of this cytokine using 22Rv1 (castration resistant) and LNCaP (castration sensitive) prostate cancer cell lines. Our results show that both CNTF and CNTFRα are expressed in prostate cancer tissues and in the above prostate cancer cell lines. In both cell lines, treatment with 10 ng/ml human recombinant (hr) CNTF activated JAK2/STAT3 signalling and inhibited both ERK and AKT pathways. Interestingly, hrCNTF treatment did not alter the expression of GLUT1, GLUT4, PCNA, MMP2 and MMP9 in LNCaP cell line but decreased the expression of GLUT1 and MMP2 in 22Rv1 cell line. Furthermore, hrCNTF treatments decreased significantly invasion capability of 22Rv1 cells.

Collectively, these data suggest a key role of CNTF in prostate cancer progression primarily modulating MMP2 and GLUT1 expression.

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The Choko-AGE study: Combining vitamin E-functionalized CHOcolate with physical exercise to reduce the risK Of protein energy malnutrition in pre-dementia AGEd people

Anna Pedrinolla[1], Massimo Venturelli[1], Federico Schena[1], Alessandra Pistilli[2], Francesco Galli[3], Desirée Bartolini[2], Mario Rende[2], Gabriele Di Sante[2] Anna Maria Stabile[2]

[1] Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Verona, Italy
[2] Department of Surgery and Medicine, Institute of Human, Clinical and Forensic Anatomy, University of Perugia
[3] Department of Pharmaceutical Science, University of Perugia

The elders are particularly vulnerable to undernutrition, resulting from defective food intake or uptake (nutrient deficiencies), leading to an altered body composition and weight loss (1). Muscle loss is the main symptom of this condition, sign of protein-energy malnutrition (PEM) and metabolic reprogramming of tissues, involved in aging process. These changes sustain insulin resistance and impaired mitochondrial metabolism of several organs and tissues, including skeletal muscle (2).

The undernutrition correlates with an accelerated and general decline in health conditions (worsening both physical and cognitive/mental aspects), thus increasing the risk of frailty and finally accelerating physical and cognitive decline. Its prevention is an unmet need in health policies.

We hypothesize that the antioxidant and cytoprotective functions of vitamin E combined with the cortisol lowering effect of chocolate polyphenols and physical activity may help to prevent the age-dependent decline of mitochondrial function and nutrient metabolism in skeletal muscle, key events underlying PEM and muscle loss in the elderly. To test this hypothesis, a vitamin E-functionalized dark-chocolate rich in polyphenols will be developed and its effects will be investigated in combination with physical activity in a 6-month randomized case-control trial on pre-dementia elderly patients, a well-defined population of subjects at risk of undernutrition and frailty.

Subjects will be maintained on protein-rich diet (0.9-1.0 g protein/Kg ideal body weight/day) and with an active physical exercise program (HIIT, High Intensity Interval Training specifically developed for these subjects). They will be randomized in 3 groups (n = 75 each): Group A (n=25) is composed by controls maintained with baseline conditions; Group B (n=25) comprised pre-dementia elders with baseline diet and HIIT, either with 30 g/day of dark-chocolate containing 500 mg total polyphenols (corresponding to 60 mg epicatechin) and 100 mg vitamin E (as RRR-alpha-tocopherol); Group C (n=25), similar to Group B but without additional vitamin E . The evaluation of the muscle mass will be the primary endpoint. We will test also neurocognitive status and previously identified biomolecular indices of frailty in pre-dementia patients (secondary endpoints). Muscle biopsies will be collected to assess myocyte contraction and mitochondrial metabolism. Laboratory endpoints will include the nutritional compliance to the proposed intervention (blood polyphenols and vitamin E status and metabolism), 24-h salivary cortisol, steroid hormones and IGF-1, and molecular indices of inflammation, oxidant stress, cell death and autophagy. These parameters will be investigated in muscle and blood cells by omics techniques. Molecular and nutritional findings will also be confirmed in vitro using skeletal myotubes, blood leukocytes and neural cell lines. Clinical and laboratory results will be processed by a dedicated bioinformatics platform to interpret the molecular response to the nutritional intervention and to personalize its application.

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Deep characterization of pro-fibrotic, pro-inflammatory and senescent signaling in the intestinal inflammatory bowel diseases (IBD)

Simona Pompili[1], Antonella Vetuschi[1], Giovanni Latella[2], Roberta Sferra[1], Alfredo Cappariello[1]

[1]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, 67100, Italy
[2]Department of Life, Health and Environmental Sciences, Gastroenterology, Hepatology and Nutrition Division, University of L'Aquila, L'Aquila, 67100, Italy

Inflammatory bowel disease (IBD), including Chron's disease (CD) and ulcerative colitis (UC), constitute a broad spectrum of chronic inflammatory disorders affecting the gastrointestinal tract. A frequent complication is the intestinal fibrosis, an irreversible process characterized by an abnormal deposition of extracellular matrix proteins (ECM), leading inevitably to complications such as obstructions and stenosis, which forces the patient to surgery. To date, no resolutive drugs in the treatment of IBD are available (1). Some molecules capable of modulating TGF-β activity, including peroxisome proliferator-activated receptor (PPAR)-γ, and its agonists (i.e., rosiglitazone), showed a promising anti-fibrotic effect (1). Although the role of TGF-β in the complex field of IBD is pivotal, the involvement of other signaling pathways corroborating the TGF-β could be a valid pharmacological target to counteract or at least mitigate the progression of IBD (2). The epithelial-mesenchymal transition process (EMT), the AGE/RAGE (advanced glycation end products/receptor of AGEs) pathway, as well as an acceleration of cellular senescence may have a concrete pathogenetic implication in IBD (3). This study aimed to evaluate the contribution of those pathways in the etiopathogenesis of IBD. We use a mouse model of colitis induced with dextran sodium sulfate (DSS, 2.5% w/v in drinking water), without or with treatments with GED-0507-34 Levo (GED, PPAR-γ agonist, 30 mg/Kg/day by oral gavage). We found that the pro-fibrotic pathway AGE/RAGE, as well as EMT signaling (E-cadherin, vimentin and β-catenin), resulted activated in DSS-treated mice compared to control, while GED counteracted their activation, confirming the ability of PPAR-γ to mitigate fibrosis. Senescence was assessed by β-gal staining, and expression of SASP members (IL-1 β, MMP1), which resulted increase in DSS-mice respect to control. Surprisingly, GED was also effective in contrasting β-gal expression and senescent degeneration. Finally, to endorse the potential translational value of these results, we evaluated biopsies of IBD patients compared to subjects undergone to follow-up colonoscopy. Evaluations reflected the data obtained from the murine model. Altogether, our results highlight the involvement of AGE/RAGE, EMT, and senescence in the disarrangement of colonic mucosa occurring in IBD, also demonstrating that PPAR-γ could represent a promising target for the IBD treatment due to its ability to ameliorating not only inflammation and fibrosis but also delaying the progression of senescence.

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Newly synthesized selective Kinesin Eg5 inhibitors as an alternative approach to counteract breast cancer progression

Alessia Ricci[1], Marialucia Gallorini[1], Valentina Di Valerio[2], Simone Carradori[1], Amelia Cataldi[1], Susi Zara[1]

[1] Department of Pharmacy, "G d'Annunzio" University of Chieti–Pescara, via dei Vestini 31, 66100 Chieti, Italy
[2] Department of Medicine and Ageing Sciences, "G d'Annunzio" University of Chieti-Pescara, 66100 Chieti, Italy

Breast cancer is one of the most diagnosed cancers in women [1]. Recently, kinesin Eg5 started to be considered a new oncogene in breast cancer due to its essential role as mitotic motor protein that allows normal bipolar spindle formation and cell replication, thus representing a new promising target for the treatment of breast cancer patients [2].

The aim of this work was to evaluate the activity of new thiadiazoline-based Eg5 inhibitors, analogues of K858, in an *in vitro* model of ER+/PR+ breast cancer (MCF7 cell line), selecting compounds **2** and **41** for their better profile as they were shown to reduce MCF7 cell viability with lower concentrations and with minimal effect on non-tumoral cells respect to their parent compound K858. **2** and **41** appeared to significantly counteract MCF7 migration by negatively modulating the NF-kB/MMP-9 pathway. The expression of HIF-1α and VEGF appears decreased by **2** and **41** administration thus preventing the recruitment of the molecular pathway involved in angiogenesis promotion. Moreover, compound **2** provokes an increased Caspase-3 activation thus triggering MCF7 apoptotic event, while **41** and K858 seem to induce the necrotic axis, as demonstrated by the augmented expression of PARP.

These results allow to argue that **2** and **41** Eg5 inhibitors can simultaneously modulate several key processes of breast cancer spread and progression, such as cell proliferation, invasiveness, migration, ECM remodelling and angiogenesis, along with apoptosis or necrosis occurrence, thus representing a new drug strategy to control breast cancer.

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KYMASIN UP as a promising natural treatment for osteo- porosis

Laura Salvadori[1,2,$], Maria L. Belladonna[3,$], Beatrice Castiglioni[4], Martina Paiella[1], Eleonora Panfili[3], Tommaso Manenti[5], Catia Ercolani[5], Luca Cor- nioli[5], Sara Chiappalupi[2,3], Giulia Gentili[2,3], Massimilano Leigheb[6,7], Gu- glielmo Sorci[2,3], Michela Bosetti[4], Nicoletta Filigheddu[1,2,†], Francesca Riuzzi[2,3,†]

[1] Department Translational Medicine, University of Piemonte Orientale, Novara 28100, Italy
[2] Interuniversity Institute of Myology (IIM), Perugia 06132, Italy
[3] Department Medicine and Surgery, University of Perugia, Perugia 06132, Italy
[4] Department Pharmaceutical Sciences, University of Piemonte Orientale, Novara 28100, Italy
[5] Laboratori Biokyma srl, Anghiari 52031, Italy
[6] Department of Health Sciences, University of Piemonte Orientale, Novara 28100, Italy
[7] Department of Orthopaedics and Traumatology, "Maggiore della Carità" Hospital, Novara 28100, Italy
[$] Contributed equally
[†] Shared senior authorship

Osteoporosis is an unresolved metabolic disease considered as a major global public health problem affecting millions of people all over the world, especially elderly. The maintenance of bone health during adult life de- pends on a correct bone remodeling process based on the balance between osteoblast (OB)-dependent bone formation and osteoclast (OC)-dependent bone resorption [1,2]. Excessive OC activity is the main cause of osteopo- rosis inducing microarchitectural deterioration of bone tissue with conse- quent increase in the risk of fractures, chronic pain and disability, ending in loss of independence and generating enormous health care costs [3]. KY- MASIN UP is a new dietary product containing *Withania somnifera*, *Si- lybum marianum* and *Trigonella foenum-graecum* extracts recently identi- fied for its strong efficacy in preventing muscle atrophy also related to ag- ing [4]. We tested KYMASIN UP in experimental models of osteoclasto- genesis (i.e., RAW 264.7 macrophages treated with RANKL) and OB differentiation (i.e., C2C12 myoblasts treated with BMP2). We found that KYMASIN UP: i) reduced the RANKL-dependent differentiation and ac- tivity of OCs by reducing Src and p38 MAPK activation; ii) maintained a physiological release of the soluble decoy receptor for RANKL, OPG, in osteoporotic conditions; and ii) increased calcium mineralization in OBs. Moreover, KYMASIN UP induced differentiation in human primary OB- like cells derived from osteoporotic subjects, conferring to our data a strong translational potential. Thus, KYMASIN UP in virtue of the strong anti- osteoporotic effects observed *in vitro* by re-balancing the activity of OBs and OCs, might be considered as a promising candidate for blunting the age-related functional decline of bone tissue.

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A standardized extract of *E. arvense* counteracts loss of mus- cle mass and strength in geriatric mice

Laura Salvadori[1,2,$], Martina Paiella[1,2,$], Tommaso Manenti[3], Catia Ercolani[3], Luca Cornioli[3], Sara Chiappalupi[2,4], Giulia Gentili[2,4], Nicoletta Filigheddu[1,2,#], Guglielmo Sorci[2,4,#] and Francesca Riuzzi[2,4,#]

[1] Department of Translational Medicine, University of Piemonte Orientale, Novara 28100, Italy
[2] Interuniversity Institute of Myology (IIM), Perugia 06132, Italy
[3] Laboratori Biokyma srl, Anghiari, Italy
[4] Department of Medicine and Surgery, University of Perugia, Perugia 06132, Italy
[$] Contributed equally [#] Shared senior authorship

Sarcopenia is an age-associated syndrome characterized by a progressive loss of skeletal muscle mass, quality and strength predisposing over-60s to frailty, bone fractures, loss of independence, morbidity and mortality [1,2]. Excessive protein breakdown and declined protein synthesis, especially of type II myosin heavy chain (MyHC-II), are observed in sarcopenia. The consequent muscle atrophy, involving preferentially type II (fast-twitch glycolytic) myofibers, together with an age-related metabolic shift towards type I (slow-twitch oxidative) myofibers strongly compromises muscle per- formance [3,4]. Due to the growing life expectancy, sarcopenia represents a major social and financial problem for Western countries, so that the identi- fication of natural active compounds to prevent or treat sarcopenia is elicit- ing increasing interest [5]. Based on our results *in vitro* showing that the of- ficinal plant, *Equisetum arvense* (EQ) protects myotubes against MyHC-II degradation under different atrophying stimuli, we administered a standard- ized extract of EQ (500 mg/kg/die) to pre-geriatric (21-month-old) C57BL/6 WT mice for 3 months. Muscles of treated mice: i) preserved mass and myofiber area; ii) improved performance; iii) showed reduced MyHC-II degradation; iv) maintained type II myofibers; v) expressed phys- iological levels of genes involved in the maintenance of muscle mass and fatty acid metabolism. Thus, EQ is a potential candidate useful to preserve muscle mass and functionality in sarcopenia, improving the quality of life of elderly and reducing sarcopenia-related health-care costs.

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Biomarkers Response In Middle Distance Runners Using Antioxidants: An Observational Study

Carmine Sellitto[1], Berenice Stefanelli[1], Marta Trucillo[2], Paolo De Blasiis[2], Amelia Filippelli[1], Germano Guerra[3], Antonio De Luca[2], Angelica Perna[3], Angela Lucariello[4], Valeria Conti[1]

[1] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Via S Allende, 84081 Baronissi, Italy
[2] Department of Mental and Physical Health and Preventive Medicine, Section of Human Anatomy, University of Campania "Luigi Vanvitelli"
[3] Department of Medicine and Health Sciences, University of Molise, 86100 Campobasso, Italy
[4] Department of Sport Sciences and Wellness, Section of Human Anatomy, University of Naples "Parthenope"

Exercise training (ET) provides health benefits to patients belonging to several clinical settings [1]. ET contrasts oxidative stress by decreasing radical oxygen species and other oxidant molecules and/or increasing antioxidant ones [1]. Antioxidants can contribute to preventing or contrasting oxidative stress and its associated cellular damage. Indeed, supplements, especially those containing vitamins and other micronutrients, are commonly used to improve athletes' wellness and performance [1]. Despite this, the effects of antioxidant supplementation have not yet been elucidated, particularly in athletes performing endurance training [1]. In this study, we investigated the effects of ET on inflammatory and oxidative response related markers in endurance athletes using or not antioxidant supplements.

An observational study enrolling middle distance runners (MDR) and age-matched sedentary volunteers (CTR) was conducted. Adult MDR performed ET for at least 6 months and signed informed consent before study initiation. MDR using antioxidant supplementation (MDR-S) took 240 mg vitamin C and 15 mg vitamin E together with mineral salts. All athletes allowed blood sample collection, reported information about dietary/consumption habits and their ET. Through mRNA levels, gene expression of COX-2, a known inflammatory biomarker, was evaluated in peripheral blood mononuclear cells; in the same way gene expression of SIRT1, MnSOD and CAT, important oxidative response related markers, was measured.

Thirty-two MDR (18 MDR-S and 14 MDR not taking supplements, MDR-NoS) and 14 CTR were enrolled. COX-2 mRNA expression levels were significantly higher in CTR than in MDR ($p=0.038$), with a higher trend in MDR-S compared to MDR-NoS. The MDR group demonstrated higher significant levels of SIRT1 mRNA compared to the CTR group ($p = 0.0387$) and, notably, MDR-NoS showed higher levels than CTR ($p = 0.0136$). MnSOD mRNA expression showed an increased trend in MDR compared to CTR and a statistically significant higher expression when we compared MDR-S to CTR ($p=0.047$) or MDR-S to MDR-NoS ($p=0.013$). No differences were observed in the evaluation of CAT mRNA expression.

The studied inflammation marker showed higher level in CTR than in athletes and on the contrary the antioxidant response markers had higher expression in athletes than in CTR. Differences in antioxidant response appeared between MDR-S and MDR-NoS. Further analyses will be carried out to confirm these results also by measuring the proteins expression and the enzymes activity whose gene expression has been analyzed.

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Sex and the Knee: Biomechanical Differences in Unilateral Landing Tests

Chiarella Sforza[1], Rodrigo Rabello[1], Federico Adragna[2], Alessandro Bonotti[1], Elodie Bouvier[3], Lucas Silva Zandonato[1], Maedeh Taghizadeh Kerman[4], Alessandro Zucchetti[1], Filippo Bertozzi[2], Claudia Brunetti[1].

[1] Università degli Studi di Milano, Milan, Italy
[2] Politecnico di Milano, Milan, Italy
[3] Polytech Marseille, Marseille, France
[4] Bu - Ali Sina University, Hamadan, Iran

Introduction. Non-contact injuries of the Anterior Cruciate Ligament (ACL) are one of the most disabling injuries that can occur in team sports [1]. The ACL anatomical structure makes it sensitive to proximal tibial anterior shear force, whose intensity is high in sport manoeuvres like cutting, landing, and pivoting [2]. In fact, these movements are related to an increased risk of sustaining ACL injuries, and the stress on the ligament becomes even more intense when executed with extended hip and knee and dynamic valgus [3]. Women have higher injury rates than men, due to anatomical, neuromuscular, and hormonal factors that exacerbate the aforementioned biomechanical risk factors [4]. Clinicians developed "functional tests" to estimate the risk of sustaining ACL injuries by simulating and analysing the kinematics of the movements related to injuries. Landing tasks are considered a great evaluation tool [5], but the lack of standardization makes it difficult to compare results, especially between sexes. This study aimed to assess sex differences in lower limb kinematics of multi-planar drop-landing tasks, customized using subject-specific characteristics and performed with the dominant lower limb.

Materials and Methods. 20 males and 20 females (22.0 ± 2.1 years) performed single-leg drop-landing tests (box height: 20% of subject's height; box-target horizontal distance: 60% of the maximal horizontal jump distance) and a subsequent jump in one of four directions (vertical, lateral, forward, medial). A set of standardised passive landmarks were positioned on the trunk and lower limbs of the subjects, and their movements were collected with a 9-cameras motion capture system. Hip and knee joint angles were evaluated at ground contact and at the peak in the next 100 ms with a 2-Way ANOVA, with sex and test as factors ($p<0.05$).

Results. When compared to men, women exhibited significantly less hip flexion and knee external rotation, and higher knee abduction at ground contact. Women had higher peak hip adduction and knee abduction, less peak hip flexion and knee flexion than men. For all differences, a medium effect size was found. Between-test comparisons showed that the lateral secondary jump generated the highest peak hip adduction with a large effect size, while the medial jump the lowest. The central secondary jump induced higher peak hip adduction than the medial one and the lowest peak knee flexion with a medium effect size.

Discussion. The proposed tests highlighted significant sex differences in lower limb kinematics, showing how women executed "stiffer" landings on the dominant lower limb, with adducted hip and abducted knee, considered primary ACL injury risk factors [3]. Comparison among tests showed that the responses were different depending on the task's characteristics, but the sex-related features were evident in all tasks. In conclusion, the current tests were customized using subject-specific morphological and functional characteristics: the introduced elements may be useful to define standard landing functional tests for ACL injury risk, whose unambiguous interpretation may help researchers and trainers to develop more efficacious preventive, rehabilitative and training programs to reduce the injury rate, especially for female athletes.

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Renal changes in farnesoid X receptor and mitochondria in heterozygous sirtuin1 mice fed high fat diet and melatonin

Alessandra Stacchiotti[1,2], Gaia Favero[3], Glen Lockwood[4], Francesca Arnaboldi[1], Maria Monsalve[5], Victoria Cogger[4], David Le Couteur[4], Rita Rezzani[3]

[1] Department Biomedical Sciences for Health, Milan University, Milan, Italy
[2] Laboratorio di Morfologia umana Applicata, IRCCS Policlinico San Donato, Milan, Italy
[3] Department of Clinical and Experimental Sciences, Brescia University, Brescia, Italy
[4] ANZAC Research Institute, Concord General Hospital, the University of Sydney, Sydney, NSW, Australia
[5] Instituto de Investigaciones Biomedicas "Alberto Sols" (CSIC-UAM), Madrid, Spain

Silent information regulator 1 (Sirt1), belongs to a family of class III histone deacetylase, which regulates the transcription of genes involved in aging, metabolism, mitochondriogenesis. Its decreased expression/activity in heterozygous Sirt1+/- mice (HET) has been previously associated to abnormal lipid metabolism, inflammation, and disrupted autophagy in the liver of obese mice [1]. Farnesoid X receptor (FXR), is a crucial member of the nuclear receptor superfamily of ligand-activated transcription factors, greatly regulated by biliary acids [2]. FXR is emerging as a potential therapeutic renal target in type 1 and 2 diabetes and ischemia/reperfusion [3,4]. Considering that proximal cortical tubules are firstly based on aerobic metabolism and are prone to autophagy/mitophagy, here we characterized proximal tubular and mitochondrial changes in HET mice placed on obesogenic diet (HFD-58% lard) plus or not melatonin (MEL) (10 mg/kg) in drinking water for 16 weeks, in comparison with C57BL/6J mice (WT) receiving the same treatment.

Interstitial fibrosis was assessed by Masson trichrome staining, FXR immunostaining was performed by ABC-peroxidase method and mitochondria in proximal tubules characterized by TOM20 immunostaining and TEM. Furthermore, to show the extent of basal autophagy/mitophagy we fasted and refeed for 24h HET mice and WT mice plus or not leupeptin, a lysosomal inhibitor.

Main results were that MEL alleviated proximal tubular changes and interstitial fibrosis in WT HFD but not in HET HFD. Basal mitochondria density was reduced in starved HET versus WT and not affected by leupeptin. Ultrastructural analysis demonstrated lipid droplets, calcification granules, and abnormal mitochondria in cortical proximal tubules in HET HFD. Finally, melatonin restored nuclear tubular FXR staining in WT HFD but not in HET HFD mice.

In conclusion, these data suggest that 1) tubular mitochondria are deeply affected in mice lacking full SIRT1 expression; 2) a dietary hypercaloric stimulus is not properly used in the kidney; 3) antioxidant melatonin is ineffective in HET mice model of obesity. However, further experimental approaches are warranted to reveal molecular mechanisms involved in mitophagy disruption and renal damage in these peculiar mice.

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Infrared thermal evaluation of the spine in sportive adults: a morphological classification

Bruno Trovato[1], Luca Petrigna[1], Martina Sortino[1], Marta Zanghì[1], Federico Roggio[1], Giuseppe Musumeci[1]

[1]Department of Biomedical and Biotechnological Sciences, Anatomy, Histology and Movement Science Section, School of Medicine, University of Catania, Via S Sofia n°87, 95123, Catania, Italy

Background: The interest in infrared thermography (IRT) applied to the human body is increasing in the last few years, considering the non-invasive and risk-free nature of the exam. With the IRT it is possible to analyze all the visible regions of interest (ROI) of the human body and evaluate, by analyzing the temperature, their metabolic and vascular activities and possible asymmetries.

Aim: the aim of this study is to use the IRT to evaluate the thermal profile of the back of healthy subjects and relate it to the type of sport practiced.

Methods: The spines of 54 participants considered healthy after careful clinical evaluation were evaluated, 14 female and 40 male, mean age of 22.3 (2.1), mean height 169.6 (9.5), mean weight 66.2 (13.4) and mean BMI of 22.8. Participants were divided into three groups by sport practiced: Fitness (F), Soccer (C), Dance (D). Thermal images were acquired with a professional FlirE60 IRT (Wilsonville, OR, USA) and three representative ROI of the cervical, thoracic and lumbar regions were identified. The statistical analysis used were the Shapiro-Wilk test to evaluate normality and Anova analysis with a post hoc Tukey test to compare groups.

Results: The data were normally distributed. Anova analysis was statistically significant for cervical ($p<0.001$), dorsal ($p<0.001$) and lumbar ($p<0.01$) area between groups. Furthermore, Tukey post hoc test showed significant differences between soccer players (temperature 34.32° SD=0.72°) and dancers (temperature 33.36° SD=0.97°) ($p < 0.01$), dancers and fitness (temperature 34.68° SD=0.96°) ($p<0.001$) for the cervical area. Significance was also found between the dorsal area of subjects practicing fitness (temperature 34.0° SD=1.03°) and dancers (temperature 32.71° SD= 0.92°) ($p <0.001$) and subjects practicing dance and football (temperature 33.70 SD=0.88°) ($p<0.05$). A statistically significant difference was found for the lumbar region only between dancers (temperature 32.17° SD=1.28°) and fitness group (temperature 33.32° SD=1.33°) ($p<0.01$).

Conclusion: To the best of our knowledge, this is the first study that defines the thermal profile of the back of healthy subjects and classifies it according to the type of sport practiced. From the statistical analysis it emerged that dancers had a statistically lower cervical average temperature than the other groups while for the dorsal tract, the fitness group had a statistically higher average temperature than the other groups. Significant differences were found for the lumbar region, but only between dancers and fitness group. These differences can be explained considering the muscles involved in the specific technical gestures of the discipline. These results will be useful for the future evaluation of thermal patterns in subjects with alterations caused by musculoskeletal pathologies.

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Development of anthropometric equations predicting DXA-measured percentage of fat mass in black belt karatekas

Valentina Cavedon[1], Franco Sacristani[1], Marco Sandri[1], Chiara Milanese[1], Carlo Zancanaro[1]

[1] Dipartimento di Neuroscienze Biomedicina e Movimento, Università di Verona, Italy

In karate, excess percentage of fat mass (%FM) may be associated with poor sport performance. Dual-energy X-ray absorptiometry (DXA) can accurately measure %FM (%FM-DXA), but cost and availability may limit its usage in practice. Anthropometry has long been used as a tool to develop predictive equations for %FM against a criterion method. The present study explored the ability of sixteen available anthropometric equations to estimate %FM (%FM-AE) in black belt karate athletes. In addition, two population-specific predictive equations to estimate %FM-DXA were developed from direct anthropometric measurement of karatekas. Forty-six male karatekas (mean age 21.7 ± 3.8 years) had a standard anthropometric profile (circumferences, skinfold thickness at nine sites), and a whole-body DXA scan.

In male karate athletes all the considered anthropometric equations revealed inaccurate in estimating the %FM-DXA (range of the limits of agreement in Bland-Altman analysis 6.43%-13.37%). Using anthropometric data from karatekas, regression analysis yielded two population-specific, statistically significant models ($P < 0.001$ for both) predicting %FM-DXA. In a first model (adjusted $R^2 = 0.883$; Standard Error of Estimate [SEE] = 1.3%), the predictors were the abdominal, triceps, calf, and biceps skinfolds; in a second model (adjusted $R^2 = 0.814$; SEE = 1.6%), the predictor was the sum of nine skinfolds (biceps, triceps, subscapular, chest, axilla, suprailiac, abdominal, anterior thigh, and calf). The current findings highlight the need for population-specific anthropometric equations to accurately estimate the %FM-DXA in male karate athletes. The two predictive anthropometric equations developed in this study may represent a useful tool for professionals dealing with body composition in this athletic population to accurately estimate the %FM-DXA by means of anthropometry.

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Storia e didattica innovativa delle discipline morfologiche

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The three University missions and the digital anatomy table: a multifasceted interactive tool to be used from school to undergraduate and postgraduate education

Rosemarie Heyn[1]

[1] Sezione di Anatomia, Dipartimento SAIMLAL, Sapienza Università di Roma, Roma, Italy

Anatomy is clearly at the basis of medical education. Identifying clinically relevant anatomical features, especially when evaluating medical imaging (CT scans; MRI; X-rays; US; 3D volume renderings; histological images), is an essential skill, from beginners (first- and second-year medical students) to postgraduate (Master, PhD) students. At the same time, biomedically-oriented school students may better appreciate what studying medicine and anatomy mean throughout the visualization of simple and clear medical images. Digital anatomy tables are now being increasingly used at medical schools as a valid interactive teaching/learning tool (1-2). These tables allow to be used both face-to-face as well as remotely from any device. During the last 4 years Sapienza University has been engaged in developing the three main University missions: teaching, research, and interaction with society, thus opening new horizons. Regarding teaching, anatomy laboratory sessions are delivered dividing the class in small groups of students (mean number of 8 students in post-COVID-19 education). Each group was about 30 to 40 minutes interacting directly with the table and with the facilitator. The facilitator prepared in advance specific clinical cases and bookmarks relevant to the session to be taught, trying to choose the best images and 3D volume renderings so the students can train their clinical eye. With the support of Sectra (Sectra AB, Linköping, Sweden), the first interactive guide was prepared, containing hyperactive links to images separated according to the order in which anatomy is taught in Italy (i.e., first year, Anatomy 1: musculoskeletal system, joints, and cardiovascular system; Anatomy 2: the lymphatic, respiratory, digestive, urinary, and reproductive systems; second year, Anatomy 3: nervous, endocrine, and integumentary systems). Besides, postgraduate medical students (i.e., Masters) as well as PhD visiting students are welcome. Regarding research, in turn, we have ongoing projects on the xiphoid process and the coccyx to better evaluate the anatomical variants. Of course, many other subjects may be studied. Finally, regarding the third mission, it is several years we are collaborating with some biomedically-oriented Roman schools within the project Paths for Transverse Skills and Orientation (*Percorsi per le Competenze Trasversali e l'Orientamento, PCTO*). Online (Zoom) meetings were organized with the classes and a summary of the human body was shown by means of the 3D volume renderings. In conclusion, the digital anatomy table is a multifasceted teaching/learning tool that can be used at all levels of biomedical education. Nevertheless, besides the licences price, facilitators need a mandatory training, a time consuming, sometimes unfriendly process, that may render difficult the entrance of new teachers.

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Anatomical laboratory: Prosections, 3D Models, Imaging and Anatomage

Matteo Moretti[1], Gabriele Ceccarelli[2], Gabriella Cusella[2]

[1] Department of Public Health, Experimental and Forensic Medicine, Unit of Legal Medicine,
[2] Department of Public Health, Experimental and Forensic Medicine, Unit of Anatomy, University of Pavia, via Forlanini 8-12, 27100, Pavia, Italy

In the academic year 2022/23, the students enrolled in the "Golgi" Medical course of our university will be approximately 330. In addition to lectures, we always involve students in practical activities to induce them to transform the concepts learned on the books into three-dimensional structures that can be finally lead back to the patient's body. To achieve this purpose, we have created a route divided into stages. (A) The first phase takes place in the anatomical room. With the help of the book, the student must recognize the structures indicated in a pre-printed sheet on the 3D plastic model. (B) The second phase involves the recognition of the same structures on a human prosection. Fortunately, we have many samples available for this stage; they belong to the collection started in Pavia by Antonio Scarpa and implemented by successors as Bartolomeo Panizza (1785-1867) and Giovanni Zoja (1833-1899). (C) In the third stage students, under the guidance of a tutor, experience the use of *Anatomage* to simulate anatomical dissection but using digitized dissection. Students are asked to reproduce the previously examined prosection and, where possible, implement it with further anatomical details. (D) The third phase involves analysing radiological anatomy images using the specific functionality offered by Anatomage Table, choosing them from those relating to the same anatomical district. (E) In the fourth stage, a clinical case among those present in the database is analysed. The case must have characteristics such as to involve a morphologically significant alteration of the same anatomical structures. F. Last stage. The tutorial ends with viewing a short cadaver dissection video. While viewing the recording, we ask the student to recognize the main visible structures.

Unfortunately, in our University the dissection from a cadaver is not feasible but, in combination with the use of 3D models and prosections, digitalyzed dissection has been incorporated into ours syllabus and we noted that it helps students in the visualization of complex anatomical systems. Most importantly, students subsequently find it much easier to tackle the study of medical and surgical clinics. Finally yet importantly, we select the students who perform the role of Tutors through specific projects, among medical students who have completed their third year exams with very high score. Having to support the activities here illustrated, the Tutors themselves must not only remember the acquired knowledge but also propose it from a preclinical point of view. In our opinion, this is a very important benefit of our teaching method.

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Medieval anatomy in Dante's works

Gianfranco Natale[1,2], Paola Lenzi[1], Paola Soldani[1], Riccardo Ruffoli[1], Francesco Fornai[1,3]

[1] Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy
[2] Museum of Human Anatomy "Filippo Civinini", University of Pisa, Pisa, Italy
[3] IRCCS Neuromed, Pozzilli, Italy

Knowledge of anatomy in the Middle Ages was very inaccurate and referred to the so-called *hystoria incisionis*, the beginning of treatises dedicated to the description of the human body. In these manuscripts nine fundamental anatomical representations were reported. The first five, referring to general systems (skeleton, muscles, arteries, veins, nerves), were easily identified in the *Fünfbilderserie* (series of five figures). A more careful search was necessary to recognize in the *Nine-System Figure Series* the complete series, including the various organs. Dante Alighieri was a member of the *Corporazione Fiorentina dei Medici e degli Speziali*. Therefore, he knew the art of medicine and could wear the long and wide red robe (*lucco*), with the head covered with a hood (*becchetto*) with the tips falling down on the sides of the face, typical clothing of the doctor. In fact, in two of his main works, *Divine Comedy* and *Convivio*, he left us many medical and anatomical testimonies. First of all, he demonstrated that he was well acquainted with some ancient medical figures, such as Hippocrates, Galen and Avicenna, as well as his contemporaries, such as Taddeo Alderotti. In *Divine Comedy* he used several anatomical terms, for example *casso* (rib cage), *humerus*, and *cerebro* (brain). The term *nuca* (nape) was used according to the meaning of Islamic anatomy, that is the cervical spinal cord, demonstrating an excellent knowledge of Islamic books (*Inferno* XXXII, 127-129). Among the schismatics of the ninth *bolgia* there is a real anatomical dissection, that of Muhammad: *rotto dal mento infin dove si trulla. / Tra le gambe pendevan le minugia; / la corata pareva e 'l tristo sacco / che merda fa di quel che si trangugia* [*cleft from the chin right down to where men fart. / Between the legs the entrails dangled. I saw / the innards and the loathsome sack / that turns what one has swallowed into shit*] (*Inferno* XXVIII, 24-27). Of particular interest is *canto* XXV of *Purgatorio*, in which Dante exposed an articulated physiological and embryological description of the human body with the theory of the generation of the soul and its relationships with the body. When in *Convivio* (I, VII) he expressed the concept of exact measurement, he took as an example some parts of the human body that have a precise number, such as the teeth or fingers of the hand. In this work (II, III, IX) there is also a detailed description of the visual system, including the optical components of the eye and the transmission of light, as well as the optical illusions. The anatomical part of this examination recalls the figures of the visual system of the *hystoria incisionis*. These numerous anatomical references by Dante, especially in *Inferno*, are not surprising, considering the large number of bodies encountered, often altered by the law of retaliation. Nor is it surprising this great medical competence of Dante, taking into account that in the past the men of culture had expertise on all areas of knowledge.

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The Need of Facial Anatomy Cadaver Lab in the Training Programs of Aesthetic Medicine

Giulio Nittari[1], Enea Traini[1], Ciro Ruocco[1] and Francesco Amenta[1]

[1] School of Pharmaceutical Sciences and Health Products, University of Camerino, via Madonna delle Carceri, 9, 62032, Camerino (MC) Italy

Aesthetic medicine differs from conventional medicine in that it is focused on improving normal structures and the patient's self-esteem. Its growing demand hinges on well-being being regarded as more than just the absence of abnormality in structure or function. Level of competency and training of health professionals practicing aesthetic medicine is still not defined. The only guideline available in Europe derives from the European Standard EN 16844 "Aesthetic medicine services - Non-surgical medical treatments". Accordingly, a practitioner shall be trained in the respective treatment and training shall include anatomy at a level allowing to minimize inappropriate treatment.

Unfortunately, postgraduate university courses do not include courses of dissection on cadaver. Safe injection and other aesthetic medicine treatments require a profound knowledge of the head and neck anatomy. This in our opinion cannot be learned without a practical cadaver/dissection training.

Our group organizes from 13 years postgraduate two-year Master courses in Aesthetic Medicine and Therapeutics with didactic involvement requiring earning 120 ECTS. From 4 years we have introduced for attendants of the international courses a compulsory cadaver lab training course.

It is a two-day full immersion course. In the first day using models and three-dimensional simulators the anatomy of facial muscles, fat compartments and blood vessels are studied. The second day on fresh cadaver included hands on practice with live injection points into cadaver, anatomical locations of the needles and topography of the injection sites, practical dissections done by attendants under teacher's supervision.

These cadaver sessions resulted in a deep knowledge of face anatomy, more detailed and with higher exam scores compared to attendants of the Italian language class for which the cadaver lab was not compulsory.

These results suggest that a cadaver practical training of aesthetic doctors is necessary to guarantee a safe and appropriate aesthetic medicine practice.

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Tecnologie innovative

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The knotty question on 3D data: are volume and surface measurements equivalent in 3D models of anatomical structures acquired with diverse optical systems?

Annalisa Cappella[1,2], Loris Marcolongo[3], Noha M. Hassan[3], Joshua Yang[3,4], Marcio de Menez[5], Ana Maria Cerón-Zapata[6], Gianluca Tartaglia[3,7]

[1] Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Italia
[2] UO Laboratorio di Morfologia Umana Applicata, IRCCS Policlinico San Donato, San Donato Milanese, Italia
[3] Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, Università degli Studi di Milano, Italia
[4] Harvard School of Dental Medicine (HSDM), Boston, MA, United States
[5] State University of Amazonas (UEA), Manaus, Brazil
[6] Pediatric Dentistry and Preventive Orthodontics, Universidad CES, Medellin, Colombia
[7] UO Chirurgia Maxillo-Facciale e Odontostomatologia, Fondazione IRCCS Cà Grande Ospedale Maggiore Policlinico, Milano, Italia

Anatomy is the foundation of biomedical disciplines which has advanced through the constant discrimination between "normal" and "non-normal" anatomical structures. The advent of quantitative approaches in anatomical and biomedical research in the last decade has increasingly involved 3D technologies. This inflated popularity did not even spare 3D optical methods, which use volumetrics and surface calculations as well as 3-dimensional point-to-point distances. However, the repeatability and reproducibility of these methods still need to be fully investigated. Regardless of the anatomical structure under study, this kind of data is fundamental for population standards, clinical and surgical follow-up, and diagnosis for certain diseases, such as cleft lip and palate (CLP).

Palates of neonates and infants with CLP represent complex anatomical structures to evaluate for the above purposes. 3D optical devices (such as stereophotogrammetry and laser scanning) are optimal systems for digitizing CLP casts to quantify all these data.

Recently, several studies in literature showed a great interest on volumetric and surface measurements of cleft palate structures providing comparative data for further clinical studies [1-3].

But are these reference data truly comparable? Can we correspond data deriving from 3D models acquired by different optical systems? Linear and angular measurements, followed by surface data, are those most examined in terms of intra-instrument reproducibility, while the reproducibility of volumetric measurements still need to be more thoroughly investigated.

With this intent, the present study aimed to compare volumetric and surface measurements on digital cleft palatal models acquired by two different systems: laser scanning (Dental Wing Series 3) vs stereophotogrammetry (Vectra 3D Imaging System). 96 palatal casts of 32 patients with unilateral cleft lip and palate (age from birth to 2 years old) were scanned by the two systems. Volumetric and area analyses were carried out on the two cleft palatal segments (minor and major), selected from the 3D models in the same software: Vectra 3D Analysis Module (VAM). Area was measured through one validated automatic protocol [4] while volume was assessed through two protocols, one automatic and the other semi-automatic [5]. Inter and intra-operator repeatability as well as inter-instrument reproducibility were verified for both measurements and all protocols, with the technical error of measurements (TEM), its relative value (rTEM) [6] and the Bland-Altman test [7].

Intra- and inter-operator repeatability were very good for area calculation (rTEM 2.4% and 2.7% respectively) and good for volume estimate (rTEM 6.6% and 5.9% respectively). According to the Bland-Altman test, the inter-instrument reproducibility proved high (87%) for surface measurements while insufficient (<46%) for the volumetric estimations (regardless which

protocol was used). No differences were found when considering either minor or major palatal segments.

Our results confirm that surface values of anatomical structures (like minor and major cleft palatal segments) correspond independently from the mesh of the 3D model and thus from the instrument used for acquisitions, which assures researchers that they can keep comparing area data published in clinical studies. In contrast, volumetric measurements greatly depend on which instrument is used for digitizing models and collecting data, highlighting a clear caveat for researchers working on biomedical studies: Be sure of comparing equivalent volumetric data, i.e. data deriving from the same instrument.

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Enriched graphene oxide-polypropylene sutures modulate the inflammatory pathway induced by Escherichia coli lipopolysaccharide

Luigia Fonticoli[1], Ylenia Della Rocca[1], Guya Diletta Marconi[2], Oriana Trubiani[1], Francesca Diomede[1], Jacopo Pizzicannella[3]

[1] Department of Innovative Technologies in Medicine & Dentistry, University "G d'Annunzio" Chieti-Pescara, Chieti, Italy
[2] Department of Medical, Oral and Biotechnological Sciences, University "G d'Annunzio" Chieti-Pescara, Chieti, Italy
[3] Ss Annunziata" Hospital, ASL 02 Lanciano-Vasto-Chieti, Chieti, Italy

Graphene oxide (GO) derived from graphene, has a remarkable chemical-physical properties such as stability, strength and thermal or electric conductivity and additionally shows antibacterial and anti-inflammatory properties[1]. The aim of this study was to evaluate the anti-inflammatory effects of the polypropylene sutures (PPSs) complexed with two different concentration of GO in the modulation of the inflammatory pathway TLR4/NFKB p 65/MyD 88/ NLRP3 induced by the Escherichia coli lipopolysaccharide (LPS-E) in an *in vitro* model of human gingival fibroblasts (hGFs). The gene and the protein expression of inflammatory markers was evaluated by respectively rt-PCR, western blot and immunofluorescence analysis, while the adhesion of hGFs on the PPSs/GO constructs was evaluated by optical microscopy and scanning electron microscopy (SEM). Both GO concentrations used in the PPSs/GO constructs appear to decrease the expression of inflammatory markers in hGFs treated with LPS-E compared to hGFs treated with LPS-E in contact with PPSs not complexed with GO. The enrichment of the PPSs with GO shows an improving effect in the wound regeneration processes, modulating the inflammatory process and accelerating its closure.

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Establishment of 3D cultures of myometrium, leiomyoma and leiomyosarcoma cells: two models compared

Pamela Pellegrino[1], Stefania Greco[1], Francesca Giampieri[2], Giovanni Delli Carpini[2], Franco Capocasa[3], Bruno Mezzetti[3], Maurizio Battino[2], Andrea Ciavattini[2], Pasquapina Ciarmela[1,4]

[1] Department of Experimental and Clinical Medicine, Universita` Politecnica delle Marche, 60020 Ancona, Italy
[2] Department of Clinical Science, Università Politecnica delle Marche, Ancona, Italy
[3] Department of Agricultural, Food, and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy
[4] Department of Information Engineering, Università Politecnica delle Marche, Ancona, Italy

Uterine leiomyomas, or fibroids, are the most common benign, monoclonal, gynecological tumors in women's uterus [1]. They affect about 77% of women of reproductive age, and 25% of them bear clinically apparent tumors causing significant morbidity, including heavy or abnormal uterine bleeding, pelvic pain or pressure, infertility, and reproductive dysfunction in rare cases [2]. Although the exact etiological mechanism remains unknown, it is known that stem cells, genetic and epigenetic factors, sex steroids, growth factors, cytokines, chemokines, and extracellular matrix (ECM) components are involved in the development and growth of leiomyomas [3,4,5]. The malignant transformation of the myometrium brings to the leiomyosarcoma, rare but very aggressive condition [6].

In the literature the model systems for studying this disease are mostly limited to primary cell cultures in monolayers, xenografts, and transgenic mouse models. Each of these approaches has provided valuable insights into leiomyoma pathophysiology, but all have their own limitations [7], the need to find an experimental model that is close to an *in vivo* study has aroused interest in the establishment of 3D cell cultures.

Furthermore, 3D tumor models are useful both for the more accurate understanding of the pathogenesis, as well as for performing solid cancer-related *in vitro* assays (migration, invasion, radiation or drug testing) [8].

For this reason, the aim of this study is to use alternative methods. In fact, in recent years there has been a great interest in tissue engineering, which involves the use of either bioprinters or matrigels for the formation of 3D environments, that allow to test the efficacy of the compound in preclinical experiment models (organoid cultures produced by 3D bioprinters), overcoming the limits of the results obtained from *in vitro* experiments with cells grown in monolayer, and improving the biological relevance of the date obtained. In this study we used two different techniques for the formation of 3D models: Agarose and 3D bioprinter.

Firstly, we choose to use agarose gel to obtain the organoids. Agarose is a natural biodegradable, non-adhesive, and non-toxic polysaccharide derived from seaweed. It has characteristic necessary for creating 3D cell culture models: high porosity, which allows for the renewal of nutrient media for 3D cell growth, and provides access to gases and small molecules, it is an optically transparent material, solidifies in molds at room temperature, which makes it possible to perform experiments under sterile conditions without significant difficulties, while the accessibility of the resulting gel wells to a pipette tip makes it possible to introduce cells and conduct other manipulations [9].

Using the 2% agarose we inoculated 10^6 cells of myometrium, leiomyoma and leiomyosarcoma obtaining organoids visible under a phase contrast optical microscope. Thanks to the use of the agarose, it was possible to investigate the cellular morphology, for example with the use of DAPI (4',6-diamidino-2-phenylindole), which is a fluorescent nuclear stain, and Haematoxylin and Eosin. The problem of this technique is linked to the molecular investigation, in fact, it

is difficult to isolate the organoids from the matrix and analyze them molecularly, for this reason we continued our studies using the second method.

In this study we used the bioprinting CELLINK INKREDIBLE+, a pneumatic extrusion-based equipped with a system with double print heads and a UV LED curing system in 6-wells as a 3D bioprinter, and we choose to print a simple and squared grid placed in the center of each well. We choose the cartridge with Alginate bioink because it stimulates the formation of an extracellular matrix which is very important for myometrial, leiomyoma and leiomyosarcoma cells. In fact, it is characterized by a high porosity that allows the easy diffusion of the solutes through the scaffold, allowing the adequate supply of nutrients and oxygen to the cells [10]. For the mix of bioink and cells we used the "dual syringe mixing method", it is a method that allows to mix 1 ml of alginate and 100 μl of cell pellets. For printing we used the following printing parameters: temperature of 21°C and a pressure of 9-11 KPa. Subsequently, once was printed the cells and alginate, we used $CaCl_2$ (50mM) as a crosslinker.

Both methods allowed the development of 3D organoids of all three cell types. Since leiomyosarcoma is a malignant tumor, it was found to be more performing in terms of growth rate and development of the 3D cellular structure. Myometrium and leiomyoma cells also aggregated to form 3D structures, confirming that these models represent a valid alternative for the study of leiomyoma. The leiomyoma cells embedded in both agarose and alginate in their growth medium formed organoids which successfully proliferate and self-organized into complex structures developing a sustainable organoid culture that emulates the condition of leiomyoma through the accumulation of extracellular matrix, suggesting the potential use of this model for a better understanding of pathophysiology, etiopathogenesis and further suggests that it could be a good model for the study and testing a new prevention methods and alternative preventive drug.

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Highly sensitive flow cytometry allows the monitoring of SARS-CoV-2-specific T cells

Pasquale Simeone[1,2], Simone Vespa[1,2], Giulia Catitti[1,2], Davide Buca[1,2], Domenico De Bellis[1,2], Laura Pierdomenico[1,2], Damiana Pieragostino[2,3], Ilaria Cicalini[2,3], Piero Del Boccio[2,4], Luca Natale[2], Marco Marchisio[1,2], Sebastiano Miscia[1,2], Vincenzo De Laurenzi[2,4], Liborio Stuppia[2,5], Paola Lanuti[1,2]

[1] Department of Medicine and Aging Sciences, University "G d'Annunzio" of Chieti-Pescara, 66100 Chieti, Italy
[2] Center for Advanced Studies and Technology (CAST), University "G d'Annunzio" of Chieti-Pescara, 66100 Chieti, Italy
[3] Department of Innovative Technologies in Medicine and Dentistry, University "G d'Annunzio" of Chieti-Pescara, 66100 Chieti, Italy
[4] Department of Pharmacy, University "G d'Annunzio" of Chieti-Pescara, 66100 Chieti, Italy
[5] Department of Psychological, Health and Territory Sciences, School of Medicine and Health Sciences, University "G d'Annunzio" of Chieti-Pescara, 66100 Chieti, Italy

During viral infections, antibodies and T cells act together to prevent pathogen spread and remove virus-infected cells. The protective and/or pathological role of virus-specific T cells in SARS-CoV-2 infection has been the focus of many studies in COVID-19 patients and in vaccinated individuals. Here, we have investigated anti-spike IgG levels and SARS-CoV-2- specific T cells in 125 donors (90 vaccinated with four different vaccine platforms, 16 individuals with a previous natural infection, and 19 not vaccinated donors who did not report previous SARS-CoV-2 infections). Our data show that anti-spike IgG titers were similar between naturally infected (NI) subjects and those vaccinated with adenoviral vector vaccines. We also observed that 100 % of all groups of immunized donors (both vaccinated and NI donors) produced memory CD4+ and/or CD8+ T cells. We also demonstrated a sustained polyfunctionality of SARS-CoV-2-specific T cells both in resolved natural infections and in vaccinated subjects. Altogether our data suggest that the natural infection produces an overall response (memory T cells/anti-spike IgG) similar to that induced by vaccination, thus these detailed immunological evaluations may be relevant to advance our understanding of the protection induced by various COVID-19 vaccines compared to protection following natural infection, as well as understand fundamental differences in immune memory to mRNA and adenoviral vector vaccine platforms in humans. These data may also be relevant for other vaccine efforts especially for the success of novel vaccines effective against emerging virus variants.

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Improved Efficacy of Quizartinib in Combination Therapy with PI3K Inhibition in Primary FLT3-ITD AML Cells

Manuela Zavatti[1*], Salihanur Darici[1*], Gillian A Horne[2], Xu Huang[2], Heather G Jørgensen[2], Sandra Marmiroli[1]

[1] Cellular Signaling Unit, Section of Human Morphology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, 41125, Italy
[2] Haemato-Oncology/Systems Medicine Group, Paul O'Gorman Leukaemia Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow, G12 0ZD, UK
* Equal contributors

Acute myeloid leukemia (AML) is a heterogeneous hematopoietic malignancy, characterized by uncontrolled clonal proliferation of abnormal myeloid progenitor cells, with poor outcomes (1-3).

The internal tandem duplication (ITD) mutation of the Fms-like receptor tyrosine kinase 3 (FLT3) (FLT3-ITD) represents the most common genetic alteration in AML, detected in approximately 30% of AML patients, and is associated with high leukemic burden and poor prognosis (1). Therefore, this kinase is an attractive druggable target for the treatment of FLT3-ITD AML, and selective small molecule inhibitors have been identified and trialled (2). However, clinical outcomes have been disappointing so far due to poor remission rates, also because of acquired resistance. Combination treatment consisting of FLT3 inhibitors with other targeted therapies is thus regarded as a strategy to overcome resistance (1-3). In this study, we report the preclinical efficacy of the combination of FLT3 inhibitor quizartinib with pan PI3K inhibitor BAY-806946 in FLT3-ITD primary cells from AML patients. We show here that BAY-806946 enhanced quizartinib cytotoxicity and, most importantly, that this combination increases the ability of quizartinib to kill CD34+ CD38- leukemia stem cells, whilst sparing normal hematopoietic stem cells (HSC). Because constitutively active FLT3 receptor tyrosine kinase is known to boost aberrant PI3K signaling, the increased sensitivity of primary cells to the above combination can be the mechanistic results of the disruption of signaling by vertical inhibition.

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Tessuto muscolare e connettivo

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Role of dysbiosis in cardiovascular diseases: a preliminary morphological study

Francesca Arnaboldi[1], Nicoletta Gagliano[1], Martina Di Modica[2], Alessandra Stacchiotti[1,3], Laura Brigida Cornaghi[1], Claudia Moscheni[4], Tiziana Triulzi[2], Valentina Arlotta[2], Giancarla Bernardo[1], Rita Rezzani[5], Lucia Sfondrini[1,2], Elda Tagliabue[2], Francesca Bianchi[1,3]

[1] Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milan, Italy
[2] Molecular Targeting Unit, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
[3] UO Laboratorio di Morfologia Umana Applicata, IRCCS Policlinico San Donato, Milan, Italy
[4] Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan, Italy
[5] Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy

Healthy gut is the habitat of trillions of bacteria, called intestinal microbiota, greatly responsive to diet changes, which lives in symbiotic relationship with the host and provides circulating metabolites, hormones, cytokines necessary for human metabolism. The gut-heart axis is a novel emerging concept based on the accumulating evidence that perturbed gut microbiota, defined as dysbiosis, plays a role -as risk factor- in the pathogenesis of most of the cardiovascular diseases.

The mechanisms involving gut microbiota in cardiovascular diseases are not yet described but experimental evidences suggest a role for microbiota signaling to mitochondria, that has been shown to affect mitochondrial metabolism, activate immune cells, induce inflammasome signaling, and impair the epithelial barrier function.

Here, we aimed at describing the effect of dysbiosis on cardiac muscle tissue at the structural and ultrastructural level to explore the eventual role of the microbiota as a possible inexpensive and feasible new avenue to prevent the onset of cardiovascular diseases and/or to improve outcomes.

In detail, Friend leukemia virus B (FVB) mice were treated with the antibiotics vancomycin dissolved in drinking water in order to induce dysbiosis in the intestine, or left untreated (CT). At the sacrifice, the heart was removed and immediately processed for light and transmission electron microscope (TEM) evaluations. The spleen has been collected for the analysis of the immune phenotype by cytofluorimetric analysis (FACS).

Preliminary results on the immune profile revealed a slight decrease in the GZMB+NK cells in vancomycin-treated mice, compared to CT. Light microscopy analysis of hematoxylin-eosin-stained sections of the heart did not reveal any evident morphological differences induced by dysbiosis. TEM analysis confirmed the preserved ultrastructure in both the experimental conditions, and mitochondria exhibited a similar structure, characterized by evident lamellar cristae.

Overall, these preliminary findings suggest that the general structure of cardiac muscle tissue is not affected by dysbiosis. However, further analysis is needed to confirm this suggestion and to better characterize the effect of dysbiosis at the ultrastructural level.

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An experimental model for fibroblasts to myofibroblasts transition on mechano-mimetic polyacrylamide hydrogels

Flaminia Chellini[1], Alessia Tani[1], Rachele Garella[2], Daniele Martella[3,4], Alice Cappitti[3], Francesco Palmieri[2], Camilla Parmeggiani[3], Sandra Zecchi[1], Roberta Squecco[2], Chiara Sassoli[1]

[1] Dept of Experimental and Clinical Medicine, Section of Anatomy and Histology, Imaging platform, University of Florence, Italy
[2] Dept of Experimental and Clinical Medicine, Section of Physiological Sciences, University of Florence, Italy
[3] European laboratory for Non-Linear Spectroscopy (LENS), University of Florence, Italy
[4] Italian National Institute of Metrology Research INRiM, Turin, Italy

Myofibroblasts are considered the cells primarily responsible for the formation of a transient scar required to restore the integrity and preserve the function of injured tissues. They derive mainly from the differentiation of fibroblasts in the extracellular matrix (ECM), promoted by the combined action of pro-fibrogenic factors, particularly transforming growth factor (TGF)-β1 and of mechanical stimuli in the damaged microenvironment. They exhibit the features of both collagen-synthetically active fibroblasts and smooth muscle cells showing *de novo* expression of α-smooth muscle actin (sma) and, although they are not regarded as electrically excitable cells, peculiar biophysical properties and trans-membrane ion currents typical of smooth muscle cells. As compared to fibroblasts, differentiated myofibroblasts are larger, have a polygonal shape and secrete higher amounts of collagen. In physiological conditions, once the tissue regeneration has accomplished, the scar will be degraded and myofibroblasts progressively disappear. By contrast, the generation of myofibroblasts and their persistence in an activated functional state are recognized as "core cellular mechanisms" of pathological fibrosis. High-throughput *in vitro* models of the fibroblast-myofibroblast transition appear mandatory for a comprehensive study of myofibroblast biology and fibrosis mechanisms, for the identification of smart therapeutic targets and novel antifibrotic therapy screening. Here we generated three mechano-mimetic substrates of polyacrylamide (PA) hydrogels by free radical polymerization with an accurate tailoring of water content and crosslinking degree to modulate the mechanical properties. The bulk compression modulus ranged from 29 to 1 kPa. NIH/3T3 fibroblasts were cultured on glass coverslips (control) and on PA hydrogels for 48 h in proliferation medium or in low serum condition in absence or presence of TGF-β1 and morpho-functionally analysed for myofibroblast differentiation. Preliminary results indicate that the optimal myofibroblastic differentiation occurs when the cells are cultured on PA hydrogels with a bulk compression modulus around 1 kPa in low serum condition plus TGF-β1, as judged by the confocal immunofluorescence analysis of α-sma expression, the cell shape evaluation and by the electrophysiological analysis of the biophysical and conductive properties. Notably, cells cultured on PA hydrogels in the absence of TGF-β1 showed stiffness-dependent differences in morpho-functional properties suggesting that mechanical stimuli especially from "tissue-soft" substrates are sufficient to induce a myofibroblastic phenotype acquisition. Experiments are ongoing to evaluate the involvement of stretch activated channels in the different cell responses and the potential cross-talk between mechano-transduction and TGF-β1 mediated pathways.

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Cancer-related cachexia: Focus of Beneficial Effect of Endurance Exercise on the Skeletal Muscle

Daniela D'amico[1], Giuseppe Donato Mangano[1], Martina Sausa[2], Filippo Macaluso[2], Francesca Rappa[1], Antonella Marino Gammazza[1], Francesco Cappello[1], Valentina Di Felice[1], Rosario Barone[1]

[1] Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo, Palermo, Italy
[2] SMART Engineering Solutions & Technologies (SMARTEST) Research Center, eCampus University, Palermo, Italy

Cancer-related cachexia is a multiorgan syndrome characterized by the loss of body weight with specific losses of skeletal muscle and adipose tissues. Skeletal muscle impairment involves inflammatory cytokines and following structural and metabolic dysregulations. Physical exercise has been proposed to the whole-body homeostasis through the modulation of anabolic and catabolic stimuli. Thus, the aim of our study was to investigate lifespan, tumor growth, cachexia onset, and skeletal muscle homeostasis on adult BALB/c AnNHsd mice subcutaneous inoculated with a fresh fragment of C26 colon carcinoma to induce cachexia and undergoing to different intensities endurance trainings. Some of them were maintained in a sedentary state (SED/I/SED), others were trained after being sedentary (SED/I/TRP), others were maintained in a trained state at low intensity after inoculation (TRP/I/TRL) and high intensity after inoculation (TRP/I/TRH). Uninoculated trained (SED/TR) and sedentary (SED/SED) mice were used as controls. Results showed a significant increase in the lifespan and in cachexia onset of the TRP/I/TRH group. Immunofluorescence were performed on tumor, gastrocnemius, plantaris, and soleus muscles to detect the expression of Heat shock protein 60 (Hsp60), Isolectin, and Peroxisome proliferator- activated receptor-gamma coactivator 1 alpha (Pgc1α) proteins as a marker of oxidative stress, vascularization, and mitochondrial biogenesis, respectively. Hsp60 expression in soleus muscle compared with plantaris and gastrocnemius muscles was higher in the trained groups. Immunoreactivity of Isolectin was significantly increased in white gastrocnemius and soleus muscles from SED/I/TRP and TRP/I/TRH groups compared to the not-bearing tumor groups. In plantaris and red gastrocnemius muscles from TRP/I/TRH group was assessed a higher level of Isolectin compared to the SED/SED group, while the amount of this protein was significantly increased in the SED/I/TRP group compared to both SED/SED and SED/TR groups in the same muscles. Pgc1α showed higher protein levels in soleus, plantaris, and red gastrocnemius muscles from SED/I/TRP and TRP/I/TRH groups than SED/SED group. Therefore, the molecular pathways triggered by the health effects of physical exercise need to be investigated to develop new pharmacological therapies for oncological individuals affected by cachexia.

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Effect of SARS-CoV-2 infection on placental vascularization

Angelica Perna[1], Eleonora Hay[2], Paolo De Blasiis[2], Marco La Verde[3], Francesca Caprio[3], Carmine Sellitto[2], Germano Guerra[1], Alfonso Baldi[4], Angela Lucariello[5], Antonio De Luca[2]

[1] Department of Medicine and Health Sciences "Vincenzo Tiberio", University of Molise, Campobasso, Italy
[2] Department of Mental and Physical Health and Preventive Medicine, Section of Human Anatomy, University of Campania "Luigi Vanvitelli", Naples, Italy
[3] Department of Woman, Child and General and Specialized Surgery, Obstetrics and Gvnecology Unit University of Campania "Luigi Vanvitelli", Naples Italv
[4] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Università degli Studi della Campania "L Vanvitelli", Caserta, Italy
[5] Department of Sport Sciences and Wellness, University of Naples "Parthenope", Naples, Italy

When a new infectious agent is discovered that leads to the outbreak of a pandemic, as in the case of Covid-19, the first thing we ask is whether it has the ability to be transmitted through the placenta, causing adverse effects, and the impact it may have on the normal physiology of the organ itself. For SARS-CoV-2 infection, transmission appears to be rare because the placenta does not appear to co-express high levels of the primary factors that facilitate virus entry, such as angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2).

What has been observed during this pandemic period is that SARS-CoV-2 infection, during pregnancy, is associated with a number of adverse outcomes, such as an increased risk of pre-eclampsia, preterm delivery, hypertensive disorders, gestational diabetes, and low birth weight, with the presence of vascular malformations in the placenta.

In the present research work we studied, by immunohistochemistry technique, the expression of CD34 and podoplanin (PDPN) as markers of vasculogenesis to find any differences between women with Covid-19 and healthy controls. Human placenta samples were obtained with informed consent from full-term women, all in a gestational period between 35-41 weeks; were collected control placentas and placentas from mothers who became infected during the first, second, and third trimesters of gestation. The most common symptoms of infection in mothers were fever, anosmia, ageusia and asthenia, and 12 were treated with paracetamol, corticosteroids and azithromycin. Fibrin deposits and lymphocyte infiltration in the villi, edema and thrombi were found as vascular changes. PDPN expression was found in the villous stroma as a plexiform network around the villous nucleus of fetal vessels; significant down-regulation is observed in the villous stroma of women infected during the III trimester compared with controls. CD34 shows no change in expression levels comparing control with pathological.

Certainly PDPN plays a key role in healthy placental vasculogenesis and thus in its proper physiology, and surely Covid-19 alters its normal expression; further studies are deemed necessary to understand what mechanisms are being altered to try to avoid possible complications for both mother and fetus in the contagions that will still occur.

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Raf-1 Kinase Inhibitor Protein (RKIP) onco-suppressor gene: A new biomarker for ovarian cancer?

Stefania Greco[1], Pamela Pellegrino[1], Federica Giantomassi[2], Giovanni Delli Carpini [3], Gaia Goteri[2], Andrea Ciavattini [3], Pasquapina Ciarmela[1]

[1]Department of Experimental and Clinical Medicine, Universita` Politecnica delle Marche, 60020 Ancona, Italy
[2] Department of Biomedical Sciences and Public Health, Universita` Politecnica delle Marche, 60020 Ancona, Italy
[3]Department of Medical Biotechnology and Department of Molecular and Developmental Medicine, Università Politecnica delle Marche, 60020 Ancona, Italy

Ovarian cancer (OvCa) is the most common and most lethal gynecological cancer in women, in fact it has a high rate of mobility and mortality. The diagnosis of OvCa is very difficult and usually occurs when the neoplasm is in an advanced stage [1-3]. The treatments currently in use are platinum-based chemotherapy and surgery, but it has been discovered that patients eventually relapse, developing a resistance to chemotherapy with a low chance of survival usually for stages III and IV [4]. The OvCa metastasizes mainly in the abdominal cavity, in particular in the omentum, it is a cancer considered highly invasive [5, 6]. The metastatic process is regulated by a complex cascade of event. A metastatic cancer cell escapes from a primary tumour, invades circulation, and it grows in a secondary area. Currently the metastasis could be reduced and stopped by inhibiting the metastasis suppressor gene, which would seem to block the signaling pathway that produces the metastasis [7, 8]. Several metastasis suppressor genes have been identified (e.g. Nm23, KISS1, KAI1, BRMS1, TIMPs, E-cadherin, MKK4, TXNIP, CRSP3, DRG-1 and RhoGDI2). Recent studies have shown that the Raf-1 Kinase Inhibitor Protein (RKIP) is a metastasis suppressor gene, infact RKIP is absent or reduced in cell lines derived from metastatic prostate cancer [9] breast cancer [10] melanoma cells [11] as well as in begnign tumoral cells such us leiomyoma cells [12].

The aim of this study was to evaluate the RKIP expression in OvCa tissues, represented by serous borderline tumors, low-grade and high-grade serous carcinomas. The results obtained with the immunohistochemical technique are in line with the results in the literature. Indeed, the expression of RKIP decreases with increasing aggression or metastasis of tumors, and in fact in OvCa the expression of RKIP was almost completely absent. Furthermore, we analyzed a second aspect. From the data in the literature it emerges that fibronectin increases expression as metastases increase [13]. We wanted to evaluate the effect of fibronectin on OvCa tissues and bordelines. Borderline ovarian tumors represent a non-invasive tumor of uncertain malignant potential with characteristic histology. They occur in younger women, are present at an early stage, and have a favorable prognosis, but symptomatic recurrence and death may be found as long as 20 years after therapy in some patients. The molecular changes in borderline ovarian tumors indicate linkage of this disease to type I ovarian tumors (low-grade ovarian carcinomas) [14]. Data showed that the expression of fibronectin increases with increasing aggressiveness of the disease, while the expression decreases or is completely absent in the borderline.

This study established that RKIP could be a good marker for identifying the aggressiveness of the gynecological pathologies studied, which are currently histopathologically difficult to understand.

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Mitochondrial morphology and bioenergetics in C2C12 myotubes

Isola R[1], Lai Y[1], Hoppel CH[2], Noli R[1]

[1] Dept Biomedical Sciences, Univ of Cagliari, Division of Cytomorphology, Italy
[2] Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA

Previous mitochondrial ultrastructural studies used two-dimensional models to observe cristae morphology and/or assessed mitochondria bioenergetics [1,2]. However, no study investigated on the relationship between the three-dimensional (3-D) structure of the cristae and the bioenergetic activity of the cells on a relevant number of mitochondria.

This preliminary study compares the 3-D structure of cristae, by high resolution scanning electron microscopy (HRSEM), with mitochondrial bioenergetics. We also tested the efficacy of two permeabilizing agents, digitonin and α-chaconine, on oxidative phosphorylation (OXPHOS) detection and on perturbation of mitochondrial morphology in whole cells.

To this end, we used mouse myoblasts cell cultures (C2C12) after myotube differentiation. Mitochondrial OXPHOS has been assessed by a Clark-type electrode on digitonin- or α-chaconine- permeabilized myotubes [3]. Bioenergetics has been tested by adding substrates against complex I, II, III, IV, a lipid substrate and an uncoupler. We applied osmium maceration technique [4] on myotubes, treated with or without permeabilizing agents, or on undifferentiated myoblasts, for three-dimensional observation by HRSEM.

Titration of both permeabilizing agents was performed by oxygraphy. The resulted best concentrations were: 0.008% for digitonin and 30 μM for $\boldsymbol{\alpha}$-chaconine. These concentrations were tested for ultrastructural morphology.

The basic mitochondrial morphology revealed pleomorphic mitochondria, that were thin and elongated, or short and roundish. Mitochondrial cristae in thin mitochondria were mainly lamellar, while in roundish mitochondria were more various. In general, a manifest change of cristae appearance was not evident after adding the permeabilizing agent, regardless of digitonin or α-chaconine administration. Untreated myoblasts displayed similar morphologies.

Bioenergetic data were consistent in digitonin and α-chaconine treated cells, but a slight additional efficacy for digitonin was observed.

In conclusion, no apparent differences in cristae morphology were detected in myotubes after either digitonin or α-chaconine cell membrane permeabilization.

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A possible phylogenetic arrangement of the Dystrophin Glycoprotein Complex in low- and high-ranking baboons

Giovanna Vermiglio[1], Davide Labellarte[1], Erika Biondo[1], Chiara Busacca[1], Valentina Randazzo[1], Maria Righi[1], Alba Migliorato[1].

[1] University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Messina, Italia

The dystrophin-glycoprotein complex is a multimeric system made up of the sarcoglycan sub-complex, the sarcomplasmatic complex and the dystroglycans complex. The sarcoglycan sub-complex is a protein transmembrane system made up of six isoforms: alpha, beta, gamma, delta, epsilon and zeta-sarcoglycans. In muscle tissue it is involved in cytoskeleton-extracellular matrix connection stabilizing the sarcolemma during muscle activity and it plays a role in force transduction. This protein system is also expressed in muscle of non-human primates such as chimpanzees and baboons and its expression change depending on the social ranking of primates. The aim of the present study was to analyse, by immunohistochemistry, the expression of other proteins that interact with sarcoglycan complex such as dystroglycans, dystrophin and laminin in masseter and sternocleidomastoid muscles of low- and high-ranking baboons. Our results have shown that the expression of beta dystroglycan changes depending on the social ranking while laminin and dystrophin are always expressed both in low- and high-ranking baboons. These data suggest us that only the expression of the transmembrane proteins of the dystrophin-glycoprotein complex is influenced by the social ranking and that could probably depend on a phylogenetic arrangement.

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Human osteoblasts cultured in the presence of U73122, inhibitor of phosphoinositide specific phospholipase C enzymes

Matteo Corradini[1], Marta Checchi[1], Serena Truocchio[1,2], Carla Palumbo[1], and Vincenza Rita Lo Vasco[1]

[1] Section of Human Morphology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Italy
[2] Biomedical and Neuromotor Sciences - DIBINEM, University of Bologna, Italy

U-73122 [1-(6-((17β-3-methoxyestra1,3,5(10)-trien-17-yl) amino) hexyl) -1H-pyrrole-2,5-dione] is an amino-steroid of clinical-translational interest, widely used for its ability to inhibit Phospholipase C specific phosphoinositide (PLC) enzymes. Beside the temporary block of the enzymatic activity, U-73122 owns off-target effects, including modulation of the expression of selected *PLC* genes in osteosarcoma cell lines and different localization of PLC enzymes in tumor cells depending on the cell line. It was demonstrated that different PLC isoforms in the nucleus, cytoplasm or both. Nuclear PLCs play important roles in gene regulation. Some isoforms were claimed to play a role in force development according to conventional models of PLCs located either at or near the plasma membrane.

As PLC enzymes were demonstrated to be involved in the differentiation of osteoblasts, we cultured human osteoblasts (hOBs) in the presence of U-73122 treatment in order to evaluate possible effects upon *PLC* gene expression and PLC enzymes localization compared to untreated hOBs.

Our results confirm literature excluding toxicity of U-73122 upon cell survival. Although further quantitative analyses are required, U-73122 treatment had no ON/OFF effect upon the expression of *PLC* genes. U-73122 treatment affected the localization of PLC enzymes. PLCs were observed at both the nuclear and cytoplasmic levels, as well as in vesicles in the cytoplasm (an event that mainly characterized the PLC β and δ subfamilies) or in vesicles at the cell membrane in pseudopod-like structures (mainly PLC β2 and PLC β3) and in membrane ruffles.

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Adipose tissue of the face. It is everywhere the same?

Marina Protasoni[1], Andrea Brambilla[1], Piero Antonio Zecca[1], Mario Raspanti[1], Dario Bertossi[2], Marcella Reguzzoni[1]

[1] Dipartimento di Medicina e Chirurgia, Università degli Studi dell'Insubria
[2] Dipartimento di Scienze chirurgiche, odontostomatologiche e materno-infantili, Università degli Studi di Verona

Subcutaneous adipose tissue of the face is organised in different fat pads subdivided in superficial and deep layer. Although on macroscopical observation the facial fat pads appear to be similar, during ageing they underwent to a different modification. Starting from this evidence, we decide to evaluate if some differences should be described at microscopical level.

To start to investigate the structure and the ultrastructure of the facial fat pads, and to obtain some details of their three-dimensional architecture, light and scanning electron microscopy were performed on samples harvested from 10 patients during maxillary surgery. 6 samples were harvested from Bichat fat pad and 4 samples were harvested from superficial fat pads.

Light and scanning microscopy demonstrate that adipose tissue of superficial and deep layers are different both for the adipocyte's dimensions and for the different organisation of the collagen fibres that envelope the adipocytes.

These results represent the first step of our study, further experiments are now undergoing in order to better evaluate the differences between superficial and deep layer, but also between patients of different age and/or different BMI.

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VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI SIAI, 2021

SIAI Società Italiana di Anatomia e Istologia

VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI DELLA SOCIETÀ ITALIANA DI ANATOMIA E ISTOLOGIA (SIAI) TENUTASI PRESSO L'AULA MAGNA DEL DIPARTIMENTO DI SCIENZE BIOMEDICHE E NEUROMOTORIE (DIBINEM), ISTITUTI ANATOMICI, UNIVERSITA' DI BOLOGNA, E ONLINE SUL LINK RISERVATO AGLI ISCRITTI.

In data 25 Settembre 2021, alle ore 15:00, in seconda convocazione, ha avuto luogo, in presenza, presso l'Aula Magna del Dipartimento di Scienze Biomediche e Neuromotorie (DIBINEM), Istituti Anatomici, Università di Bologna, e online sul link riservato agli iscritti, l'Assemblea Generale dei Soci della Società Italiana di Anatomia e Istologia per discutere il seguente Ordine del Giorno:

1) Comunicazioni del Presidente.
2) Relazione del Tesoriere sui rendiconti finanziari del 2019 e 2020 e sulle previsioni finanziarie per il 2021 e 2022. Relazione dei Revisori dei Conti.
3) Modifiche dello Statuto e del Regolamento.
4) Attività dei Collegi dei Docenti di Anatomia e di Istologia ed Embriologia. Relazione dei Presidenti o dei loro Delegati.
5) Aggiornamento sull'Italian Journal of Anatomy and Embryology.
6) Aggiornamento sul Sito web della SIAI.
7) Assegnazione Premio alla Carriera.
8) Assegnazione Premi Ricercatori under 40.
9) Assegnazione Premio Migliore Comunicazione Orale.
10) Assegnazione Premi Poster.
11) Prossimi Congressi Nazionali della SIAI: proposte di temi di relazione.

12) Proposta di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.

13) Commemorazione dei Soci scomparsi.

14) Risultati elettorali e composizione del nuovo Consiglio Direttivo.

15) Varie ed eventuali.

Presiede la riunione il Presidente della SIAI, Prof. Giuseppe Anastasi; funge da Segretario Verbalizzante il Prof. Roberto Di Primio.
La Segreteria Organizzativa informa il Presidente che alle ore 15:10 sono presenti in Aula 90 Soci, mentre sono collegati in via telematica 83 Soci (**Allegati N.1** e **2**), per un totale di 173 Soci.
Il Presidente dichiara aperta l'Assemblea e procede alla discussione dell'Ordine del Giorno.

1. **Comunicazioni del Presidente.**

Il Presidente saluta i Soci presenti e quelli collegati online. Dichiara che non ci sono comunicazioni e passa alla discussione del secondo punto all'OdG.

2. **Relazione del Tesoriere sui rendiconti finanziari del 2019 e 2020 e sulle previsioni finanziarie per il 2021 e 2022. Relazione dei Revisori dei Conti.**

Il Presidente dà la parola al Prof. Gianpaolo Papaccio, Tesoriere della SIAI. Il Prof. Papaccio illustra all'Assemblea sia i Bilanci Consuntivi degli anni 2019 e 2020 che le Previsioni Finanziarie degli anni 2021 e 2022 con relative Relazioni di Accompagno (**Allegati N. 3-6**). Tali Bilanci sono già stati inviati a tutti i Soci in modo che essi potessero averne contezza ed analizzarli prima dell'Assemblea. Il Tesoriere informa l'Assemblea che i Revisori dei Conti hanno già depositato la loro relazione (**Allegato N.7**) dalla quale si evince che non ci sono irregolarità nei Bilanci, che la SIAI è in attivo e che il numero dei Soci morosi risulta in drastica riduzione.
Il Tesoriere inoltre si sofferma sul fatto che molti Soci:

- non provvedono al pagamento annuale della quota, che è modesta, nel mese di Gennaio o nei primi mesi dell'anno, onde permettere una normale gestione della Società e una distribuzione delle spese;
- tendono a regolarizzare la loro posizione solo in prossimità delle elezioni del nuovo Direttivo, ossia ogni tre anni;
- pagano mediante i Dipartimenti, i cui bonifici sono sovente indecifrabili e talvolta non indicano quale sia il o i Soci cui attribuire la somma versata.

Tutto quanto sopra determina evidenti problemi e squilibri nella programmazione delle spese e obbliga il Tesoriere a tenere una somma di denaro come riserva, ben superiore a quella che sarebbe necessaria, ad evitare di andare in passivo.

Orbene ciò non è più possibile anche per riuscire a perseguire fino in fondo i fini sociali della SIAI e poter prendere delle iniziative per incentivarne l'attività scientifica.

Il Tesoriere ricorda di aver dovuto pregare il Presidente di limitare i fondi dedicati alla attribuzione di eventuali premi, proprio perché negli anni 2019 e 2020 la raccolta delle quote era stata scarsa. Allo stato, dopo l'invio di numerose mail, molti Soci hanno regolarizzato la loro posizione e quasi 300 sono in regola.

Tuttavia, si dovrà continuare con un regolare pagamento annuale, altrimenti occorrerà trovare altre soluzioni visto che l'attuale attivo, mai avuto, di circa 60.000,00 Euro non può fare vivere la SIAI per ben 3 anni.

Il Presidente si congratula con il Tesoriere per il lavoro svolto e per i risultati ottenuti nella riscossione delle quote arretrate. Egli ricorda all'Assemblea l'esistenza della norma statutaria che prevede la cancellazione dall'Elenco dei Soci a fronte di una morosità di oltre due anni.

Indi il Presidente chiama l'Assemblea a votare sul punto 2 all'OdG.

L'Assemblea approva all'unanimità.

3. Modifiche dello Statuto e del Regolamento.

Il Presidente ricorda ai presenti che il Consiglio Direttivo ha proposto alcune Modifiche dello Statuto e, conseguentemente, del Regolamento ed ha dato incarico alla Prof. Gigliola Sica di farsi carico di consultare un Notaio di sua fiducia per controllare la stesura di tali Modifiche ed introdurre tutte quelle necessarie per l'accreditamento della SIAI presso il Ministero della Salute, secondo il Decreto Ministeriale del 2/08/2017.

La Prof. Sica, collegata online, è invitata dal Presidente ad illustrare le Modifiche dello Statuto e del Regolamento delle quali si è interessata, sottoponendole alla supervisione del Notaio, e quelle introdotte dallo stesso Notaio, Modifiche che vengono oggi proposte all'approvazione dell'Assemblea (**Allegati N. 8** e **9**). La documentazione relativa è stata già portata a conoscenza dei Soci, affinché avessero il tempo di esaminarla. La Prof. Sica ricorda che lo Statuto dell'Associazione è stato registrato a Roma, Ufficio Atti Pubblici il 18/2/1999; ha visto modifiche del 30/12/2004 e, successivamente, ulteriori modifiche approvate dall'Assemblea Generale dei Soci in data 21/9/2012. Su questa base si è proceduto a rivedere il testo per cui, allo stato, nel testo attuale, figurano **delle parti non in grassetto** relative alle Modifiche già passate in Assemblea nel 2012 (che vedono solo alcune piccole correzioni), da confermare; **delle parti in grassetto** relative a delle Modifiche richieste dal Direttivo della SIAI e **delle parti in grassetto sottolineato** introdotte dal Notaio, secondo il Decreto Ministeriale del 2/08/2017, utili all'accreditamento della Società presso il Ministero della Salute. La Prof. Sica procede alla lettura di tutti gli Articoli dello Statuto e, relativamente alle Modifiche richieste dal Direttivo, sottolinea l'introduzione nell'Art. 5 dello Statuto di una frase che si riferisce **al fatto che la SIAI cura la pubblicazione dell'Italian Journal of Anatomy and Embryology** e nell'Art.14 di una frase **che include nel patrimonio**

dell'Associazione gli incassi del contributo per la stampa degli articoli pubblicati sull'Italian Journal of Anatomy and Embryology e ogni altra entrata ammessa dalle norme vigenti in materia. La Prof. Sica informa l'Assemblea che tra gli Organi dell'Associazione **sono stati introdotti il Collegio dei Probi Viri e il Comitato Scientifico**. Ella comunica all'Assemblea che è necessario, all'Art.4 dello Statuto, indicare la sede legale dell'Associazione che non può essere nella città di residenza del Tesoriere e propone che tale sede sia nel Dipartimento di Scienze della Vita e Sanità Pubblica, Sezione di Istologia ed Embriologia, Università Cattolica del Sacro Cuore, Largo Francesco Vito, 1- 00168 Roma. Successivamente legge e commenta anche il Regolamento, dove, su input del Direttivo, sono state introdotte Modifiche relative all'Art.4 (indicando **che l'Italian Journal of Anatomy and Embryology è edito a cura di Firenze University Press, proprietà dell'Università degli Studi di Firenze, e che esso sarà diffuso online con free access)** e all'Art. 7 (**pagamento delle quote sociali tramite il Sito web della Società**). E' stato infine aggiunto l'Art.12 **concernente i Probi Viri.**

Dopo alcuni chiarimenti richiesti dai Soci, lo Statuto e il Regolamento vengono approvati all'unanimità non prima che il Presidente abbia ringraziato, anche a nome del Direttivo, la Prof. Sica per il gravoso lavoro svolto e si sia congratulato per la chiara esposizione delle Modifiche. Il Presidente chiede all'Assemblea di poter delegare la Prof. Sica a depositare gli atti presso il Notaio. L'Assemblea approva all'unanimità.

4. **Attività dei Collegi dei Docenti di Anatomia e di Istologia ed Embriologia. Relazione dei Presidenti o dei loro Delegati.**

Il Presidente invita il Prof. Raffaele De Caro e il Prof. Gianpaolo Papaccio, in qualità rispettivamente di Presidenti del Collegio dei Docenti di Anatomia e del Collegio dei Docenti di Istologia ed Embriologia, ad aggiornare l'Assemblea sul lavoro svolto e sulle attività in corso.

Il Prof. De Caro, Presidente del Collegio dei Docenti di Anatomia, presenta la relazione sull'attività svolta dal Collegio nell'anno accademico 2020/2021. Le riunioni si sono svolte in modalità telematica in data:

3 Giugno 2020 con argomento: Riflessioni sulla didattica online.

25 Settembre 2020 con i seguenti argomenti:

a) Riflessioni sulla nuova legge sulla donazione del corpo a scopo didattico e scientifico con riaffermazione della centralità dell'Anatomia Umana.
b) Attività didattica del nuovo anno accademico. E' stata ribadita l'importanza del rapporto diretto Docente/Studenti per l'insegnamento dell'Anatomia ed è stata ribadita la necessità della modalità sincrona nel caso in cui si debba ricorrere alla didattica a distanza.

1° Dicembre 2020 con argomento: aggiornamento sulla didattica blended e sulla legge della donazione del corpo a scopo didattico.

12 Febbraio 2021 con argomento: ulteriore aggiornamento sulla didattica blended e sulla legge sulla donazione del corpo a scopo didattico.

26 Aprile 2021 con argomento: stesura del contributo del Collegio in tema di Abilitazione Scientifica Nazionale.

Il Presidente ha inoltre partecipato alle riunioni dell'Intercollegio di Area Medica, tenutesi in modalità online in data 8 Giugno 2020 e 15 Dicembre 2020, mentre il Segretario del Collegio, Prof. Andrea Montella, ha partecipato alla riunione dell'8 Giugno 2021 svoltasi in presenza a Roma.

Riunioni plenarie del Collegio in modalità telematica si sono svolte in data 11 Giugno e 27 Luglio 2021. I principali documenti prodotti sono stati:

a) Stesura dell'articolo: The Italian law on body donation: A position paper of the Italian College of Anatomists, di De Caro, R. et al., pubblicato su Ann Anat 2021 Nov; 238: 151761. Doi: 10.1016/j. aanat.2021.151761.
b) Contributo del Presidente al Tavolo Tecnico della SIAI sulla legge "Norme in materia di disposizione del proprio corpo e dei tessuti post-mortem a fini di studio, formazione e di ricerca scientifica".
c) Contributo del Presidente al Tavolo Tecnico Ministeriale per l'individuazione dei Centri di Riferimento e del Regolamento e Decreti Attuativi per l'applicazione della legge di cui sopra.

Altri argomenti affrontati sono stati:

Riflessioni sull'attività didattica, aa 2021/2022.

Censimento e presentazione della formazione post-lauream in Anatomia.

Il Presidente, Prof. Anastasi, ringrazia il Prof. De Caro e dà la parola al Prof. Papaccio.

Il Prof. Papaccio, Presidente del Collegio dei Docenti di Istologia ed Embriologia, illustra all'Assemblea quanto il Collegio ha fatto dal Settembre 2019 al Settembre 2021.

Il Collegio dei Docenti d'Istologia ed Embriologia, in questo biennio, caratterizzato dall'impossibilità a vedersi di persona, ha da subito adoperato piattaforme quali Zoom o Meet.

Pertanto l'attività non si è mai interrotta e si è proceduto ad effettuare:

- n. 11 Riunioni della Giunta;
- n. 2 Assemblee di tutti gli Associati (26 ottobre 2020 e 26 febbraio 2021);
- n. 6 partecipazioni alle Riunioni dell'Intercollegio;
- n. 10 documenti/lettere ufficiali per varie fattispecie.

In particolare, nell'Assemblea del 26 Ottobre 2020:

- è stato approvato il Regolamento, che si affianca allo Statuto e che si prega di voler inserire nel nuovo sito della SIAI;
- si sono svolte le elezioni di n. 2 Membri della Giunta, il cui primo mandato era in scadenza;
- sono stati istituiti premi per le migliori presentazioni che si sono tenute, come di consueto, nella Seduta Scientifica associata, nel pomeriggio, all' Assemblea dei Soci del Collegio.

Nell'Assemblea del 26 febbraio 2021:

- si è proceduto alla elezione del Presidente per scadenza del suo primo mandato triennale. Il Prof. Papaccio è stato rieletto per acclamazione. Indi sono stati allo stesso modo rieletti n. 4 componenti della Giunta, anch'essi per scadenza del loro primo mandato triennale;
- si è proceduto alla modifica della Declaratoria del Settore in vista di una possibile revisione dei SSD, declaratoria che è poi stata inviata al Prof. Pedone, rappresentante dei Professori Ordinari (P.O.) dell'Area 05 al CUN;
- è stato istituito il Premio Monesi-Rizzoli per la migliore ricerca in Istologia ed Embriologia Medica (invitati i Proff. Lia Guidotti e Mario Molinaro in ricordo dei loro Maestri).

La prima edizione del Premio è stata vinta da una giovane dottoranda di Roma «La Sapienza», che ha prevalso nel giudizio della giuria su un totale di 19 comunicazioni;

- si è discusso, infine, delle sedi, dei vari problemi e della numerosità di P.O. In particolare, si è sottolineato che si è avuto un ricambio generazionale profondo e che sono ben 11 i P.O. di nuova nomina su 32 attuali P.O. del Settore. Sono previsti altri 10-12 bandi negli anni 2022 e 2023 e 4 passaggi di Settore a fronte di 2 soli pensionamenti, per cui il periodo di discesa sembrerebbe superato.

Nella Giunta del 31 marzo 2021:

- si è proceduto alla nomina del nuovo Segretario/Tesoriere nella persona della Prof. Monica Mattioli Belmonte da Ancona;
- alla pianificazione della Riunione dei P.O. per ASN 21/23, Riunione che si è poi concretizzata in Maggio;
- alla istituzione di un Seminario da tenersi da parte dei nuovi P.O. in servizio: primo Seminario, dopo la Giunta, per il 1° Ottobre 2021 (Prof. Maurilio Sampaolesi);
- si è discusso sulle modifiche della legge 240/2010 (proposte su reclutamento ed interlocuzioni con CUN, CRUI ed Intercollegio);
- si è proceduto all' analisi dei passaggi da SSD BIO/16 ed altri a BIO/17;
- alla programmazione 2022 per SEDI.

5. Aggiornamento sull'Italian Journal of Anatomy and Embryology.

Il Presidente invita il Prof. Domenico Ribatti, in qualità di Editor in Chief della rivista Italian Journal of Anatomy ed Embryology, a riferire sulla situazione della rivista.

Il Prof. Ribatti comunica che nel triennio 2019-2021 in cui Egli, come Direttore Scientifico, ed il Prof. Ferdinando Paternostro, come Direttore Esecutivo, hanno diretto la rivista, sono stati pubblicati 4 numeri (Volume 123, N.3, 2018; Volume 124, N. 1, 2 e 3, 2019) per un numero complessivo di 84 articoli. Il Comitato Tecnico Scientifico è stato completamente rinnovato e sono stati pubblicati anche contributi di Soci prestigiosi della SIAI, come i Proff. Cinti, Sforza, Manzoli, Gulisano, Orlandini, Familiari, Mazzarello, Natali e Cocco. Allo stato attuale, 20 articoli sono stati accettati per la pubblicazione e altri 30 sono in standby. Nel contempo, si sono susseguite una serie di controversie

di carattere amministrativo con la FUP che hanno impedito una regolare pubblicazione del giornale. Queste questioni hanno recentemente trovato una soluzione attraverso la sottoscrizione con la FUP di un nuovo contratto di edizione che prevede che gli autori dei lavori accettati per la pubblicazione paghino i costi di stampa degli articoli alla Società, che provvederà a pagare alla FUP un importo forfettario per la stampa del numero della rivista. Inoltre, il nuovo contratto prevede la pubblicazione di due numeri per anno insieme ad un supplemento che raccoglie gli Abstracts del Congresso della Società.

Il Presidente, unitamente all'Assemblea, prende atto degli sforzi in cui si sono prodigati i Proff.: Ribatti e Paternostro per conservare la rivista e proiettarla verso un futuro più stabile e adeguato al suo ruolo quale organo ufficiale della SIAI.

6. Aggiornamento sul Sito web della SIAI.

La Prof.ssa Zecchi viene invitata dal Presidente della SIAI a relazionare sul sito Web della Società. Il sito di SIAI necessita di una riorganizzazione del menu di navigazione e dei suoi contenuti interni in modo da renderli fruibili e accessibili all'utente. Ha un layout che può essere migliorato e reso più accattivante e creativo ma allo stesso tempo istituzionale. E' stata proposta un'opera di restyling e refactoring del sito avendo come punto di riferimento i suoi destinatari: i Soci. Sulla base dei contenuti attuali del sito la navigazione è stata resa più semplice anche attraverso un layout in linea con gli standard di web design attuali. Il nuovo menu di navigazione prevede delle voci principali, due Call to action ben evidenti e delle voci secondarie ma sempre visibili. Sia la SIAI che i due Collegi sono stati evidenziati, senza dimenticare i contributi dei Soci, i Congressi, le News e gli Eventi proposti. Nella voce "SIAI" si trovano tutte le informazioni relative alla Società con dei rimandi al contenuto interno. Viene illustrata la Storia e presentati l'Elenco dei Soci, i Membri del Consiglio Direttivo, le Sedi, lo Statuto e il Regolamento. Tutte le informazioni sono inserite in un'unica pagina con link interni così che l'utente sia guidato nella navigazione e scopra i contenuti di cui ha bisogno. In particolare per le Sedi, è prevista una pagina specifica dove vederle tutte. È stata stilata una lista di tutte le informazioni standard che ogni Sede deve compilare e aggiornare. Ci sono due possibili modelli per la descrizione della Sede. Per entrambi i Collegi sono state realizzate due pagine distinte ma con gli stessi contenuti: una descrizione del Macrosettore, Collegio Docenti, Struttura, Statuto, Regolamento, Sede e una Call to action dove si invita a visitare la pagina "Diventa Socio". La pagina "Diventa Socio" presenta tre possibilità con cui associarsi: iscrizione generica alla Società, iscrizione al Collegio dei Docenti di Anatomia oppure al Collegio dei Docenti di Istologia ed Embriologia. Cliccando in uno dei tre box, si aprirà una pagina dedicata con un form in cui poter inviare la domanda di iscrizione che poi sarà approvata dal Collegio. Di seguito sono riportate alcune informazioni sui referenti e sul pagamento della quota annuale. Per il pagamento o il rinnovo della quota sociale è prevista una pagina dedicata in cui poter fare il versamento direttamente sul sito

web. E' necessario condividere i gateway di pagamento Paypal e Stripe in modo da poter sviluppare la pagina e renderla funzionante.
Il Presidente ringrazia la Prof. Zecchi anche a nome dell'Assemblea.

7. Assegnazione Premio alla Carriera.

Il Presidente prende la parola e riferisce sui lavori della Commissione composta dai Proff.: Amelio Dolfi, Sandra Zecchi, Carlo Tacchetti ed Antonio Filippini, preposta all'attribuzione del Premio alla Carriera e nominata dal Consiglio Direttivo in data 12/07/2021. La Commissione, dopo aver preso in esame le candidature pervenute (Prof. Roberto Di Primio, Prof. Rosario Donato, Prof. Elisabetta Falcieri), sottolinea l'eccellente percorso accademico che caratterizza le carriere di tutti i candidati. Tuttavia, dopo ampio dibattito, all'unanimità propone l'assegnazione del Premio alla Carriera al Prof. Roberto Di Primio. Il premio viene conferito con la seguente motivazione: "Oltre all'eccellente curriculum accademico, il Prof. Di Primio si è dimostrato sempre molto attivo nel perseguire le finalità della SIAI sia come Socio sia ricoprendo le cariche di Membro del Direttivo e di Segretario. (**Allegato N. 10**).
L'Assemblea approva la proposta della Commissione ed il Presidente si congratula con il Prof. Di Primio.

8. Assegnazione Premi Ricercatori under 40.

Il Presidente dà lettura dei risultati della Commissione nominata dal Consiglio Direttivo, in data 12/07/2021, composta dai Proff.: Gigliola Sica, Stefania Montagnani e Gianpaolo Papaccio per l'attribuzione di due premi a Ricercatori under 40. La Commissione ha preso atto delle candidature pervenute ed ha dapprima controllato l'iscrizione dei Candidati alla SIAI e la regolarità del versamento delle quote di iscrizione. Successivamente ha valutato approfonditamente i curricula, la produzione scientifica dei Candidati (in particolare le tematiche di ricerca per verificarne la congruenza e la non frammentarietà) e i loro indici bibliometrici. Sulla base di tale valutazione, la Commissione dichiara all'unanimità vincitrici le Dott.sse: Diomede Francesca e D'Amico Agata Grazia (**Allegato n. 11**).
L'Assemblea approva i lavori della Commissione e il Presidente si congratula con le vincitrici, che vengono invitate a ritirare la pergamena oltre che il premio in denaro di duemila Euro.

9. Assegnazione Premio Migliore Comunicazione Orale.

Il Presidente dà lettura dei risultati della Commissione nominata dal Consiglio Direttivo, in data 22/09/2021, composta dai Proff.: Guido Cavaletti, Roberta Di Pietro, Fabrizio Michetti, Paolo Onori, Luca Tamagnone e Sandra Zecchi Orlandini, per l'attribuzione del Premio alla Migliore Comunicazione Orale. La Commissione dichiara all'unanimità vincitrice la Dott.ssa Elena Stocco (**Allegato N. 12**).

L'Assemblea approva i lavori della Commissione e il Presidente si congratula con la vincitrice che avrà un premio in denaro di mille Euro.

10. Assegnazione Premi Poster.

Il Presidente dà lettura dei risultati della Commissione nominata dal Consiglio Direttivo, in data 22/09/2021, per l'attribuzione di due Premi per il miglior Poster, composta dai Proff.: Antonio De Luca, Giuseppe Santoro e Pietro Gobbi. Risultano vincitori i Dott: Stacchiotti e Sgarzi (**Allegato N.13**).

L'Assemblea approva i lavori della Commissione e il Presidente si congratula con i vincitori che usufruiranno dell'iscrizione gratuita al prossimo Congresso Nazionale della SIAI.

11. Prossimi Congressi Nazionali della SIAI: proposte di temi di relazione.

Il Presidente comunica che il Prof. De Caro ha riconfermato la disponibilità di ospitare il Congresso Nazionale della SIAI del 2022 a Padova in occasione degli 800 anni dalla costituzione dell'Università di Padova. Il Prof. De Caro, invitato dal Presidente, espone le motivazioni della richiesta e le potenzialità dell'Ateneo a poter organizzare il nostro prossimo Congresso. Il Presidente ringrazia il Prof. De Caro e l'Assemblea approva all'unanimità la sede del Congresso per l'anno 2022.

Il Presidente comunica che ha ricevuto una lettera dalle Proff. Carla Palumbo e Sandra Marmiroli dell'Università di Modena–Reggio Emilia, le quali si dicono disponibili ad organizzare uno dei prossimi Congressi nella loro sede.

Il Presidente si compiace della disponibilità delle Proff. Palumbo e Marmiroli ad organizzare quanto prima un Congresso della SIAI nella loro sede e le ringrazia sentitamente.

12. Proposta di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.

Il Presidente dà la parola al Prof. Di Primio che, in qualità di Segretario, aggiorna l'Assemblea sulla situazione delle domande di ammissione alla SIAI, pervenute durante gli anni 2020 e 2021. Le domande sono in tutto 42:

1. **Amelio Daniela**
2. **Barchi Marco**
3. **Bartolini Desirèe**
4. **Basile Giampaolo Antonio**
5. **Bianchi Francesca**
6. **Bielli Pamela**
7. **Boschetti Elisa**
8. **Caggiati Alberto**
9. **Canciani Elena**
10. **Cappella Annalisa**
11. **Centofanti Antonio**
12. **Chellini Flaminia**
13. **Consalez Giangiacomo**
14. **De Blasiis Paolo**

15. **De Felici Massimo**
16. **De Luca Ciro**
17. **Di Agostino Silvia**
18. **Di Giacomo Viviana**
19. **Di Sante Gabriele**
20. **Dobrowolny Gabriella**
21. **Dolci Susanna**
22. **Falletta Paola**
23. **Franco Caterina**
24. **Galli Carlo**
25. **Grimaldi Paola**
26. **Guizzardi Stefano**
27. **La Noce Marcella**
28. **Lo Vasco Vincenza Rita**
29. **Madaro Luca**
30. **Meregalli Cristina**
31. **Moretti Matteo**
32. **Panaro Maria Antonietta**
33. **Papa Veronica**
34. **Papaccio Federica**
35. **Pozzi Giulia**
36. **Riuzzi Francesca**
37. **Sancilio Silvia**
38. **Sciamanna Giuseppe**
39. **Taurone Samanta**
40. **Varano Gabriele**
41. **Vermiglio Giovanna**
42. **Virtuoso Assunta**

Il Prof. Di Primio richiama l'Assemblea alle regole dello Statuto che normano le modalità di iscrizione e sollecita ad uniformarsi ad esse al fine di evitare disguidi o ritardi nell'iscrizione.

L'Assemblea approva all'unanimità tutte le domande pervenute e dà mandato al Tesoriere di inviare ai nuovi Soci le norme per il pagamento della quota per l'anno 2022.

Il Segretario passa poi ad illustrare l'elenco dei 9 più 1 dei Soci Emeriti, dei quali due sono deceduti e ricorda che coloro ai quali è stato assegnato il Premio alla Carriera entrano di diritto nell'elenco dei Soci Emeriti. Attualmente solo il Prof. Damiano Zaccheo risulta insignito del titolo al di fuori dell'ottenimento del Premio.

Il Segretario mostra quindi l'elenco attuale dei 13 Soci Onorari. Tutti sono Ricercatori stranieri di grande prestigio. Il prossimo Consiglio Direttivo avrà il compito di rivedere tale elenco ed eventualmente aggiornarlo.

L'Assemblea prende atto degli elenchi proposti.

13. Commemorazione dei Soci scomparsi.

Il Presidente elenca i nominativi dei Colleghi deceduti negli ultimi due anni invitando a commemorarli i Soci che sono stati a più stretto contatto con loro.

La Prof. Elisabetta Falcieri commemora il Prof. Antonio Lauria, scomparso nel Giugno del 2020.

La Prof. Rita Rezzani commemora il Prof. Luigi Rodella, scomparso nel Settembre del 2020.

Il Prof. Lucio Cocco commemora la Prof.ssa Giuliana Piccari Scarpa, deceduta nel Dicembre del 2020.

La Prof.ssa Chiarella Sforza commemora il Prof. Giuliano Pizzini, scomparso nel Dicembre del 2020.

il Prof. Saverio Cinti commemora il Prof. Francesco Osculati, scomparso nel Maggio del 2021

Il Prof. Francesco Cappello commemora il Prof. Vincenzo Tessitore, scomparso nel Settembre del 2021.

Il Prof. Gianpaolo Papaccio commemora il Prof. Alberto Calligaro, scomparso nel Settembre del 2021.

14. **Risultati elettorali e composizione del nuovo Consiglio Direttivo**.

Il Presidente prima di dare la parola al Prof. Sergio Morini, Presidente della Commissione Elettorale per il rinnovo dei Componenti del Consiglio Direttivo per il triennio 2022-2024, costituita anche dai Proff. Sergio Castorina e Virginia Tirino e nominata in data 9/09/2021, si congratula con il Prof. Lucio Cocco per la sua elezione a nuovo Presidente del Consiglio Direttivo. L'Assemblea applaude alla comunicazione e quindi il Prof. Morini illustra i risultati ottenuti dai vari candidati come riportato nell' **Allegato N. 14.**

Il Presidente si congratula con la Commissione Elettorale e l'Assemblea approva all'unanimità i risultati presentati. Il Presidente infine esprime le sue felicitazioni ai Membri costituenti il nuovo Direttivo che entrerà nei suoi pieni poteri il 1° Gennaio 2022.

15. **Varie ed eventuali**.

Il Presidente, non essendoci argomenti da discutere al punto 15) all'OdG, dichiara conclusi i lavori dell'Assemblea. Ringrazia tutti i Soci in presenza e online e augura un buon lavoro al novo Consiglio Direttivo.

Il presente Verbale viene approvato seduta stante dall'Assemblea.

Il Presidente	Il Segretario Verbalizzante	Il Tesoriere
Prof. Giuseppe Anastasi	Prof. Roberto Di Primio	Prof. Gianpaolo Papaccio

ALLEGATO N.1

SOCI PRESENTI IN SEDE:

1.	Paola	Alberti
2.	Giuseppe Pio	Anastasi
3.	Francesca	Arnaboldi
4.	Pasquale	Bandiera
5.	Sara	Bandiera
6.	Valeria	Bertagnolo
7.	Eugenio	Gaudio
8.	Elisa	Borsani
9.	Rafael	Boscolo-Berto
10.	Iacopo Junio Valerio	Branca
11.	Alberto	Cacciola
12.	Alessandra	Cappellini
13.	Francesco	Cappello
14.	Simone	Carotti
15.	Valentina Alda	Carozzi
16.	Amelia	Cataldi
17.	Saverio	Cinti
18.	Lucio I. M.	Cocco
19.	Dario	Coletti
20.	Velia	D'Agata
21.	Agata Grazia	D'amico
22.	Raffaele	De Caro
23.	Carlo	Dell'Orbo
24.	Angela	Di Baldassarre
25.	Mariasevera	Di Comite
26.	Roberta	Di Pietro
27.	Roberto	Di Primio
28.	Michelino	Di Rosa
29.	Francesca	Diomede
30.	Irene	Faenza

31. Mirella Falconi
32. Giuseppe Familiari
33. Francesco Fazi
34. Antonio Filippini
35. Roberta Fiume
36. Paolo Flace
37. Matilde Y. Follo
38. Antonio Franchitto
39. Nicoletta Gagliano
40. Raffaele Geremia
41. Marco Gesi
42. Barbara Ghinassi
43. Antonio Giordano
44. Giuliana Gobbi
45. Pietro Gobbi
46. Massimo Gulisano
47. Paola Lanuti
48. Mattia Lauriola
49. Veronica Macchi
50. Guido Macchiarelli
51. Romina Mancinelli
52. Lucia Manzoli
53. Nadir Maraldi
54. Marco Marchisio
55. Guya Diletta Marconi
56. Giulia A. Mariani
57. Antonella Marino Gammazza
58. Sandra Marmiroli
59. Selenia Miglietta
60. Daniela Mirandola
61. Stefania Montagnani
62. Andrea Montella
63. Sergio Morini
64. Claudia Moscheni

65. Vanessa Nicolin
66. Gabriella Nicolini
67. Paolo Onori
68. Maria Grazia Palmerini
69. Michele Papa
70. Gianpaolo Papaccio
71. Laura Pierdomenico
72. Daniela Quacci
73. Giulia Ramazzotti
74. Stefano Ratti
75. Marcella Reguzzoni
76. Stefano Relucenti
77. Mario Rende
78. Giuseppina Rizzo
79. Alessandra Ruggeri
80. Giuseppe Santoro
81. Andrea Sbarbati
82. Chiarella Sforza
83. Carolina Simioni
84. Alessandra Stacchiotti
85. Gabriella Teti
86. Marcello Trucas
87. Alessandro Vercelli
88. Marco Vitale
89. Sandra Zecchi
90. Maria Zingariello

ALLEGATO N.2

SOCI COLLEGATI ONLINE:

1. Alida Amadeo
2. Francesco Amenta
3. Alessandra Barbiera
4. Nunzia Bernardini
5. Francesca Bonomini
6. Paola Brun
7. Barbara Buffoli
8. Silvano Capitani
9. Graziella Cappelletti
10. Guido Carpino
11. Arianna Casini
12. Sergio Castorina
13. Paola Castrogiovanni
14. Guido Cavaletti
15. Gabriele Ceccarelli
16. Pasquapina Ciarmela
17. Giovanni Cirillo
18. Michelangelo Cordenonsi
19. Gabriella Cusella
20. Giuseppina Cutroneo
21. Antonio De Luca
22. Vincenzo Desiderio
23. Claudia Della Via
24. Silvia Di Agostino
25. Valentina Di Felice
26. Anna Di Vito
27. Claudia Dolci
28. Amelio Dolfi
29. Elena Bianca Donetti
30. Mariella Errede
31. Cinzia Fabrizi

32. Angelo	Favaloro
33. Gaia	Favero
34. Matteo	Giovarelli
35. Giulia	Guarnieri
36. Rosemarie	Heyn
37. Rosa	Imbesi
38. Raffaella	Isola
39. Angela	Lucariello
40. Angela Bruna	Maffione
41. Ludovico	Magaudda
42. Emanuela	Marcenaro
43. Paola Lorena	Marmiroli
44. Ilenia	Martinelli
45. Elena	Masselli
46. Demetrio	Milardi
47. Prisco	Mirandola
48. Stefania	Moscato
49. Giuseppe	Musumeci
50. Stefania Annarita	Nottola
51. Alessandra	Pacini
52. Giancarlo	Panzica
53. Stefano	Papa
54. Ferdinando	Paternostro
55. Carolina	Pellegrini
56. Simona	Pergolizi
57. Angelica	Perna
58. Franca	Piras
59. Elena	Pompili
60. Simona	Pompili
61. Chiara	Porro
62. Andrea	Porzionato
63. Marina	Quartu
64. Francesca	Rappa
65. Mario	Raspanti

66. Alessandro	Riva
67. Federica	Riva
68. Chiara	Sassoli
69. Bianca Maria	Scicchitano
70. Maria Pina	Serra
71. Claudio	Sette
72. Roberta	Sferra
73. Gigliola	Sica
74. Simona	Sivori
75. Maria Alessandra	Sotgiu
76. Carlo	Tacchetti
77. Luca	Tamagnone
78. Seyed Khosrow	Tayebati
79. Virginia	Tirino
80. Daniele	Tomassoni
81. Rosa	Vaccaro
82. Antonella	Vetuschi
83. Maria Teresa	Viscomi

ALLEGATO N.3

Bilancio consuntivo anno 2019

CAUSALE DELLE ENTRATE	ENTRATE Euro	CAUSALE DELLE USCITE	USCITE Euro
Quote sociali incassate nel corso dell'anno 2019 (n° 337) incluse le quote arretrate, le quote incassate non al netto e in attesa di integrazioni e le quote non riconducibili allo stato di alcun socio	**€ 20.273,69**	**Elenco spese per attività statutarie**	
		Quote di Iscrizione al Congresso SIAI 2019 di due Soci vincitori dei premi poster anno 2018	€ 674,42
		Contributo per l'organizzazione del Convegno G.I.S.N. anno 2019	€ 500,00
		Spese varie (mantenimento conto corrente bancario, spese bollo e commissioni bancarie ecc.) anno 2019	€ 865,53
		Pagamento F24 anno 2019	€ 293,03
		Premi "Giovane Ricercatore", anno 2019	€ 4.000,00
		Premio "Miglior comunicazione orale " , anno 2019	€ 1.000,00
		Contributo Congresso Napoli	€ 5.000,00
		Quota associativa annuale IFAA ed EFEM, anno 2019	€ 519,72
		Contributo all' It J. Anat. Embryol. anno 2019	€ 408,21
		Elenco spese di funzionamento	
		Spese di rimborso viaggi estero dei soci delegati per congressi EFEM ed IFAA, anno 2019	€ 2.164,42
		Spese relative all'utilizzo del server per il sito web SIAI anno 2019 società Phoops	€ 4.880,73
		Spese per il funzionamento del Consiglio Direttivo, anno 2019	€ 1.199,29
TOTALE DELLE ENTRATE	**€ 20.273,69**	**TOTALE DELLE USCITE**	**€ 21.505,35**

DISAVANZO DELL'ESERCIZIO FINANZIARIO 2019	**€ 1231,66**		

CAUSALE DELLE ENTRATE	ENTRATE Euro	CAUSALE DELLE USCITE	USCITE Euro
Saldo Conto corrente Bancario al 31/12/2018	€ 28.620,93		
TOTALE SALDO FINANZIARIO AL 31/12/2018	€ 28.620,93		
DISAVANZO DELL'ESERCIZIO FINANZIARIO 2019	**€ 1231,66**		
SALDO FINANZIARIO AL 31/12/2019	**€ 27.389,27**		
STANZIAMENTI IMPEGNATI AL 31/12/2019			**Euro**
Accantonamento premi poster e comunicazione anno 2019			€ 2.000,00
Spese per sito WEB SIAI anno 2019			€ 700,00
Contributo all'It.J. Anat. Embryol. anno 2019			€ 410,00
Spese per ECM anno 2019			€ 500,00
Spese per il funzionamento della Presidenza anno 2019			€ 1.000,00
Spese per il funzionamento della Segreteria anno 2019			€ 1.000,00
Spese per il funzionamento della Tesoreria anno 2019			€ 1.000,00
Compenso per Consulenza Commercialista relativa alla stesura del bilancio consuntivo anno 2018 e bilancio previsionale anno 2020			€ 1.500,00
TOTALE IMPEGNO DI SPESA			**€ 8.110,00**
SALDO DISPONIBILE	**€ 19.279,27**		

Relazione di accompagnamento al rendiconto economico e finanziario per l'anno 2019

Come risulta dal bilancio consuntivo, il saldo finanziario al 31/12/ 2019 è pari ad **€ 27.389,14.** Tale saldo comprende l' importo pari ad € 5.278,69 del conto corrente postale liquidato nell'anno 2018.

A tale importo devono essere sottratti **€ 8.110,00** impegnati nel bilancio previsionale del 2019, ma non ancora effettivamente utilizzati alla data del 31/12/2019 per le seguenti voci di spesa:

- **Accantonamento premi poster e comunicazione anno 2019: € 2.000,00;**
- **Contributo all'It.J. Anat. Embryol. per l'anno 2019: € 410,00;**
- **Spese per il sito web della Società anno 2019: € 700,00;**
- **Spese per ECM anno 2019: € 500,00;**
- **Spese per il funzionamento della Presidenza: € 1.000,00;**
- **Spese per il funzionamento della Segreteria: € 1.000,00;**
- **Spese per il funzionamento della Tesoreria: € 1.000,00;**
- **Consulenza Commercialista anno 2018: € 1.500,00.**
-

Pertanto l'anno 2019 si chiude con un **saldo disponibile** di **€ 19.279,27.**

Durante il 2019 le quote associative incassate sono state 337 comprese alcune quote arretrate ed integrazioni di versamenti di quote non corretti, per un totale di **€ 20.273,69,** che sommate al saldo finanziario al 31/12/2018 pari ad **€ 28.620,93** hanno dato la disponibilità di **€ 48.894,62.**

Le entrate hanno permesso di coprire le spese previste e non previste, includendo i fondi impegnati e non erogati.

La rispondenza dei Soci ai solleciti da parte del Tesoriere in merito alla regolarizzazione dei pagamenti delle quote associative si è rivelata buona; comunque, al 31 dicembre 2019, rimane ancora un certo numero di Soci che debbono regolarizzare la loro posizione. Il Tesoriere sottolinea che l'eventuale recupero delle quote arretrate consentirebbe alla SIAI di effettuare una adeguata programmazione delle attività statutarie e di intraprendere nuove iniziative.

Il Tesoriere

Prof. Gianpaolo Papaccio

ALLEGATO N.4

Bilancio consuntivo anno 2020

CAUSALE DELLE ENTRATE	ENTRATE Euro	CAUSALE DELLE USCITE	USCITE Euro
Quote sociali incassate nel corso dell'anno 2020 (n°123) incluse le quote arretrate, le quote incassate non al netto e in attesa di integrazioni e le quote non riconducibili allo stato di alcun socio	**€ 7407,56**	**Elenco spese per attività statutarie**	
		Contributo per l'organizzazione del Convegno G.I.S.N. anno 2020	€ 500,00
		Spese varie (mantenimento conto corrente bancario, spese bollo e commissioni bancarie ecc.) anno 2020	€ 670,60
		Quota associativa annuale IFAA ed EFEM, anno 2020	€ 144,63
		Elenco spese di funzionamento	
		Compenso per consulenza commercialista relativo alla stesura del bilancio consuntivo 2018 e previsionale 2020	€ 1.201,97
		Spese per il funzionamento del Consiglio Direttivo, anno 2020	€ 250,00
TOTALE DELLE ENTRATE	**€ 7407,56**	**TOTALE DELLE USCITE**	**€ 2767,2**

AVANZO DELL'ESERCIZIO FINANZIARIO 2020	**€ 4.640,36**		

CAUSALE DELLE ENTRATE	ENTRATE Euro	CAUSALE DELLE USCITE	USCITE Euro
Saldo Conto corrente Bancario al 31/12/2019	**€ 27.389,27**		
TOTALE SALDO FINANZIARIO AL 31/12/2019	**€ 27.389,27**		
AVANZO DELL'ESERCIZIO FINANZIARIO 2020	**€ 4.640,36**		
SALDO FINANZIARIO AL 31/12/2020	**€ 32.029,63**		
STANZIAMENTI IMPEGNATI AL 31/12/2019			**Euro**

Spese per rinnovamento integrale sito WEB SIAI anno 2020			€ 5.000,00
Contributo all'It.J. Anat. Embryol. anno 2020			€ 410,00
Spese per ECM anno 2020			€ 500,00
Spese per il funzionamento della Presidenza anno 2020			€ 1.000,00
Spese per il funzionamento della Segreteria anno 2020			€ 1.000,00
Spese per il funzionamento della Tesoreria anno 2020			€ 1.000,00
Compenso per Consulenza Commercialista relativa alla stesura del bilancio consuntivo anno 2019 e bilancio previsionale anno 2021			€ 1.500,00
TOTALE IMPEGNO DI SPESA			**€ 10.410,00**
SALDO DISPONIBILE	**€ 21.619,63**		

Relazione di accompagnamento al rendiconto economico e finanziario per l'anno 2020

Come risulta dal bilancio consuntivo, il saldo finanziario al 31/12/ 2020 è pari ad **€ 32.029,63**
A tale importo devono essere sottratti **€ 10.410,00** impegnati nel bilancio previsionale del 2020, ma non ancora effettivamente utilizzati alla data del 31/12/2020 per le seguenti voci di spesa:

- **Contributo all'It.J. Anat. Embryol. per l'anno 2020: € 410,00;**
- **Spese per rinnovamento integrale sito web della Società anno 2020: € 5000,00 ;**
- **Spese per ECM, anno 2020 € 500,00;**
- **Spese per il funzionamento della Presidenza € 1.000,00;**
- **Spese per il funzionamento della Segreteria € 1.000,00;**
- **Spese per il funzionamento della Tesoreria € 1.000,00;**
- **Consulenza Commercialista anno 2019: € 1.500,00.**
-

Pertanto l'anno 2020 si chiude con un **saldo disponibile** di **€ 21.619,63.**

Durante il 2020 le quote associative incassate sono state 123 comprese alcune quote arretrate ed integrazioni di versamenti di quote non corretti, per un totale di **€ 7.407,56,** che sommate al saldo finanziario al 31/12/2019 pari ad **€ 27.389,27** hanno dato la disponibilità di **€ 34.796,83.**

Va rivelato che, a causa della pandemia, durante l'anno 2020 le spese a carico della Società sono state ovviamente notevolmente inferiori.

Le entrate hanno permesso di coprire le spese previste e non previste, includendo i fondi impegnati e non

erogati.

Il Tesoriere sottolinea che alcune spese ssono state procrastinate all' anno 2021 e che, per le quote arretrate, i soci saranno invitati a procedere alla regolarizzazione nell'anno 2021

Il Tesoriere

Prof. Gianpaolo Papaccio

SIAI Società Italiana di Anatomia e Istologia

ALLEGATO N.5

Previsione finanziaria 2021

SOCI NEL 2019:	**391**
SOCI NEL 2020:	**395**
SOCI ORDINARI 2020*:	**372**
SOCI DIMISSIONARI/CANCELLATI/DECEDUTI 2020:	**16**
SOCI EMERITI/ONORARI:	**23**

*Compresi i nuovi soci in numero di 20 ammessi all'assemblea di settembre 2019

Quote Sociali anno 2021	**372**	€	22.320,00
Quote Sociali arretrate 2015 – 2020		€	8.180,00
Totale entrate		€	**30.500,00**

USCITE

Contributo al 74° Convegno Nazionale 2021, atti di convegni, altri contributi a convegni, partecipazione a convegni, organizzazione eventi scientifici, borse di studio, etc.	€	10.000,00
Accantonamento per premi poster dell'anno 2021 e per premio comunicazione assegnato nell'anno 2021	€	2.000,00
Accantonamento per premi SIAI (Premio alla Carriera e n.2 Premi Ricercatori under 40), anno 2021	€	4.000,00
Contributo all' Italian Journal of Anatomy and Embryology, anno 2021	€	2.000,00
Spese per sito web della Società, anno 2021	€	2.000,00
Spese per la partecipazione Meeting Comitato Internazionale per la Terminologia Anatomica e Istologica, FICAT, anno 2021	€	1.000,00
Quota adesione all'European Federation for Experimental Morphology, EFEM, anni 2020 e 2021	€	900,00
Quota adesione all'International Federation of Anatomical Associations, IFAA, anno 2021	€	200,00
Spese varie (bancarie, postali, necrologi, etc.), anno 2021	€	600,00
Spese impreviste, anno 2021	€	1 .000,00

permetterebbero alla SIAI di migliorare ulteriormente tali scopi, come peraltro già messo in atto dal Presidente e da tutto il Direttivo.

Il Tesoriere

Prof. Gianpaolo Papaccio

SIAI Società Italiana di Anatomia e Istologia

ALLEGATO N.6

Previsione finanziaria 2022

SOCI NEL 2020:	**395**
SOCI NEL 2021:	**394**
SOCI ORDINARI 2021*:	**372**
SOCI DIMISSIONARI/CANCELLATI/DECEDUTI 2021:	**1**
SOCI EMERITI/ONORARI:	**23**

Quote Sociali anno 2022	**372**	€	22.320,00
Quote Sociali arretrate 2015 – 2020		€	9.780,00
Totale entrate		€	**32.100,00**

USCITE

Contributo al 75° Convegno Nazionale 2022, atti di convegni, altri contributi a convegni, partecipazione a convegni, organizzazione eventi scientifici, borse di studio, etc.	€	10.000,00
Accantonamento per premi poster dell'anno 2022 e per premio comunicazione assegnato nell'anno 2022	€	2.000,00
Accantonamento per premi SIAI (Premio alla Carriera e n.2 Premi Ricercatori under 40), anno 2022	€	4.200,00
Contributo all' Italian Journal of Anatomy and Embryology, anno 2022	€	2.000,00
Spese per sito web della Società, anno 2022	€	2.000,00
Spese per la partecipazione Meeting Comitato Internazionale per la Terminologia Anatomica e Istologica, FICAT, anno 2022	€	1.000,00
Quota adesione all'European Federation for Experimental Morphology, EFEM, anni 2021 e 2022	€	900,00
Quota adesione all'International Federation of Anatomical Associations, IFAA, anno 2022	€	400,00
Spese varie (bancarie, postali, necrologi, etc.), anno 2022	€	600,00
Spese impreviste, anno 2022	€	1 .000,00

Totale spese per attività statutarie	***€***	***24.100,00***
Spese per il funzionamento (Segreteria, Tesoreria, Presidenza e Direttivo)	€	6.000,00
Spese per consulenza commercialista anno 2021	€	2.000,00
Totale spese di funzionamento	***€***	***8.000,00***
Totale uscite	**€**	**32.100,00**

Relazione di accompagnamento alla previsione finanziaria per l'Anno 2022

La chiusura del bilancio consuntivo del 2020 con un saldo disponibile di **€ 21.619,63** ha permesso al Tesoriere di sostenere alcune spese indicate nella Previsione Finanziaria del 2021.

Al 31 Agosto 2021, sono state incassate 592 quote sociali comprensive di quelle relative all'anno in corso e arretrate (dal 1 settembre 2020 al 31 agosto 2021).

Al 31 Agosto 2021, il totale delle entrate è pari a **€ 46.310,21** e comprende le quote riscosse finora.

Comunque il piano previsionale del 2021 prevedeva entrate pari a **€ 30.500,00**, dovute alla riscossione delle quote dell'anno in corso, più una cifra forfettaria concernente il recupero delle quote arretrate.

In particolare, in tale previsione, come in quelle degli anni precedenti, è stata indicata questa cifra forfetaria sulla base dell'esperienza relativa alle difficoltà di ottenere il pagamento degli arretrati da tutti i Soci non in regola.

La Società conta attualmente 395 Soci, di cui 372 Soci Ordinari e 22 Soci Emeriti o Onorari (esonerati dal pagamento della quota Sociale).

Alla data del 31 agosto 2021, dei 372 Soci Ordinari che sono tenuti a pagare la quota associativa:

- 199 Soci sono in regola fino al 2021;
- 18 Soci sono in regola fino al 2020, devono la quota 2021
- 65 Soci sono in regola fino al 2019, devono le quote del 2020 e 2021;
- 48 Soci in pari con il 2018, devono le quote del 2019, 2020 e 2021;
- 29 Soci in pari con il 2017, devono le quote del 2018, 2019, 2020 e 2021;
- 8 Soci in pari con il 2016, devono le quote del 2017, 2018, 2019, 2020 e 2021
- 5 Soci in pari con il 2015, devono le quote del 2016, 2017, 2018, 2019, 2020 e 2021.

Il Tesoriere fa presente che cercherà di raggiungere la parità di bilancio e di fare previsioni finanziarie quanto più possibile aderenti alla realtà. Riferisce inoltre che nel corso del 2021, un discreto numero di Soci ha risposto positivamente all'azione di richiamo per il recupero delle quote arretrate.Rimane un piccolo numero di Soci che non ha mai risposto ai solleciti di pagamento; pertanto, in base a quanto stabilito nello Statuto e al parere del Direttivo SIAI, si è già provveduto alla revisione dell' Elenco dei Soci che sarà ancora revisionato ove mai tali soci non provvedessero secondo le norme statuarie al pagamento delle quote arretrate.

Il Tesoriere ricorda che gli scopi istituzionali della Società Italiana di Anatomia e Istologia sono essenzialmente la promozione della ricerca e della didattica nel campo delle discipline anatomiche e istologiche, pertanto l'incasso puntuale delle quote annuali ed il recupero delle quote arretrate

permetterebbero alla SIAI di migliorare ulteriormente tali scopi, come peraltro già messo in atto dal Presidente e dal Direttivo.

Il Tesoriere

Prof. Gianpaolo Papaccio

ALLEGATO N.7

Associazione "Società Italiana di Anatomia ed Istologia"
Verbale dei revisori dei conti

L'anno 2021 il giorno 23 del mese di settembre alle ore 9:30 in modalità telematica utilizzando la piattaforma Teams si sono riuniti i revisori dei conti, nominati dal Direttivo della SIAI in data 9/9/2021

nelle persone dei professori:

- Guido Macchiarelli, Presidente;

- Roberta Di Pietro, Membro effettivo;

- Simona Sivori, Membro effettivo con funzioni di Segretario

Dopo aver preso visione della documentazione relativa ai Rendiconti finanziari degli anni 2019 e 2020 ed alle previsioni finanziarie relative agli anni 2021 e 2022 dell'Associazione, messa a disposizione della Commissione stessa, si è proceduto all'esame della suesposta documentazione, la quale non evidenzia irregolarità.

Sia i rendiconti sia i preventivi finanziari evidenziano che l'Associazione è in attivo e che tale attivo è risultato incrementato nell'esercizio 2020.

Si mette inoltre in evidenza che i soci morosi risultano in diminuzione.

La seduta viene tolta alle ore 10:30.

Letto, confermato e sottoscritto.

I REVISORI

Guido Macchiarelli (Presidente)

Roberta Di Pietro (Membro)

Firmato digitalmente da
SIMONA SIVORI
Università degli Studi di Genova
Firmato il 23/09/2021 10:43
Seriale certificato : 657897

Simona Sivori (Membro Segretario)

ALLEGATO N. 8

STATUTO DELL'ASSOCIAZIONE

DENOMINATA "SOCIETA' ITALIANA DI ANATOMIA E ISTOLOGIA"

Art. 1

L'Associazione si denomina "Società Italiana di Anatomia e Istologia" ed ha come scopi istituzionali la promozione e l'incremento della ricerca e della didattica nel campo delle discipline anatomiche e istologiche.

Art. 2

L'Associazione *non ha fini* di lucro ed è aperta a tutti i cultori delle discipline morfologiche sia italiani che stranieri.

L'Associazione ed i suoi legali rappresentanti perseguono le finalità sociali con autonomia ed indipendenza anche con riferimento al non esercizio di attività imprenditoriali o partecipazione ad esse, ad eccezione delle attività svolte nell'ambito del Programma nazionale di formazione continua in medicina (ECM).

L'Associazione non ha tra le finalità istituzionali la tutela sindacale dei Soci e comunque non svolge, direttamente o indirettamente, attività sindacale.

L'Associazione, nel perseguimento degli scopi istituzionali, si impegna ad intrattenere corrette relazioni con i terzi evitando qualsiasi rapporto che possa generare vantaggi personali a favore dei Soci o di membri degli organi sociali i quali sono obbligati a dichiarare senza indugio al Presidente, in forma scritta,

l'esistenza di qualsiasi situazione che possa ingenerare conflitto di interessi con l'Associazione.

Art. 3

Le domande di ammissione dei nuovi Soci, corredate da un succinto curriculum vitae, devono essere controfirmate da due membri dell'Associazione.

Sono ammessi come Soci ordinari, senza limitazioni, tutti i soggetti in possesso dei requisiti previsti da questo Statuto, appartenenti alle discipline morfologiche, sia italiani che stranieri, con attività lavorativa nel settore che l'Associazione rappresenta o che operano nelle strutture e settori di attività del servizio sanitario nazionale ovvero in regime libero-professionale.

Oltre ai Soci ordinari fanno parte dell'Associazione «Soci emeriti» e «Soci onorari», individuati fra cultori di discipline morfologiche che si siano distinti per la loro attività e il loro impegno rispettivamente i primi tra i Soci della Società Italiana di Anatomia e Istologia e i secondi tra studiosi stranieri.

La nomina dei Soci emeriti e dei Soci onorari *è deliberata dall'Assemblea Generale su proposta del Consiglio Direttivo.*

Art. 4

L'Associazione ha sede legale nel Comune di Roma, Dipartimento di Scienze della Vita e Sanità Pubblica, Sezione di Istologia ed Embriologia, Università Cattolica del Sacro Cuore, Largo Francesco Vito, 1- 00168 Roma

Art. 5

In ottemperanza ai propri scopi istituzionali, l'Associazione provvede a favorire la diffusione delle conoscenze nell'ambito dell'Anatomia e dell'Istologia promuovendo *incontri, dibattiti, conferenze, manifestazioni didattiche e congressi, curando la pubblicazione e diffusione di libri e riviste ed istituendo ed assegnando borse di studio a giovani laureati, borse di perfezionamento presso istituti, centri o enti in Italia e all'estero* **e conferendo premi relativi all'attività di ricerca.** *In particolare, l'Associazione provvede ad indire l'annuale Congresso della Società Italiana di Anatomia e Istologia* e può, una volta riconosciuti la notevole rilevanza scientifica e l'impegno previsto per l'organizzazione di questa e di altre manifestazioni congressuali, prevedere la concessione di un contributo, *compatibilmente con le esigenze del Bilancio.*

L'Associazione cura la pubblicazione dell'Italian Journal of Anatomy and Embryology.

Art. 6

Organi dell'Associazione sono:

1) *il Presidente*

2) *il Segretario*

3) *il Tesoriere*

4) il Consiglio Direttivo

5) l'Assemblea dei Soci

6) **il Collegio dei Probiviri**

7) il Comitato Scientifico

E' esclusa la retribuzione delle cariche sociali.

Art. 7

L'Assemblea dei Soci è convocata con *e-mail* recante l'ordine del giorno e inviata all'indirizzo dei singoli Soci almeno venti giorni prima della data fissata per l'adunanza. *L'Assemblea è valida in prima convocazione con la presenza di almeno la metà più uno dei Soci; in seconda convocazione con qualsiasi numero di Soci.*

Tutte le deliberazioni dell'Assemblea sono prese a maggioranza di voti dei presenti, **salvo che sia diversamente previsto dalla legge o da questo Statuto.**

A ciascun socio iscritto nel Libro Soci spetta un voto.

I Soci possono farsi rappresentare alle Assemblee da altri Soci mediante delega scritta. Ogni socio non può presentare più di *due* deleghe.

La riunione assembleare, ove se ne ravvisi la necessità ed a discrezione del Presidente, può avvenire tramite video-conferenza, alle seguenti condizioni e nel rispetto della vigente normativa in materia:

a) che siano presenti nello stesso luogo il Presidente ed il Segretario della riunione, se nominato, che provvederanno alla formazione e sottoscrizione del verbale, dovendosi ritenere svolta la riunione in detto luogo, salvo che la normativa vigente consenta lo svolgimento della riunione esclusivamente tramite

video-conferenza nel rispetto in ogni caso delle condizioni di cui in appresso;

b) che sia consentito al Presidente della riunione di accertare l'identità degli intervenuti, regolare lo svolgimento della riunione, constatare e proclamare i risultati della votazione;

c) che sia consentito al soggetto verbalizzante di percepire adeguatamente gli eventi della riunione oggetto di verbalizzazione;

d) che sia consentito agli intervenuti di partecipare alla discussione ed alla votazione simultanea sugli argomenti all'ordine del giorno, nonché visionare, ricevere o trasmettere documenti.

Art. 8

In occasione di ogni Congresso nazionale è convocata, in via ordinaria ed in seduta amministrativa, l'Assemblea Generale dei Soci.

In sede di seduta amministrativa l'Assemblea dei Soci decide la programmazione dell'attività dell'Associazione, provvede alla scelta delle sedi dei futuri Congressi e ne designa i relativi Presidenti, *approva* l'ammissione dei nuovi Soci e le eventuali dimissioni, procede alla elezione delle cariche sociali *(quando previsto)*, alla approvazione del bilancio consuntivo dell'anno precedente e del bilancio preventivo dell'anno successivo che dovranno essere controllati da tre Revisori legali nominati dal Presidente; delibera su eventuali proposte di modifica dello

Statuto e del Regolamento.

I bilanci consuntivi e preventivi approvati dall'Assemblea andranno pubblicati sul sito web istituzionale dell'Associazione unitamente ad eventuali incarichi retribuiti.

Art. 9

Il Consiglio Direttivo è composto da undici membri. L'Assemblea dei Soci elegge direttamente, con votazione a scrutinio segreto, il Presidente, il Segretario ed il Tesoriere e, contemporaneamente, otto consiglieri scelti nella misura di quattro Anatomici e di quattro *Istologi.*

L'elezione dei componenti il Consiglio Direttivo è possibile per un massimo di tre mandati consecutivi (ciascuno di tre anni).

L'elezione alla stessa carica (Presidente-Segretario-Tesoriere) è possibile per non più di due mandati consecutivi (ciascuno di tre anni).

Il Presidente uscente è membro di diritto per un triennio del nuovo Consiglio Direttivo come Past President.

I legali rappresentanti e gli amministratori dell'Associazione non devono aver subito sentenze di condanna passate in giudicato in relazione all'attività dell'Associazione.

Art. 10

Il Consiglio Direttivo cura quanto è necessario per il funzionamento e l'incremento dell'Associazione e mantiene relazioni con Associazioni italiane e straniere affini.

Il Consiglio Direttivo è convocato dal Presidente, mediante

avviso spedito ai membri almeno tre giorni prima dell'adunanza mediante mezzi che garantiscano l'avvenuta ricezione, quando occorra o quando la convocazione sia richiesta da almeno tre dei suoi componenti.

Le riunioni del Consiglio sono regolarmente costituite quando vi sia la presenza della maggioranza dei suoi membri.

Il Consiglio delibera con il voto favorevole della maggioranza dei presenti.

Su invito possono partecipare alle riunioni del Consiglio Direttivo anche il/i Presidente/i del successivo Congresso della Società e l'Editor in Chief dell'Italian Journal of Anatomy and Embryology, per le sole questioni attinenti alla organizzazione del Congresso stesso e all'attività editoriale della rivista.

Le riunioni del Consiglio Direttivo, ove se ne ravvisi la necessità ed a discrezione del Presidente, possono avvenire tramite video-conferenza, alle seguenti condizioni e nel rispetto della vigente normativa in materia:

a) che siano presenti nello stesso luogo il Presidente ed il Segretario della riunione, se nominato, che provvederanno alla formazione e sottoscrizione del verbale, dovendosi ritenere svolta la riunione in detto luogo, salvo che la normativa vigente consenta lo svolgimento della riunione esclusivamente tramite video-conferenza nel rispetto in ogni caso delle condizioni di cui in appresso;

b) che sia consentito al Presidente della riunione di accertare

l'identità degli intervenuti, regolare lo svolgimento della riunione, constatare e proclamare i risultati della votazione;

c) che sia consentito al soggetto verbalizzante di percepire adeguatamente gli eventi della riunione oggetto di verbalizzazione;

d) che sia consentito agli intervenuti di partecipare alla discussione ed alla votazione simultanea sugli argomenti all'ordine del giorno, nonché visionare, ricevere o trasmettere documenti.

Art. 11

Il Consiglio Direttivo è tenuto a redigere un regolamento per il funzionamento dell'Associazione, la cui osservanza, una volta ottenuta l'approvazione dell'Assemblea, è obbligatoria per tutti i Soci.

Art. 12

Il Presidente rappresenta a tutti gli effetti l'Associazione di fronte a terzi ed in giudizio. Convoca e presiede le sedute del Consiglio e le Assemblee dei Soci; in caso di impedimento può delegare a rappresentarlo, di volta in volta, un membro del Consiglio Direttivo.

Il Segretario mantiene i rapporti con i Soci, custodisce l'archivio sociale, provvede all'aggiornamento del libro dei Soci, alla stesura dei verbali delle riunioni e degli atti ufficiali e cura la pubblicazione degli atti della Società.

Il Tesoriere provvede alla esazione delle quote sociali ed alla

amministrazione del fondo sociale.

Art. 13

Il Collegio dei Probiviri è costituito da tre membri eletti dall'Assemblea Generale dei Soci in occasione delle elezioni del Consiglio Direttivo; contestualmente viene eletto il Presidente del Collegio dei Probiviri.

I Probiviri durano in carica tre anni e sono rieleggibili.

Qualsiasi Socio con una anzianità di associazione di almeno dieci anni consecutivi ed in regola con il pagamento delle quote associative può candidarsi alle elezioni, purchè non ricopra altre cariche sociali.

Il Collegio dei Probiviri tutela il rispetto delle norme statutarie, etiche e deontologiche da parte dei Soci e decide in via arbitrale le controversie che intercorrono tra i singoli associati e tra questi ultimi e l'Associazione.

Le decisioni del Collegio dei Probiviri, le cui riunioni sono convocate dal Presidente mediante avviso spedito almeno tre giorni prima dell'adunanza mediante mezzi che garantiscano l'avvenuta ricezione, sono adottate a maggioranza assoluta.

Art. 14

Il patrimonio dell'Associazione è costituito:

a) dalle quote sociali;

b) da eventuali contributi di Enti o di privati;

c) da eventuali lasciti o donazioni;

d) dal fondo costituito dai fondatori e da contributi volontari

degli associati versati a tal titolo;

e) dagli incassi del contributo per la stampa degli articoli pubblicati sull'Italian Journal of Anatomy and Embryology e da ogni altra entrata ammessa dalle norme vigenti in materia.

Art. 15

Il Socio è tenuto a corrispondere una quota annuale il cui ammontare è fissato dall'Assemblea e può essere soggetto a revisione.

Il Socio che, per quanto sollecitato, non provvede al pagamento della quota sociale per due anni consecutivi è considerato dimissionario. La sua riammissione è consentita previa presentazione di apposita domanda e contestuale regolarizzazione dei pagamenti dovuti per i relativi crediti dell'Associazione, ove certi, liquidi ed esigibili.

La suindicata procedura di riammissione si applica anche ai Soci dimissionari per altre ragioni verso i quali l'Associazione vanti crediti certi, liquidi ed esigibili per quote annuali non versate.

Art. 16

Il Comitato Scientifico dell'Associazione ha funzioni di verifica e controllo della qualità di tutte le attività svolte e della produzione tecnico-scientifica della Società, che sarà effettuata secondo gli indici di produttività scientifica e bibliometrici validati dalla comunità scientifica internazionale.

Il Comitato è composto da quattro membri più il Presidente scelti

dal Consiglio Direttivo dell'Associazione sulla base della comprovata esperienza, notoria indipendenza ed imparzialità e della elevata professionalità.

I componenti del Comitato decadono allo scadere del Consiglio Direttivo che li ha nominati.

L'Associazione cura, con i mezzi dei quali dispone e nelle forme e con le modalità stabilite dal Regolamento, la pubblicazione dei contributi scientifici presentati ai Congressi ed alle assisi direttamente promosse.

L'Associazione si obbliga in ogni caso a pubblicare l'attività scientifica attraverso il proprio sito web, aggiornato costantemente.

Art. 17

L'Associazione ha durata di tempo illimitata.

Art. 18

L'eventuale scioglimento dell'Associazione è deciso dall'Assemblea dei Soci con le maggioranze previste dall'art. 21 c.c.

In caso di scioglimento, il patrimonio residuo dell'Associazione dovrà essere destinato, su decisione dell'Assemblea dei Soci, agli scopi dell'Associazione od a scopi affini, rimanendo in ogni caso esclusa qualsiasi *ripartizione* tra i Soci.

Art. 19

Per tutto quanto non previsto dal presente Statuto si fa riferimento alle norme di legge in materia.

ALLEGATO N. 9

REGOLAMENTO

Art. 1

I Congressi Nazionali della Società Italiana di Anatomia e Istologia hanno cadenza annuale.

La Società può erogare contributi, oltre che agli organizzatori del Congresso Nazionale, anche *ai promotori di* altri Convegni, i responsabili dei quali dovranno inviare al Consiglio Direttivo della Società apposito rendiconto della utilizzazione del contributo concesso.

In base al proprio bilancio un contributo può essere concesso dalla Società Italiana di Anatomia e Istologia anche per la organizzazione di Corsi di aggiornamento, promossi sotto l'egida e con il patrocinio della Società *stessa.*

Art. 2

Per ogni Congresso nazionale il Consiglio Direttivo indica, con almeno un anno di anticipo il tema preferenziale, scelto con opportuna alternanza fra quelli eventualmente segnalati dai gruppi di ricerca dei vari Atenei. Del tema preferenziale viene data tempestivamente comunicazione ai Soci.

Art. 3

Il Congresso nazionale si articola in due relazioni, comunicazioni orali, sessioni poster ed altri tipi di eventi scientifici proposti dagli organizzatori del Congresso (tavole rotonde, simposi satelliti, ecc.). Delle due relazioni, la prima

è affidata dal Consiglio Direttivo, sulla base del tema preferenziale prescelto, ad un Socio o ad un Ricercatore esterno in considerazione della loro specifica competenza scientifica. La seconda relazione, su tema libero, viene scelta dal Consiglio Direttivo anche fra quelle proposte al Presidente con almeno un anno di anticipo. Le comunicazioni orali ed i poster possono essere inerenti al tema preferenziale del Congresso oppure a tema libero.

Per le relazioni è prevista la durata di 45 minuti con 15 minuti di discussione, mentre per le comunicazioni la durata è di 7 minuti con 3 minuti di discussione. Agli Organizzatori del Congresso è lasciata la facoltà di concedere un tempo maggiore ad alcune comunicazioni inserite all'inizio delle varie sessioni.

Art. 4

L'"Italian Journal of Anatomy and Embryology", **edito a cura di Firenze University Press, proprietà dell'Università degli Studi di Firenze**, è la Rivista ufficiale della Società Italiana di Anatomia e Istologia e su essa verranno pubblicati lavori scientifici sottoposti a una revisione *di* ***esperti selezionati*** *dall'Editor-in-Chief.*

L'Italian Journal of Anatomy and Embryology sarà diffuso online con *free access*.

Art. 5

Il *riassunto* delle Relazioni ufficiali e gli **Abstract** dei Congressi della Società sono stampati in lingua inglese in un

volume pubblicato come Supplemento della rivista "Italian Journal of Anatomy and Embryology" e la stampa dovrà adeguarsi al formato della Rivista.

Nello stesso Volume saranno stampati in lingua italiana i verbali dell'Assemblea generale dei Soci e della seduta amministrativa del Congresso precedente.

In forma sintetica possono essere pure inseriti, sempre in lingua inglese, gli Atti dei Simposi eventualmente tenuti nell'ambito dei Congressi.

Le spese per la stampa del supplemento sono a carico del Comitato organizzatore del Congresso che provvede alla sua distribuzione gratuita a tutti gli iscritti all'apertura del Congresso.

Art. 6

Sono a carico della Società, ma vengono limitate ad un numero di *15* pagine, comprese figure e schemi in bianco e nero, le spese per la stampa di ciascuna relazione ufficiale in lingua inglese su un fascicolo dell'Italian Journal of Anatomy and Embryology. Il rimanente della spesa resterà a carico del Relatore, ivi comprese tavole e figure a colori e gli estratti della relazione. Se il Relatore non consegnerà il testo completo della relazione entro il mese di febbraio dell'anno successivo al Congresso svolto, non potrà più godere della franchigia prevista.

I necrologi, in lingua inglese e nel limite di 2 pagine per ciascuno, saranno anch'essi pubblicati a carico della Società su un fascicolo dell'Italian Journal of Anatomy and Embryology.

Art. 7

All'esazione delle quote sociali si provvede annualmente a mezzo di versamento, sia diretto sia tramite intermediari, in conto corrente bancario intestato alla Società o tramite il sito web della Società.

Art. 8

La Società Italiana di Anatomia e Istologia, nella persona del Presidente, è titolare, allo stato, del dominio web "www.siaionline.it" che ospita il sito ufficiale della Società comprendente anche l'elenco dei Soci.

Tale dominio è suscettibile di modifiche che verranno tempestivamente comunicate ai Soci, una volta che l'assetto definitivo del sito sarà stabilito.

Art. 9

Viene proposto come "Socio emerito" un Socio italiano che si sia distinto particolarmente nelle attività didattiche e scientifiche dell'area morfologica e che sia stato Socio per lunghi anni della Società Italiana di Anatomia e Istologia. Viene ammesso come "Socio onorario" uno studioso straniero, che per alti meriti nel campo scientifico e in quello didattico sia considerato degno di tale riconoscimento.

Le candidature debbono essere presentate da almeno due Soci in regola con il pagamento delle quote sociali. I Soci proponenti dovranno documentare la loro richiesta per un Socio emerito o per un Socio onorario attraverso la presentazione di un curriculum

vitae del candidato. *Il* numero dei Soci emeriti e di quelli onorari non può superare l'8% dei Soci della Società. La nomina dei Soci "emeriti" e dei Soci "onorari" *è deliberata dall'Assemblea generale su proposta del Consiglio Direttivo.*

I Soci "emeriti" e i Soci "onorari" sono esentati dal pagamento della quota sociale annuale.

Art. 10

Il Consiglio Direttivo propone all'Assemblea Generale dei Soci in sede di seduta amministrativa l'elenco dei Soci morosi da oltre due anni per la **presa d'atto** della dimissione dalla Società ai sensi dell'art. 15 dello Statuto. Solo ai Soci in regola con le quote annuali previste saranno inviate le comunicazioni sulle attività della Società e le notizie inerenti i Congressi nazionali ed internazionali.

Per i Soci non in regola con il pagamento della quota sociale annuale, la quota di iscrizione al Congresso Nazionale è quella prevista *"per i non Soci"*.

Art. 11

I Componenti del Consiglio Direttivo della Società Italiana di Anatomia e Istologia di cui all'art. 9 dello Statuto sono eletti tra coloro che hanno fatto pervenire la loro disponibilità a candidarsi al Presidente della Società, almeno trenta giorni prima della data di inizio del Congresso annuale in cui è previsto il rinnovo di tali cariche. Non saranno ammessi a candidarsi i Soci non in regola con il pagamento **delle quote sociali, inclusa**

quella dell'anno in corso.

Le elezioni possono svolgersi anche utilizzando modalità telematiche.

L'elezione dei componenti il Consiglio Direttivo è possibile per un massimo di tre mandati consecutivi (ciascuno di tre anni).

L'elezione nella stessa carica (Presidente-Segretario-Tesoriere) è possibile per non più di due mandati consecutivi *(ciascuno di tre anni)*.

Il Presidente uscente resterà per un triennio nel nuovo Consiglio Direttivo come *Past-President*.

Art. 12

I Componenti del Collegio dei Probiviri della Società Italiana di Anatomia e Istologia di cui all'art. 13 dello Statuto sono eletti tra coloro che hanno fatto pervenire la loro disponibilità a candidarsi al Presidente della Società, almeno trenta giorni prima della data di inizio del Congresso annuale in cui è previsto il rinnovo di tali cariche.

Non saranno ammessi a candidarsi i Soci non in regola con il pagamento delle quote sociali, inclusa quella dell'anno in corso.

ALLEGATO N.10

La Commissione designata dal Direttivo della Società Italiana di Anatomia e Istologia (di seguito indicata come: la Commissione) con lo scopo di valutare le candidature al premio alla Carriera SIAI per l'anno 2021, composta dai Proff: Sandra Zecchi, Carlo Tacchetti, Antonio Filippini e Amelio Dolfi, dopo aver preso in esame le candidature pervenute, sottolinea l'eccellente percorso accademico che caratterizza le carriere di tutti i candidati e delibera di proporre al Consiglio Direttivo per il premio alla Carriera 2021 il Prof. Roberto Di Primio.

Laureato in Medicina e Chirurgia. Specializzato in Ematologia Generale.
Dal 1978 al 1983: tecnico laureato presso l'Istituto di Patologia Generale dell'Università di Chieti.
Dal 1983 al 1987: ricercatore presso l'Istituto di Anatomia Umana Normale dell'Università di Genova.
Negli anni 1984, 1985 e 1990 è stato Research Associate presso il Department of Biochemistry, Uniformed Service University of Health Sciences, Bethesda, USA.
Dal 1987 al 1994 Professore Associato di Istologia presso l'Istituto di Morfologia Umana Normale dell'Università di Chieti.
Dal 1994, professore prima straordinario e quindi ordinario di Istologia la Facoltà di Medicina e Chirurgia dell'Università Politecnica delle Marche (UNIVPM).
Dal 1994 al 2019 Professore Ordinario di Istologia dell'UNIVPM.
Ha ricoperto diverse cariche gestionali:
Nel 1998 Vice Direttore Scientifico dell'IRCS INRCA di Ancona,
dal 2010 al 2018 Direttore del Dipartimento di Scienze Cliniche e Molecolari (DISCLIMO) dell'UNIVPM.
Dal 2013 al 2018 Presidente CdL Infermieristica Ascoli Piceno, UNIVPM.
Dal 2015 al 2018 componente del Senato Accademico, UNIVPM.
Dal 2015 al 2018 ha partecipato al Collegio dei Dottorati UNIVPM
Ha fatto parte di Commissioni di valutazione:
Dal 2016 al 2018 Presidente Commissione Abilitazione Scientifica Nazionale, SSD BIO/17
Nel 2020 è stato presidente della commissione ANVUR per l'accreditamento di nuovi Corsi di Laurea dell'area 05 BIO
Ha ricevuto Fondi CNR, AIRC, MURST, FIRB e PRIN per la ricerca scientifica.
E' Autore di oltre 160 pubblicazioni scientifiche su riviste internazionali
Socio della SIAI dal 1984, membro del Direttivo dal 2008, nonché Segretario dal 2017 a tutt'oggi.
Socio della Società Italiana di Istochimica dal 1984 e membro del Consiglio Direttivo del quale è stato vicepresidente nel triennio 2016/2019.
Fa parte, dalla sua costituzione, del Collegio dei docenti di Istologia e membro del Direttivo nel quale ha ricoperto il ruolo di Segretario/Tesoriere nel triennio 2018/2021
Ha organizzato il LXVIII Congresso SIAI, Ancona, 18-20 settembre 2014.
Ha organizzato il 65° Congresso Nazionale della Società Italiana di Istochimica, Ancona , 24-27 Giugno 2019.
Oltre all'eccellente Curriculum Accademico, il Prof. Di Primio si è dimostrato sempre molto attivo nel perseguire le finalità della SIAI sia come Socio sia ricoprendo le cariche di membro del Direttivo e di Segretario.

Firmato digitalmente da
SANDRA ZECCHI
O = Università degli Studi di Firenze
Firmato il 16/11/2021 10:31
Seriale Certificato: 1002000

ALLEGATO N.11

VERBALE DELLA RIUNIONE DELLA COMMISSIONE PER L'ATTRIBUZIONE DEI N. 2 PREMI AI RICERCATORI "UNDER 40", NOMINATA DAL CONSIGLIO DIRETTIVO DELLA SIAI IN DATA 12 LUGLIO 2021

La Commissione per l'attribuzione dei Premi ai Ricercatori under 40, nominata dal Consiglio Direttivo della SIAI in data 12 Luglio 2021, e formata dai Proff. Gianpaolo Papaccio, Stefania Montagnani e Gigliola Sica, si è riunita telematicamente ed ha nominato la Prof. Gigliola Sica quale Presidente ed il Prof. Gianpaolo Papaccio quale Segretario.
Indi la Commissione ha preso atto delle candidature pervenute nelle persone di:

Branca Jacopo Junio Valerio
D'Amico Agata Grazia
Diomede Francesca
Madaro Luca
Miglietta Selenia
Szychlinska Marta Anna

La Commissione ha dapprima controllato la posizione dei Candidati rispetto al pagamento delle quote di iscrizione alla SIAI ed ha rilevato che:

- Szychlinska Marta Anna non risulta essere in regola con il pagamento delle quote sociali per più annualità;
- Madaro Luca non risulta essere iscritto alla SIAI.

Pertanto tali due candidati sono stati espunti dalla valutazione.

Successivamente, la Commissione ha valutato attentamente i curricula dei quattro candidati rimanenti, la loro produzione scientifica (in particolare le tematiche di ricerca per verificarne la congruenza e la non frammentarietà) ed i loro indici bibliometrici.

Sulla base di tale valutazione, all'unanimità, la Commissione propone di assegnare i due premi a:

Diomede Francesca
D'Amico Agata Grazia

Del che è verbale addì 20 settembre 2021

Prof. Gigliola Sica

Prof. Stefania Montagnani

Prof. Gianpaolo Papaccio

ALLEGATO N.12

74mo Congresso SIAI Bologna

Premio migliore comunicazione orale

La commissione incaricata di attribuire il premio alla migliore comunicazione orale nell'ambito delle sessioni di Neuroscienze 1, 2 e 3 ha rilevato un ottimo livello scientifico di tutte le comunicazioni orali presentate e ha individuato la comunicazione dal titolo "Oxidized polyvinylalcohol-based nerve conduits for peripheral nerve regeneration in severe injury: a comparative pre-clinical study" (autori: Elena Stocco, Silvia Barbon, Enrico De Rose, Diego Faccio, Lucia Petrelli, Anna Rambaldo, Deborah Sandrin, Monica Dettin, Veronica Macchi, Cesare Tiengo, Raffaele De Caro, Andrea Porzionato), presentata da Elena Stocco, come meritevole del premio per qualità scientifica e brillantezza dell'esposizione.

La commissione

Prof. Guido Cavaletti

Prof.ssa Roberta Di Pietro

Prof. Fabrizio Michetti

Prof. Paolo Onori

Prof. Luca Tamagnone

Prof.ssa Sandra Zecchi Orlandini

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SANDRA ZECCHI
O = Università degli Studi di Firenze
Firmato il 20/11/2021 17:27
Seriale Certificato: 1002000

Bologna, 25/09/2021

ALLEGATO N.13

Verbale della Commissione Poster

La Commissione di valutazione Poster, costituita dai Professori Antonio De Luca, Giuseppe Santoro e Pietro Gobbi, ha proceduto ai lavori di selezione per identificare i due poster meritevoli di essere premiati, come consuetudine oramai consolidata nei Congressi della nostra Società. Procedendo collegialmente e considerando unicamente i contributi effettivamente consultabili in quanto caricati sul Sito congressuale ed escludendo altresì i poster nei quali risultavano, tra i coautori, i componenti della stessa Commissione di valutazione, è stata effettuata una prevalutazione tra i 90 contributi presenti sul sito.

La valutazione è stata effettuata considerando, contestualmente, l'impatto grafico e la rilevanza dei contenuti, privilegiando, in ogni caso, i contributi che presentavano informazioni di carattere morfologico e morfologico sperimentale. La Commissione desidera far rilevare l'elevatissimo livello qualitativo medio dei dati presentati e l'ampio spettro delle tematiche morfologiche affrontate.

Questi motivi impongono la sottolineatura del fatto che la Commissione ha avuto effettivamente delle difficoltà sia nel lavoro di preselezione che nella successiva identificazione dei vincitori, volendo presentare, comunque, il più vivo apprezzamento per la vivacità culturale del nostro intero Settore Scientifico Disciplinare.

15 poster (05, 10, 16, 23, 27, 37, 40, 43, 56, 57, 80, 81, 83, 88, 90) sono stati quindi selezionati in questa prevalutazione. Tra questi, dopo un'ulteriore valutazione collegiale, sono dunque emaersi i due poster risultati vincitori e, segnatamente, il

P5: CARDIAC CHANGES IN SIRTUIN1 HETEROZYGOUS MICE ON LARD DIET PLUS MELATONIN DI: STACCHIOTTI ET AL., (MILANO, BRESCIA, BELGRADO, MADRID)

ed il

P88: MET RECEPTORREGULATES PERINUCLEAR ACTIN CAP REMODELING THROUGH YAP1 INACTIVATION DI: SGARZI ET AL., (BOLOGNA, BUDAPEST, PADOVA, TORINO)

Con I più fervidi complimenti ai vincitori ed ai contributi tutti,

La Commissione Poster

P. Gobbi Giuseppe Santoro

Antonio De Luca

ALLEGATO N.14

ELEZIONI PER IL RINNOVO DELLE CARICHE DEL CONSIGLIO DIRETTIVO DELLA SOCIETA' ITALIANA DI ANATOMIA E ISTOLOGIA

Verbale

Il giorno 25 settembre alle ore 9:30 si è riunita in modalità telematica la Commissione Elettorale per il Rinnovo delle Cariche del Consiglio Direttivo della Società Italiana di Anatomia e Istologia.

La Commissione, nominata dal Direttivo nella sua seduta del 9.9.2021, è presente al completo nelle persone di:

- Prof. Sergio Morini
- Prof. Sergio Castorina
- Prof. Virginia Tirino

La Commissione unanime propone il Professore Sergio Morini quale presidente e la Professoressa Virginia Tirino quale Segretaria.

Il Presidente dichiara aperta la seduta.

La Commissione prende atto che le candidature pervenute sono le seguenti:

Presidente:
-Prof. Giuseppe Pio Anastasi - Università di Messina
-Prof. Lucio Cocco – Università di Bologna

Segretario:
-Prof.ssa Gigliola Sica - Università Cattolica *del Sacro Cuore*, Roma

Tesoriere:
-Prof. Gianpaolo Papaccio - Università della Campania *Luigi Vanvitelli*, Napoli

Consiglieri Anatomia:
-Prof. Antonio De Luca - Università della Campania *Luigi Vanvitelli*, Napoli
-Prof. Paolo Onori - Università "La Sapienza", Roma
-Prof.ssa Carla Palumbo - Università di Modena e Reggio Emilia
-Prof. Carlo Tacchetti - Università Vita-Salute San Raffaele, Milano
-Prof. Alessandro E. Vercelli - Università di Torino
-Prof.ssa Sandra Zecchi Orlandini- Università di Firenze

Consiglieri Istologia:
-Prof. Michelangelo Cordenonsi - Università di Padova
-Prof. Antonio Filippini - Università "La Sapienza", Roma
-Prof.ssa Monica Mattioli Belmonte- Università Politecnica delle Marche, Ancona
-Prof.ssa Oriana Trubiani - Università di Chieti

La Commissione inoltre prende atto dell'invio, con comunicazione del 14/09/2021 da parte del Tesoriere della SIAI, dell'elenco completo dei Soci ordinari in regola per partecipare alle elezioni del rinnovo delle cariche del Consiglio Direttivo, e dell'elenco dei Soci emeriti, risultando che il numero complessivo dei Soci aventi diritto al voto è 294, di cui 288 soci ordinari e 6 soci emeriti.
Il giorno 24 settembre 2021, alle ore 12:59, la Commissione riceve comunicazione dal Tesoriere a mezzo e-mail di considerare la Prof.ssa Barbara Ghinassi nell'elenco dei Soci Ordinari in regola per la votazione.

Di conseguenza, il numero complessivo dei Soci aventi diritto al voto è 295, di cui 289 soci ordinari e 6 soci emeriti.

La Commissione dà atto che le votazioni si sono svolte secondo quanto previsto dall'art. 9 dello Statuto. La votazione è avvenuta il giorno 24 settembre 2021 con apertura del seggio alle ore 10.00 e chiusura alle ore 17.00; la procedura è stata implementata on line tramite la piattaforma http://siai.leanevent.it/. La raccolta dei dati è avvenuta attraverso la piattaforma JOTFORM che è HIPAA-compliant con server cloud con base in Europa che definisce i requisiti per il trattamento dei dati sanitari protetti dei privati. La compliance HIPAA è regolamentata dal Department of Health and Human Services e l'OCR. L' esportazione su Google Sheet è stata riservata ed in sola visualizzazione con gli utenti della Commissione elettorale. Responsabile di tutta la procedura è stata la Dott.ssa Luana Martuzzi.

Il voto è stato espresso da n. 270 votanti totali (91,5 % degli aventi diritto).

Le preferenze validamente espresse risultano così attribuite:

Presidente

1. Prof. Lucio Ildebrando Cocco n. 124 voti
2. Prof. Giuseppe Pio Anastasi n. 114 voti

Le Schede bianche sono state in numero di 32

Segretario

1. Prof.ssa Gigliola Sica n. 224 voti

Le Schede bianche sono state in numero di 46

Tesoriere

1. Prof. Gianpaolo Papaccio n. 227 voti

Le Schede bianche sono state in numero di 43

Consiglieri Anatomia

1. Prof.ssa Sandra Zecchi Orlandini n. 170 voti
2. Prof.ssa Carla Palumbo n. 154 voti
3. Prof. Paolo Onori n. 148 voti
4. Prof. Alessandro E. Vercelli n. 147 voti
5. Prof. Antonio De Luca n. 125 voti
6. Prof. Carlo Tacchetti n. 125 voti

Le Schede bianche sono state in numero di 9

Consiglieri Istologia

1. Prof.ssa Monica Mattioli Belmonte n. 156 voti
2. Prof.ssa Oriana Trubiani n. 154 voti
3. Prof. Antonio Filippini n. 145 voti
4. Prof. Michelangelo Cordenonsi n. 142 voti

Le Schede bianche sono state in numero di 80

Pertanto, in base al numero di preferenze, la Commissione dichiara eletti i seguenti componenti nel Consiglio Direttivo della Società Italiana di Anatomia e Istologia per il triennio 2022-2024:

Presidente

1. Prof. Lucio Ildebrando Cocco n. 124 voti

Segretario

1. Prof.ssa Gigliola Sica n. 224 voti

Tesoriere

1. Prof. Gianpaolo Papaccio n. 227 voti

Consiglieri Anatomia

1. Prof.ssa Sandra Zecchi Orlandini n. 170 voti
2. Prof.ssa Carla Palumbo n. 154 voti
3. Prof. Paolo Onori n. 148 voti
4. Prof. Alessandro E. Vercelli n. 147 voti

Consiglieri Istologia

1. Prof.ssa Monica Mattioli Belmonte n. 156 voti
2. Prof.ssa Oriana Trubiani n. 154 voti
3. Prof. Antonio Filippini n. 145 voti
4. Prof. Michelangelo Cordenonsi n. 142 voti

Alle ore 11.00, il Presidente dichiara chiusa la seduta.

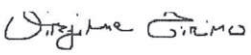
Il presente verbale viene redatto, letto dal Presidente della Commissione e sottoscritto, firmandolo di proprio pugno, dai Componenti della Commissione, e contestualmente viene inviato a mezzo e-mail al Presidente e al Segretario della SIAI.

LA COMMISSIONE

LA COMMISSIONE

Prof. Sergio Morini  Presidente

Prof. Sergio Castorina  Componente

Prof.ssa Virginia Tirino  Segretario

ALLEGATO
dati aggregati

TOTALI VOTANTI		270
CARICA PRESIDENTE		
Prof. Giuseppe Pio Anastasi - Università di Messina	114	270
Prof. Lucio Cocco – Università di Bologna	124	
Scheda bianca	32	
CARICA SEGRETARIO		
Prof.ssa Gigliola Sica - Università Cattolica del Sacro Cuore, Roma	224	270
Scheda bianca	46	
CARICA TESORIERE		
Prof. Gianpaolo Papaccio - Università della Campania Luigi Vanvitelli, Napoli	227	270
Scheda bianca	43	
CARICA CONSIGLIO DIRETTIVO ANATOMIA		
Prof. Antonio De Luca - Università della Campania Luigi Vanvitelli, Napoli	125	
Prof. Paolo Onori - Università "La Sapienza", Roma	148	
Prof.ssa Carla Palumbo - Università di Modena e Reggio Emilia	154	
Prof. Carlo Tacchetti - Università Vita-Salute San Raffaele, Milano	125	
Prof. Alessandro E. Vercelli - Università di Torino	147	
Prof.ssa Sandra Zecchi Orlandini- Università di Firenze	170	
Scheda bianca	9	
CARICA CONSIGLIO DIRETTIVO ISTOLOGIA		
Prof. Michelangelo Cordenonsi - Università di Padova	142	
Prof. Antonio Filippini - Università "La Sapienza", Roma	145	
Prof.ssa Monica Mattioli Belmonte - Università Politecnica delle Marche, Ancona	156	
Prof.ssa Oriana Trubiani - Università di Chieti	154	
Scheda bianca	80	

Ferdinando Paternostro, Direttore responsabile
Registrato presso il Tribunale di Firenze con decreto n. 850 del 12 marzo 1954

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