SEMM at Campus I Via Adamello, 16 Milan, Italy

Scientific Program

Monday, September 12, 2016

h. 13.00 to 18.00 *Registration and Poster set up*

h. 14.30 to 16.30 *Workshop "**Role of Vitamin D in Skin Cancer Risk and Prognosis**"*

h. 14.00 to 17.00 *ESPCR Council Meeting*

h. 17.00 *to* **17.15** **Opening Remarks**

Session 1: Development and Migration of Melanocytes – Chair: Eirikur Steingrimsson

(Dept. of Biochemistry and Molecular Biology, Reykjavik, Iceland)

h. 17.15 *to* 17.45 **Lukas Sommer**
Dept. of Oncology, University Hospital Zurich, Zurich, Switzerland "A Neural Crest Stem Cell Program Controlling Melanoma Formation"

h. 17.45 *to* 18.00 **Corinna Köhler**
Lab. for Molecular Cancer Biology, Center for the Biology of disease, VIB, Leuven, Belgium
"Long-lived Fully Differentiated Melanocytes can Serve as Cellular Origin of Cutaneous Melanoma"

h. 18.00 *to* 18.15 **Yusuke Nagao**
Biosci. Biotech. Ctr., Nagoya Univ., Japan; Dept. of Biol. & Biochem., Univ. of Bath, UK
"Sox5 Modulates Sox10-mediated Pigment Cell Fate Specification in Medaka amd Zebrafish"

h. 18.15 *to* 18.30 **Kyriakos Orfanidis**
Dept. of Dermatology and Venerology, and Dept. of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden
"Evaluation of TUBB3 as a Novel Diagnostic Marker to Distinguish Nevi from Melanoma"

h. 18.30–19.00 **Margret H. Ogmundsdottir**
School of Health Sciences – Faculty of Medicine, Reykjavik, Iceland
"The Transcriptional Regulation of Autophagy in Melanoma"

h. 19.30 *to* ***21.00*** ***Welcome Aperitif***

Tuesday, September 13, 2016

Session 2: Pigmentation Disorders – Chair: Mauro Picardo

(San Gallicano Dermatologic Institute, Rome, Italy)

h. 09.00 *to* 09.30 **Julien Seneschal**
INSERM U1035, Université de Bordeaux, Bordeaux, France
"New Insights on Innate and Adaptative Immune Response in Vitiligo"

h. 09.30 *to* 09.45 **Doreen Schwochow-Thalmann**
Dept. of Animal Breeding anf Genetics, Swedish University of Agricultural Sciences, Sweden; GABI, INRA, AgroParisTech, Universitè Paris-Saclay, Jouy-en-Josas, France
" Towards Understanding the Molecular Basis of Pigment Pattern Formation in Birds"

h. 09.45 *to* 10.15 **Maria Vittoria Schiaffino**
Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy
"Role of the Master Lysosomal Regulator TFEB in the Pathogenesis of Ocular Albinism"

h. 10.15 *to* 10.30 **Marta Abrisqueta González**
Dept. of Biochemistry, Molecular Biology and Immunology, University of Murcia, Spain, and Instituto Murciano de Investigación Biomédica (IMIB)
"Human Melanocortin 1 Receptor (MC1R)-dependent Post-translational Modification of β-Arrestins (ARRBs)"

h. 10.30 *to* 10.45 **Ian J. Jackson**
University of Edinburgh, Edinburgh, UK
"The Genetics and Genomics of Human Hair Colour Variation"

h. 10.45 to 11.10 *Coffee break*

Session 3: Melanins and Melanogenesis: Mechanisms of Control and Applications – Chair: Alessandra Napolitano
(Department of Chemical Sciences, University of Naples Federico II, Naples, Italy)

h. 11.10 *to* 11.40 **Shosuke Ito**
Dept. of Chemistry, Fujita Health University, Nagoya, Japan
"Biochemical Mechanism of Rhododendrol-induced Leukoderma"

h. 11.40 *to* 11.55 **Patrick Bogdanowicz**
Dermo Cosmetic Center, Toulouse, France
"Preclinical and Clinical Efficacy of a Dermo-Cosmetic Skin Lightening Cream in Women Suffering from Melasma"

h. 11.55 *to* 12.10 **Miguel C. Seabra**
CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisbon, Portugal
"Melanin Processing by Keratinocytes After Transfer"

h. 12.10 *to* 12.25 **Lucia Panzella**
Dept. of Chemical Sciences, University of Naples "Federico II", Naples, Italy
"The Role of Carboxyl Groups in Eumelanin and Pheomelanin Properties"

h. 12.25 *to* 12.40 **Tadeusz Sarna**
Dept. of Biophysics, Jagiellonian University, Krakow, Poland
"Photogeneration and Quenching of Singlet Oxygen and Superoxide Anion by Synthetic Eumelanins and Pheomelanins"

h. 12.40 to 13.45 *Lunch*

h. 12.40 to 13.45 ***Round table**: "Science **is Not** Gender Neutral, Fortunately"*
Chair: Luisa Lanfrancone *(European Institute of Oncology, IEO, Milan, Italy)*
Discussant: Susanna Chiocca, Marie-Dominique Galibert, Sara Gandini, Marisol Soengas

h. 14.00 *to* 15.00 **Keynote Lecture: Andrea Ballabio**
Telethon Institute of Genetics and Medicine, TIGEM, Naples, Italy
"The Awesome Lysosome"

h. 15.00 to 15.20 *Coffee break*

Session 4: Molecular Epidemiology – Chair: Sara Gandini
(European Institute of Oncology, IEO, Milan, Italy)

h. 15.20 *to* 15.50 **Mathieu Boniol**
International Prevention Research Institute, Lyon, France
"Melanoma Mortality Worldwide, Toward a Decreasing Burden in the Next Decades"

h. 15.50 *to* 16.05 **Roberta Colucci**
Dept. of Surgery and Translational Medicine, Section of Dermatology, University of Florence, Florence, Italy
"Association of Vitamin D Status with Clinical Features and Repigmentation in Vitiligo Patients: Preliminary Results from an Observational Study"

h. 16.05 *to* 16.20 **Chiara Martinoli**
Melanoma Medical Oncology Unit, European Institute of Oncology, Milan, Italy
"Prognostic Relevance of Baseline Hematological Profiles in Melanoma Patients"

h. 16.20 *to* 16.50 **Susana Puig**
Dermatology Department, IDIBAPS, Universitat de Barcelona, Barcelona, Spain
"Clinical Implications of Genetics of Melanoma"

Wednesday, September 14, 2016
Session 5: Melanocytes and Melanoma Molecular Pathways – Chair: Marie-Dominique Galibert
(IGDR, University of Rennes, Rennes, France)

h. 09.00 *to* 09.30 **Laura Machesky**
Cancer Research UK Beatson Institute, Glasgow, UK
"Role of the Actin Cytoskeleton and Rho GTPases in Melanoblast Migration"

h. 09.30 *to* 09.45 **Vittoria Maresca**
San Gallicano Dermatologic Institute, Rome, Italy
"Role of A-MSH in the Control of Proliferation in Melanocytes and Melanoma Cells"

h. 09.45 *to* 10.00 **Gian Marco De Donatis**
INSERM, U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), team 12, Nice, France
"NIK Inhibition Potentiates Anti-PD1 Treatment by Inducing IFN-y Release by Cancer Cells"

h. 10.00 *to* 10.30 **Yardena Samuels**
Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel
"Towards Deciphering the Functional Genetic and Neo-Antigenic Landscape in Melanoma"

h. 10.30 to 10.50 *Coffee break*

Session 6: Melanocytes and Melanoma Molecular Pathways (continued) – Chair: Colin Goding
(Ludwig Institute for Cancer Research, University of Oxford, Oxford, UK)

h. 10.50 *to* 11.20 **Daniel Peeper**
Department of Molecular Oncology, NKI, Amsterdam, The Netherlands
"Function-based Genetic Perturbations to Reveal Novel Melanoma Vulnerabilities"

h. 11.20 *to* 11.35 **Marie-Dominique Galibert**
CNRS UMR 6290, IGDR, Université de Rennes; CHU Rennes, Laboratoire de Génomique Médicale, Rennes, France
"Tailored Small Oligonucleotides Abolish MiRNA Sequestration and Restore MicroRNA Tumour Suppressor Activity"

h. 11.35 *to* 11.50 **Marine Melixetian**
Dept. of Experimental Oncology, European Institute of Oncology, Milan, Italy
"Loss of Long Non Coding RNA TINCR Promotes Melanoma Growth and Metastasis Formation by Inducing a Switch to an Invasive Phenotype"

h. 11.50 *to* 12.20 **Ze'ev Ronai**
Sanford Burnham Prebys Medical Discovery Institute, La Jolla, USA
"ATF2 Variants in Pigmentation and Melanomagenesis – the Unexpected Turn"

h. 12.20 to 13.00 *Lunch*

h. 13.00 *to* 14.45 **Poster session**

h. 14.45 *to* 15.30 **Flash talks – Chair: Yardena Samuels** *(Dept. of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel)*

h. 14.45 *to* 14.50 **Jacques Rouanet**
UMR990 INSERM UdA Clermont-Ferrand; Dermatology Dept., CHU Estaing Clermont-Ferrand, France
"Melanoma Targeted Radionuclide Therapy with Melanin-Ligands: Molecular Mechanisms and Potentialisation"

h. 14.50 *to* 14.55 **Ahmad Najem**
LOCE, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium
"Opposite Roles for MITF and P53 in the Resistance of Mutant NRAS Melanoma to MEK Inhibition"

h. 14.55 *to* 15.00 **Ilse Hurbain**
Institut Curie, PSL Research University, CNRS, UMR 144,; Sorbonne Université, Paris, France
"A Systematic Analysis of Melanosome Structure and Distribution in Different Skin Color Phenotypes: Identification of Melanocore Clusters as Lysosome Related Organelles"

h. 15.00 *to* 15.05 **Seung Hyun Bang**
Dept. of Dermatology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea
"A Novel Ion Channel Activator, CyPPA Inhibits Melanogenesis via the GSK3β/β-catenin Pathway"

h. 15.05 *to* 15.10 **Justine Leclerc**
INSERM U1065, Université de Nice Sophia-Antipolis, Nice, France
"Oncogenic MITFE318K Promotes Senescence Delay and Melanoma Progression"

h. 15.10 *to* 15.15 **Josue Ballesteros**
Dept. of Biochemistry and Molecular Biology, Biomedical Center, Faculty of Medicine, University of Iceland, Reykjavik, Iceland
"MITF, TFEB and TFE3 in Melanoma – Regulation and Interaction"

h. 15.15 *to* 15.20 **Lluís Montoliu**
Centro Nacional de Biotecnología (CNB-CSIS) y Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER-ISCIII), Madrid, Spain
"New Mouse Models of Albinism Generated with CRISP-Cas9 Technology"

h. 15.20 *to* 15.30 Open Discussion

Interactive Session with Researchers and Clinicians
Melanoma Models: What Is Needed to Move from Bench to Bedside?
Chair: Sara Gandini & Luisa Lanfrancone
(European Institute of Oncology, IEO, Milan, Italy)

h. 15.30 *to* 15.50 **Lionel Larue**
Institut Curie, Orsay, France
"Relevance of In Vivo and In Vitro Models for Melanoma"

h. 15.50 *to* 16.10 **Pier Francesco Ferrucci**
European Institute of Oncology, IEO, Milan, Italy
"Immunotherapy and New Targets in the Treatment of Advanced Melanoma"

h. 16.10 *to* 16.30 **Daniela Massi**
Division of Pathological Anatomy, University of Florence, Florence, Italy
"Tissue Biomarkers' Development"

h. 16.30 *to* 17.00 **Open discussion**

h. 17.00 to 17.20 *Coffee break*

Session 7: Cell Metabolism Dysregulation in Melanoma – Chair: Corine Bertolotto
(INSERM U1065, Université de Nice Sophia-Antipolis, Nice, France)

h. 17.20 *to* 17.50 **Colin Goding**
Ludwig Institute for Cancer Research, University of Oxford, Oxford, UK
"An Evolutionarily Conserved Driver of Melanoma Invasiveness"

h. 17.50 *to* 18.05 Stefania Guida
Dermatology Unit, University of Modena and Reggio Emilia, Modena, Italy

"Metabolic Reprogramming in Melanoma: the Role of Peif2alpha"

h. 18.05 *to* 18.20 **Stéphane Rocchi**
INSERM, U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), Equipe Biologie et Pathologie des cellules mélanocytaire: de la pigmentation cutanée au mélanome, Nice; Université de Nice Sophia Antipolis, UFR de Médecine, Nice; Service de Dermatologie, Hôpital Archet II, CHU Nice, France
"New Compounds Triggering Endoplasmic Reticulum Stress Exert Anti-Melanoma Effects and Overcome BRAF Inhibitor Resistance"

h. 18.20 *to* 18.50 **Jean Christophe Marine**
Flanders Interuniversity Institute for Biotechnology, Leuven, Belgium
"Integrative and Comparative Genomics Analyses Identify Genetic and Epigenetic Drivers of Melanoma"

h. 20.30 ***Social Dinner***

Thursday, September 15, 2016

Session 8: Melanoma Microenvironment and the Role of Inflammation – Chair: Lionel Larue
(Institut Curie, Orsay, France)

h. 9.00 *to* 9.30 **Thomas Tueting**
Department of Dermatology University Hospital Magdeburg, Germany
"The Role of Neutrophils in Melanoma Pathogenesis and Resistance to Immunotherapy"

h. 9.30 *to* 9.45 **Thomas Rouillé**
Institut National de la Santé et de la Recherche Médicale (INSERM), U938, Saint-Antoine Research Center, Paris; Université Pierre et Marie Curie-Paris VI, Paris, France
"Varying Proliferative and Clonogenic Potential in NRAS-mutated Congenital Melanocytic Nevi According to Size"

h. 9.45 *to* 10.00 **Petra Wäster**
Experimental Pathology, Department of Clinical and Experimental Medicine, Faculty of Medicine, Linköping University, Linköping, Sweden
"The Relationship Between UV Induced Lysosomal Exocytosis and Melanosome Transfer in Melanocytes"

h. 10.00 to 10.20 *Coffee break*

h. 10.20 *to* 10.50 **Marisol Soengas**
Molecular Pathology Programme, CNIO, Madrid, Spain
"Imaging and Targeting Pre-metastatic Niches in Melanoma"

h. 10.50 *to* 11.50 **Fritz Anders Memorial Lecture: Richard Marais**
Cancer Research UK Manchester Institute, Manchester, UK
"Precision Medicine for Melanoma"

h. 11.50 to 12.50 ***ESPCR Assembly***

h. 13.00 to 14.00 *Light lunch*

Session 9: Highlights from the Other Pigment Cell Societies – Chair: Lluis Montoliu
(Department of Molecular and Cellular Biology, National Centre for Biotechnology, Madrid, Spain)

h. 14.00 *to* 14.15 **Kyoung-Chan Park**
Dept. of Dermatology, Seoul National University Bundang Hospital/ASPCR, Seongnam, Korea
"New Aspects of Melasma Treatment in Asians"

h. 14.15 *to* 14.30 **Boon Kee Goh**
Mount Elizabeth Medical Centre/ASPCR, Singapore, Singapore
"When a Leukoderma is not Vitiligo"

h. 14.30 *to* 14.45 **Kazumasa Wakamatsu**
Dept. of Chemistry, Fujita Health University, School of Health Science Toyoake/JSPCR, Aichi, Japan
"The Metabolic Fate of Ortho-quinones Derived from Catecholamine Metabolites"

h. 14.45 *to* 15.00 **Caroline Le Poole**
Dept. of Microbiology and Immunology, Loyola University Chicago/PASPCR, Chicago, USA
"Update on Vitiligo Research"

Closing remarks
End of the meeting

September 12, 2016
Session 1: Development and Migration of Melanocytes

Yy1 is a central regulator of metabolism and growth in neural crest stem cells and melanoma

Sandra Varum, Lukas Sommer

Institute of Anatomy, University of Zurich, Zurich, Switzerland

Melanoma arises from the melanocyte lineage, which originates during embryonic development from neural crest stem cells (NCSCs). There is increasing evidence that factors known to control NCSCs are also implicated in melanoma formation. Accordingly, interfering with mechanisms normally regulating NCSC development influences tumorigenesis both in genetic melanoma mouse models in vivo and in human melanoma cells. For instance, the transcription factor Sox10 regulates both maintenance of NCSCs as well as melanoma formation and expansion. Likewise, we recently identified the transcription factor Yy1 to promote proliferation both in NCSCs as well as in melanoma cells in vivo. Mechanistically, Yy1 controls a set of genes involved in various metabolic pathways; Yy1 inactivation thus leads to drastic changes in the metabolome of embryonic neural crest cells and tumor cells. In sum, Yy1 appears to be a central node of a metabolic program associated with cell type-specific growth during embryogenesis and tumorigenesis.

O-1

Long-lived fully differentiated melanocytes can serve as cellular origin of cutaneous melanoma

Corinna Köhler[1,2], David Nittner[1,2], Enrico Radaelli[3], Flavie Luciani[1,2], Jasper Wouters[4], Joost van den Oord[4], Jean-Christophe Marine[1,2]

[1]Laboratory for Molecular Cancer Biology, Center for the Biology of disease, Leuven, Belgium; [2]Laboratory for Molecular Cancer Biology, Department of Human Genetics, KULeuven, Leuven, Belgium; [3]Mouse Histopathology Core Facility, Center for the biology of disease, VIB-KULeuven, Belgium; [4]Laboratory of Translational Cell and Tissue Research, Department of Pathology, KULeuven and UZ Leuven, Leuven, Belgium

Whether cutaneous melanoma originates from melanocyte stem/progenitor cells and/or differentiated melanocytes is still a matter of debate. In order to search for melanoma's cellular origin we used a lineage tracing approach using the Tyr::CreERT2;BrafLSLV600E/+ melanoma mouse model as a genetic background. Tumors were initiated on the back or tail skin. We observed that back skin lesions originate from melanocyte progenitor cells and/or fully functional/differentiated melanocytes located in the hair bulb, which expand, eventually leading to its rupture and the dissemination of melanoma cells into the dermis. Histologically, these lesions lack epidermal involvement and exhibit schwannian features. In contrast, targeting the interfollicular melanocytes located in the tail skin results in an expansion of melanoma lesions within the epidermis. These lesions enter the dermis and expand vertically. Notably, inactivation of Pten leads to the formation of polyclonal tumors. In contrast, lesions were monoclonal on the Ink4A-null background. Importantly, tight kinetic lineage tracing experiments established that differentiated/melanocytic, but not undifferentiated/amelanocytic melanocytes were at the origin of the lesions.

Our study provides the first comprehensive lineage tracing analysis of the widely used BRafV600E-driven melanoma mouse model. This work establishes that fully differentiated interfollicular melanocytes can serve as the cell of origin in cutaneous melanoma.

O-2

Sox5 modulates Sox10-mediated pigment cell fate specification in medaka and zebrafish

Yusuke Nagao[1,2], Hiroyuki Takada[3], Motohiro Miyadai[3], Ryoko Seki[1], Yasuhiro Kamei[4], Ikuyo Hara[5], Yoshihito Taniguchi[6], Kiyoshi Naruse[5], Masahiko Hibi[1,3], Robert N. Kelsh[2], Hisashi Hashimoto[1,3]

[1]Biosci. Biotech. Ctr., Nagoya Univ; [2]Department of Biol. & Biochem., Univ. of Bath; [3]Grad. Sch. of Sci., Nagoya Univ; [4]Spectrography and Bioimaging Facility; [5]Lab. of Biores., NIBB; [6]Dept. of Preventive Medicine and Public Health, Sch. of Med., Kyorin Univ

Neural crest-derived pigment cells in teleosts are diverse, three types (melanophore, iridophore and xanthophore) in zebrafish and four types in medaka with fourth leucophore. They provide an excellent model for studying cell fate specification. Previously we demonstrated that medaka Sox5 functions as a molecular switch determining xanthophore versus leucophore fate choice by promoting xanthophore specification. In zebrafish, Sox10 is required for specification of all pigment cell types. In mouse, Sox5 antagonises melanocyte specification mediated by Sox10. Here, we introduce the interactions between Sox5 and Sox10 during pigment cell development in medaka and zebrafish.

We engineered sox10a and sox10b mutants of medaka and sox5 mutant of zebrafish with using TALEN or CRISPR/Cas9 systems. In zebrafish, loss of Sox5 partially rescued the reduced melanophore and xanthophore in sox10/baz1 mutant. In medaka, antagonistic function of Sox5 against Sox10 was also found in melanophore and iridophore. In contrast, decrease of Sox5 function aggravated the reduced xanthophore formation in sox10 mutants. Double loss of Sox5 and Sox10a caused further increase in leucophore numbers compared with sox5 single mutant. Our findings suggest conserved role for Sox5 in repressing Sox10 function of melanophore and unique cooperative activity of Sox5 and Sox10 in xanthophore and leucophore in medaka.

O-3

Evaluation of TUBB3 as a novel diagnostic marker to distinguish nevi from melanoma

Kyriakos Orfanidis[1,2], Petra Wäster[3], Katarzyna Lundmark[4,5], Karin Öllinger[3]

[1]Department of Dermatology and Venereology, Linköping University, Linköping, Sweden; [2]Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden; [3]Experimental Pathology, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden; [4]Department of Clinical Pathology and Clinical Genetics, Linköping University, Linköping, Sweden; [5]Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

High number of nevi is a risk factor for development of malignant melanoma. In addition, it is in many cases clinically and histologically challenging to distinguish melanoma from nevi and specific biomarkers are lacking. Senescence is a universal barrier against malignant transformation and tumor development, which is regulated by epigenetic changes of gene expression. Considering nevi as growth arrested benign melanocytic lesions that could develop into malignant melanoma, we designed a

model system consisting of young and senescent melanocytes isolated from the same individual and compared the gene expression with microarray analyses derived from nevi and melanoma. A concordant altered gene expression was identified in 84 genes when combining the growth-arrested samples and compared to the proliferating. TUBB3 encoding the microtubule protein tubulin β-3, was selected for further studies. Depletion of tubulin β-3 in young melanocytes and melanoma cell lines decreased proliferation and migration. Immunohistochemical assessment of tubulin β-3 in benign lesions showed dense staining in the superficial parts of dermis, which faded with the depth. In contrast, primary melanomas showed strong staining without gradient partly in a disordered pattern. Detailed studies of tubulin-β-3 expression might improve future diagnostic performance and improve the accuracy when assessing atypical and dysplastic lesions.

The transcriptional regulation of authophagy in melanoma

Margret H. Ogmundsdottir

School of Health Sciences – Faculty of Medicine, Reykjavik, Iceland

Autophagy can promote tumor formation by increasing cell survival under stress conditions such as hypoxia and nutrient starvation. Genes involved in autophagy are highly expressed in melanoma cells and the process has been proposed to play a critical role in melanoma. The transcription factors TFEB and TFE3 play a key role in autophagy regulation in various cell types, and are close relatives of MITF, the master regulator of melanocyte development. Analysis of Cancer Genome Atlas data reveals that MITF expression correlates with autophagy gene expression in metastatic melanoma. When MITF is over-expressed in melanoma cells, transcription of certain autophagy genes is activated and more autophagosomes are formed although this does not affect autophagy flux. Surprisingly, CRISPR-mediated MITF knockout cells exhibit increased expression of autophagy genes and increased autophagy flux. Our results suggest that this is due to increased expression of TFE3 in the absence of MITF. In short, our results show that MITF and its close relatives, TFEB and TFE3, regulate autophagy in melanoma and form a regulatory loop. Regulation of autophagy is therefore maintained, despite the absence of one family member.

September 13, 2016
Session 2: Pigmentation Disorders

New insights on innate and adaptative immune responses in Vitiligo

Julien Seneschal, Katia Boniface

Department of Dermatology, INSERM U1035 Immuno-Dermatology Laboratory, ATIP-AVENIR, University of Bordeaux, France

Vitiligo is the most common depigmenting disorder, affecting 0.5% of the population. A loss of epidermal melanocytes is the pathogenic hallmark of vitiligo. Factors involved in the initiation of vitiligo remain largely unknown, but include both genetic and environmental factors. Many observations highlight the role of both innate and adaptative immune cells in the development of Vitiligo. We have previously demonstrated that progressive vitiligo is associated with the infiltration of plasmacytoid dendritic cells (pDCs). More recently, we have observed that Heat Shock Protein (HSP)-70, secreted in vitiligo skin, could potentiate the activation of pDCs, leading to the production of the pro-inflammatory cytokine Interferon (IFN)α. This was associated with epidermal expression of CXCL9 and CXCL10 and the recruitment of CXCR3$^+$ T cells. Infiltrated T cells in peri-lesional skin of vitiligo expressed an effector memory phenotype with a significant proportion of cells bearing markers specific for resident memory T cells. Multiparametric analysis of the cytokine profile of these skin TEM subsets identified pro-inflammatory cytokines involved in the loss of melanocytes. Therefore our findings add a more precise understanding of the role of the innate and adaptative immune responses in vitiligo.

O-4
Towards understanding the molecular basis of pigment pattern formation in birds

Doreen Schwochow-Thalmann[1,2], Susanne Bornelöv[3,4], Henrik Ring[3], Jingyi Li[5], Erika Manlig[6], Ben Dorshorst[5], Bertrand Bed'Hom[2], David Gourichon[7], Michèle Tixier-Boichard[2], Leif Andersson[3,8]

[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Sweden; [2]GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France; [3]Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden; [4]Wellcome Trust Medical Research Council Stem Cell Institute, University of Cambridge, UK; [5]Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, USA; [6]Department of Medical Sciences, Uppsala University, Uppsala, Sweden; [7]PEAT, INRA, Nouzilly, France; [8]Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Chickens have been selected for a variety of morphological traits including their appearance. This human-mediated selection has created numerous feather colorations and patterns, which make the chicken a model species for pigmentation genetics. The Patterning (Pg) locus in chickens plays an important role in organizing black pigment on individual feathers. On its own it creates concentric, dark lines called penciling but together with other loci it forms a greater variety of pattern such as autosomal barring. Despite being considered the major driver of individual feather pattern formation in chickens, its molecular basis is unknown. To reveal the genetic variant(s) underlying Patterning, we set up a two-generation backcross pedigree involving wild-type Brown Leghorn crossed with Fayoumi (Pg/Pg) and analyzed pooled whole genome sequence data from progenies of defined plumage patterning. Our analysis did not confirm the previous mendelian assignment of Pg to chromosome 1 but we did observe strong linkage to a 1 Mb region on a different chromosome, which includes two genes involved in pigmentation. Our data furthermore suggests that Patterning might be genetically heterogeneous in chicken. Further functional studies are underway to better understand how the Patterning locus is driving pigment patterning by affecting melanocyte migration and/or pigment production.

Role of the master lysosomal regulator TFEB in the pathogenesis of ocular albinism

Angela Palmigiano[1], Anna Rosaria De Vito[1], Andrea Ballabio[2], Maria Vittoria Schiaffino[1]

[1]Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy; [2]Telethon Institute of Genetics and Medicine TIGEM, Naples, Italy

In addition to their traditional catabolic roles, lysosomes play a plethora of other functions, including repair of plasma membrane wounds, modulation of Ca^{2+} fluxes, and mTORC1 signaling. Lysosomes also directly communicate with the nucleus, by an autoregulatory loop generated by the transcription factor TFEB, which is activated in conditions of lysosomal stress, and stimulates the expression of genes involved in lysosome biogenesis and function. In order to accomplish specific tasks, several specialized cell types contain lysosome-related organelles (LROs), which share features of conventional lysosomes, yet are characterized by unique morphological and biochemical characteristics. Among LRO, melanosomes in pigment cells are devoted to the synthesis, distribution and transfer of melanin. Their biogenesis is altered in Ocular albinism type 1, an X-linked genetic disorder characterized by severe visual anomalies and giant melanosomes. The disease is due to loss-of-function of OA1, a G protein-coupled receptor, exclusively localized to the membrane of intracellular organelles, and necessary for attaining the proper number, size and distribution of melanosomes. We found that in Oa1-KO melanocytes TFEB is upregulated and constitutively translocated to the nucleus. The mechanisms underlying these abnormalities and their possible implications for melanosome biogenesis and the pathogenesis of albinism will be discussed.

O-5

Human melanocortin 1 receptor (MC1R)-dependent post-translational modification of β-Arrestins (ARRBs)

Marta Abrisqueta, Concepción Olivares, Julia Sirés-Campos, María Castejón-Griñán, Cecilia Herraiz, Jose Carlos García-Borrón, Celia Jimenez-Cervantes

Department of Biochemistry, Molecular Biology and Immunology, University of Murcia, Spain, Instituto Murciano de Investigación Biomédica IMIB

Interaction of MC1R, a Gs protein-coupled receptor (GPCR) crucial for melanocyte proliferation and differentiation, with the Gs protein is regulated by cytosolic β-arrestins (ARRBs) which mediate desensitization and endocytosis. MC1R signaling is also modulated by the E3-ubiquitin ligase Mahogunin Ring Finger-1 (MGRN1), whose mutation causes hyperpigmentation and neurodegeneration. When co-expressed in heterologous cells, MC1R interacts stably with ARRB1 or ARRB2 even in the absence of agonists, and ternary MC1R-ARRB2-MGRN1 complexes are readily detected. Since agonist-induced GPCR-ARRB interactions frequently trigger ubiquitination, we analysed MC1R-dependetn occurrence of this post-translational modification of ARRBs. We detected MC1R-dependent, agonist-independent polyubiquitination of ARRB1/2, most likely involving the C-terminal Lys400 of ARRB2 and leading to proteolytic cleavage. Native ARRB1/2 expressed without MC1R migrated as two major bands of apparent molecular weight (Mr) 52 and 40 kDa in SDS-PAGE. A third intermediate mobility band was also detected. Immunochemical analysis of epitope-tagged ARRB2, site directed mutagenesis, MALDI-TOF and functional studies suggested that the low Mr species is mono- or diubiquitinated in the absence of MC1R, thus accounting for the triplet observed in SDS-PAGE. In the presence of MC1R the 40 kDa native protein is favoured. This form interacts preferentially with MC1R and appears responsible for its desensitization and sequestration.

O-6

The genetics and genomics of human hair colour variation

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The genetics of human hair colour is a complex interaction between numerous genes. The knowledge we have of the biology of melanocytes and their interactions presents an excellent opportunity to functionally dissect this genetic system. UK Biobank is a project in which 500 000 individuals have been recruited and data collected for many clinical and physiological parameters, including hair colour. We have performed a genome wide association study of UK Biobank for red and blonde hair colour. The red hair analysis reveals the well-established association with multiple alleles of MC1R, including novel variants outside the coding region. We show that the low penetrance ("r") missense alleles of MC1R interact with variants at the ASIP locus, encoding the agouti protein. We find 3 further loci which contribute to red hair colour. By contrast we find over 25 separate loci associated with blonde hair colour, including many genes known to be involved in melanocyte biology but not previously shown to contribute to human hair colour variation. We also identify novel, pigment cell enriched, genes of previously unknown function. We are functionally analysing these genes, including making mouse mutants, as candidates for new pathways in pigment cell biology.

Session 3: Melanins and Melanogenesis: Mechanisms of Control and Applications

Biochemical mechanism of rhododendrol-induced leukoderma

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Ortho-Quinones possess a high reactivity and undergo an addition reaction with thiols. On melanosomes, an o-quinone, dopaquinone that is produced from L-tyrosine, gives rise to melanin. If a pseudo substrate of tyrosinase interferes with this process, an extrinsic o-quinone is produced, thus exerting melanocyte-specific toxicity. Rhododendrol (RD; 4-(4-hydroxyphenyl)-2-butanol), was used as a skin-whitening agent because of its tyrosinase inhibitory activity. However, in July 2013, cosmetics containing RD were recalled because a considerable number of consumers developed leukoderma. We aimed to clarify the biochemical mechanism of melanocyte toxicity caused by RD by examining tyrosinase-dependent metabolism of RD. It was found that RD serves as a good substrate for not only mushroom tyrosinase but also human tyrosinase to produce RD-quinone products. We then examined metabolism of RD in B16 mouse melanoma cells in vitro. A moderate level of RD pheomelanin was detected in RD-treated cells. We also confirmed the covalent binding of RD-quinone to non-protein and protein thiols through cysteinyl residues. The covalent binding of RD-quinone to proteins was much greater than dopaquinone. In addition, studies in progress suggest a

potent pro-oxidant activity of RD-eumelanin. These results suggest that the tyrosinase-induced production of RD reactive metabolites causes melanocyte toxicity of RD.

O-7
Preclinical and clinical efficacy of a dermo-cosmetic skin lightening cream in women suffering from melasma

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Melanin is a bio-pigment, synthesized within melanocytes localised in the basal layer of the epidermis. The quantity, type and distribution of melanin in keratinocytes are one determinant of human skin color. Melanin plays a critical role to protect skin from UV but excess melanin synthesis can lead to hyper-pigmentation disorders like melasma. Therefore, development of whitening products is of great interest to improve clinical and cosmetic concerns.

The aim of this study was to evaluate the efficacy of a skin lightening cream containing azelaic acid (12%) and glycolic acid (5.2%) with different approaches: in vitro with a pigmented reconstructed epidermis, in vitro with human skin explants and a clinical intra individual study. The results showed a significant visual whitening effect after repeated topical applications on pigmented reconstructed epidermis and human skin explants with efficient barrier. Clinical studies included 31 subjects with melasma. The statistical analysis showed a significant whitening effect at the end of treatment versus the beginning.

To conclude, we showed our skin lightening cream had a significant whitening effect in vitro and in vivo and can be used to improve melasma. The data suggested also that in vitro preclinical models could be used to predict clinical pigmentation read-out.

O-8
Melanin processing by keratinocytes after transfer

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Melanosome transfer from melanocytes to keratinocytes and subsequent redistribution to the supra-nuclear region is a critical process in skin pigmentation and protection against ultraviolet radiation. However, the molecular mechanisms underlying these processes remain elusive. We have previously proposed that exo-endocytosis of melanocores, the pigmented content of melanosomes, is the main mechanism responsible for pigment transfer. We have now focused on the processing of pigment by keratinocytes after transfer. We analyzed the role of endocytic Rab GTPases in melanin uptake by XB2 keratinocytes. We found that silencing of Rab5b, but not Rab7a or Rab9a, significantly impairs melanocore uptake by XB2. We evaluated the expression of late endosome/lysosome markers, namely LAMP2 and cathepsin D, and observed that XB2 keratinocytes express them in amounts comparable to HeLa cells. Moreover, we used DQ-BSA and LysoTracker to demonstrate that XB2 cells possess degradative and acidic compartments. Nevertheless, we observed that transferred melanin resides in compartments that co-localize with early and late endocytic markers but are neither highly degradative nor acidic. Hence, our results suggest that melanin is stored in specialized endocytic compartments within keratinocytes that are not highly acidic or degradative, allowing them to resist degradation for long periods.

O-9
The role of carboxyl groups in eumelanin and pheomelanin properties

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Recent advances in the chemistry of melanins have disclosed the importance of the monomer composition in determining significant differences in the pigment properties of crucial relevance to their biological role, including skin (photo)protection and UV-susceptibility. In this scenario, a key role seems to be played by carboxyl groups. Depending on the extent of decarboxylation during the rearrangement of dopachrome, different contents of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) can be incorporated into eumelanin pigments. Rearrangement of 5-S-cysteinyldopa o-quinoneimine likewise can lead to 1,4-benzothiazine (BTZ) or its 3-carboxylic acid (BTZCA) derivative. The DHICA-to-DHI ratio markedly affects the antioxidant and chromophoric properties of eumelanins: in particular, high amounts of DHICA-derived units decrease visible light absorption relative to DHI-based melanins, but markedly enhance antioxidant properties. In the same way carboxylated benzothiazines can confer pronounced visible and UVA absorption features to pheomelanins, accounting for light-dependent reactive oxygen species (ROS) production, whereas BTZ-related units seem to be more effective in inducing ROS production by redox cycling mechanisms in the dark. The possible biological consequences of carboxyl group retention in the eumelanin and pheomelanin pathways will be discussed.

O-10
Photogeneration and quenching of singlet oxygen and superoxide anion by synthetic eumelanins and pheomelanins

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Although melanin is commonly viewed as a photoprotective pigment, its residual photochemistry can lead to generation of melanin radicals and reactive oxygen species. Photoreactivity and photochemical instability of pheomelanin could be responsible for its postulated photomutagenic and phototoxic action. A thorough qualitative and quantitative analysis of aerobic photoreactivity of synthetic models of eumelanin and pheomelanin was carried out in this study. Melanins synthesized from DOPA, DHICA, 5-S-cysteinyldopa and 1:1 cysteine/DOPA were analyzed in neutral pH buffer for their ability to photogenerate and quench singlet oxygen and superoxide anion employing direct time-resolved detection of 1270 nm phosphorescence, and electron paramagnetic

resonance (EPR) oximetry and spin trapping. When irradiated with blue light, the two synthetic eumelanins photoconsumed oxgen faster than the pheomelanins and they also exhibited more efficient photogeneration of superoxide anion. All tested melanins photogenerated singlet oxygen albeit with a very low quantum yield (~0.0001) in the visible part of the spectrum. The efficiency of the melanins, particularly pheomelanins, to photogenerate singlet oxygen significantly increased in UVA. The observed photogeneration and quenching of reactive oxygen species by the melanin pigments suggest that such aerobic photochemistry could contribute to their photodegradation and photoaging. Supported by National Science Center, Poland (project 2013/08/W/NZ3/00700).

Keynote Lecture

The awesome lysosome

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We discovered that lysosomal biogenesis and autophagy are transcriptionally regulated by the master gene TFEB. In cooperation with the mTORC1 kinase complex, TFEB is involved in a lysosome-to-nucleus signaling pathway that controls the transition between biosynthetic and catabolic pathways in response to nutrient availability. Thus, the lysosome acts as a signaling hub that controls cell homeostasis. Modulation of the lysosomal-autophagic pathway via TFEB resulted in the clearance of accumulating substrates in cells and tissues from mouse models of Lysosomal Storage Diseases (LSDs) and common neurodegenerative diseases. These data have opened a new field of investigation (i.e. transcriptional control of lysosomal function) and conceptualized a novel therapeutic strategy (i.e. modulation of cellular clearance) with potential applicability to many diseases.

Session 4: Molecular Epidemiology

O-11

Association of vitamin D status with clinical features and repigmentation in vitiligo patients: preliminary results from an observational study

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Reduced serum 25-hydroxyvitamin D [25(OH)D] levels have been reported in vitiligo patients and correlate with clinical features of the disease.A possible role of such imbalance in vitiligo repigmentation however has not been investigated.Thus, we performed an observational cross-sectional study to compare serum 25(OH)D levels of 101 vitiligo patients and 101 age, gender and season matched controls, and to correlate such values with clinical features and repigmentation of vitiligo.We found that the majority of vitiligo patients had deficient levels of 25(OH)D (≤20 ng/ml), as defined by most international guidelines.Moreover, such deficiency was more frequently observed in vitiligo patients than in healthy controls ($P = 0.024$).An extension of vitiligo involving more than >20% of total body surface was found to be associated with insufficient (21–29 ng/ml) or deficient 25(OH)D levels ($P = 0.036$), while a stability of the disease was found to be associated with sufficient 25(OH)D levels (30–100 ng/ml, $P = 0.043$).Patients with serum sufficient 25(OH)D levels achieved more frequently a repigmentation of lesions, with better outcomes on the head/neck district (good improvement, $P = 0.001$) and on the trunk (moderate, $P = 0.018$).Our study confirms an imbalance of serum 25(OH)D levels in vitiligo patients and provides preliminary evidences of a possible beneficial role of vitamin D in achieving vitiligo repigmentation.

O-12

Prognostic relevance of baseline hematological profiles in melanoma patients

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Cancer-related inflammation may promote disease progression and affect patient outcome, and could be estimated through surrogate markers derived from routine blood tests. Here, we analyzed peripheral blood cell counts and their ratios in 584 melanoma patients included in an Institutional tumor registry within the period 2000–2010. Survival was estimated with the Kaplan-Meier method, and analyzed using Log-rank test, Cox regression and multivariate Cox proportional hazard models. We show that patients with an advanced disease had higher leukocyte, neutrophil and monocyte counts, and lower lymphocyte counts compared to stage I-III patients. Furthermore, we confirm at a single-patient level that on disease progression from regional to distant metastatic, significant changes in absolute and relative blood cell counts occur. In early-stage patients, peripheral blood cell counts were not associated with the survival. Instead, in stage IV patients, higher leukocytes ($P = 0.001$), neutrophils ($P = 0.0002$), monocytes ($P = 0.002$) and ratio of neutrophils to lymphocytes (NLR, $P < 0.0001$) were all significantly associated with increased risk of mortality, independently of other known prognostic factors. Finally, by analyzing an independent cohort of 720 patients receiving the checkpoint inhibitor ipilimumab, we provide evidences of the potential clinical utility of peripheral blood cell markers in this clinical setting.

September 14, 2016
Session 5: Melanocytes and Melanoma Molecular Pathway

RhoGTPases control melanoblast migration

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The individual molecular pathways downstream of Cdc42, Rac and Rho GTPases are well documented, but we know surprisingly little about how these pathways are coordinated. In the developing embryo, melanoblasts originating from the neural crest traverse the dermis to the reach the epidermis of the skin and hair follicles. We previously established that Rac1 signals via Scar/WAVE and Arp2/3 to effect pseudopod extension and migration of melanoblasts in skin. We will discuss our new findings that RhoA is redundant in the melanocyte lineage, but Cdc42 controls multiple motility systems independently of Rac1. Cdc42 null melanoblasts were elongated and displayed large, bulky pseudopods with active actin dynamics. Despite assuming a shape usually associated with fast motility, Cdc42 knockout melanoblasts migrated slowly and inefficiently in the epidermis, with nearly static pseudopods. In-depth molecular analysis, including global RNA-sequencing, revealed defects in adhesion pathways, mislocalisation of active myosin and a failure to regulate dynamic integrin-based adhesion in Cdc42 null cells. Thus, while Rac1 has a very specific role in signaling to branched actin network generation, Cdc42 coordinates multiple systems for efficient movement.

*These two authors contributed equally.

O-13

Role of A-MSH in the control of proliferation in melanocytes and melanoma cells

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Peroxisome Proliferator Activated receptors (PPARs) are transcription factors which control several functions including proliferation. We demonstrated that α-MSH induces PPAR-γ through the phosphatidylinositol pathway, a signalling typical of G-Protein Coupled Receptors (GPCRs). GPCRs modulate mitogenic/anti-mitogenic signals but fragmentary data are described regarding the role of MC1R in this regulation. Here, we explored the link between α-MSH stimulation and proliferative behavior in melanocytes and melanoma cells, evaluating proliferation through cell cycle analyses and the expression of associated proteins in the presence of different inhibitors and activators of the ancestor cAMP/ PKA and phosphatidylinositol pathways. Our results show that: (a) in the presence of a wild type MC1R, α-MSH promoted hyper-proliferation in melanocytes and down-proliferation in melanoma cells; (b) in response to α-MSH, the cAMP/PKA pathway was found to be mainly implicated in conditioning the proliferative response in melanocytes, but resulted only partially involved in regulating the proliferative behavior of melanoma cells. In these cells, α-MSH drove the reduction of proliferation primarily through the phosphatidylinositol signal pathway, employing PPAR-γ as an effector element. The α-MSH/PPAR-γ/ anti-proliferation axis, within the transduction pathways related to the activity of MC1R, can offer perspectives for new therapeutic approaches for melanoma.

O-14

NIK inhibition potentiates anti-PD1 treatment by inducing IFN-γ release by cancer cells

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We recently showed that the non-canonical NF-kB pathway and NIK, its upstream kinase, is a major regulator of EZH2.

Methods We used cell lines from different types of cancer and syngeneic mouse models and we tested the possibility that inhibition of the non-canonical-NF-kB pathway could promote the release of IFN-γ and IFN-γ-related cytokines by cancer cells.

Results We designed a NIK inhibitor called DMBP5 that is as effective as NFkB2-siRNA in down-regulating the non-canonical-NFkB pathway and we tested it for its ability to reduce EZH2 levels without causing side effects or toxicity in normal cells. The inhibition of the non-canonical-NF-kB pathway by siRNA or DMBP5 in melanoma, colon-cancer and lung-cancer caused the release of IFN-γ and its downstream factors in vitro and in syngeneic mice. That secretome was a potent attractor of immune cells and caused macrophage M1-polarization and T-cell and dendritic-cell activation. DMBP5 reduced the size of subcutaneous tumors, and when combined with anti-PD1 treatment, it led to a dramatic reduction in tumor size.

Conclusions The use of effective NIK inhibitors such as DMPB5 reduces tumor growth directly and potentiates anti-PD1 treatment by inducing an IFN-γ response by the cancer cells, suggesting a powerful therapeutic approach for solid cancers.

‡These authors contributed equally to this work.

RASA2 is a novel tumor suppressor in melanoma

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Most approved drugs that target genetically altered proteins in cancer are towards kinases. However, a majority of the proteins mutated in cancer are tumor suppressors which cannot be re-activated by small molecules. A possible solution is exploiting the fact that tumor suppressor gene inactivation results in activation of a downstream growth pathway. We sought to systematically identify tumor suppressor genes in melanoma and characterize the downstream pathways activated by their loss of function.

To this end, we compiled and analyzed a database of 501 melanoma whole exomes, to identify novel melanoma suppressor genes. This analysis revealed *RASA2* as a melanoma tumor suppressor for the first time. *RASA2*, encoding a RasGAP, is mutated in 5% of melanomas. We examined recurrent mutations in RASA2 and found that they increased RAS activation, increasing melanoma cell growth. RASA2 expression was lost in 30% of melanomas and was associated with reduced patient survival.

These finding, together with functional data indicating its effect on cell growth and migration, suggest that *RASA2* is an important tumor suppressor in human melanoma. Particularly important is the fact that RASA2 suppression provides an alternative mechanism of RAS activation. This study highlights the importance of Ras-GAPs in cancer.

Session 6: Melanocytes and Melanoma Molecular Pathways (continued)

Systematic genetic perturbations to reveal melanoma vulnerabilities

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For a long time, advanced stage melanomas were refractory to the available therapeutic options, but recent developments have begun offering better perspectives. The small molecule inhibitor vemurafenib, specifically targeting the mutant $BRAF^{V600E}$ kinase, was the first standard of personalized care for patients diagnosed with mutant BRAF metastatic melanoma. Although this compound initially reduces tumor burden dramatically, eventually most melanomas become resistant and progress while on treatment. This occurs by acquisition of additional mutations or other alterations most of which reactivate the mitogen-activated protein kinase (MAPK) pathway.

Therefore, in spite of these new perspectives, there is a dire need to identify additional targets amenable to therapeutic intervention, to be used in combination with vemurafenib or other specific inhibitors (e.g., immune checkpoint blockade) to overcome or prevent drug resistance and achieve more durable clinical responses.

We are studying (lack of) sensitivity to targeted treatment using patient biopsies, patient-derived xenografts (PDX) and low-passage cell lines. These systems are used for systematic function-based genetic screens to identify melanoma factors that are required for proliferation and survival of melanoma cells. Similar screens are done to modulate the response to targeted agents. The results from these and related studies will be discussed.

O-15

Tailored small oligonucleotides abolish mirna sequestration and restore microrna tumour suppressor activity

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microRNAs (miRNAs) are central players regulating virtually every biological processes. Dysregulation of miRNA expression and/or activity is thus associated to many diseases including cancer. We identified an original mechanism that dampens the tumour suppressor activity of miRNAs in melanoma. We showed that non-canonical miRNA Responsive Element (MRE) foster long-term miRNA sequestration and demonstrated that small oligonucleotides masking specifically the sequence of non-canonical MRE reverse miRNA-sequestration. This study uncovered thereby an original oncogenic mechanism and a tailored therapeutic solution to restore miRNA tumour suppressor activity.

O-16

Loss of long non coding RNA TINCR promotes melanoma growth and metastasis formation by inducing a switch to an invasive phenotype

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Despite recent advances in identifying oncogenic drivers of melanomagenesis, complex epigenetic events underlying initiation and progression of melanoma remain poorly understood. Growing evidence suggests that long non coding RNAs (lnc RNAs) are key regulators of melanoma growth. By means of unsupervised consensus clustering of TCGA melanoma datasets, we identified a transcriptomic signature that distinguishes primary melanomas on their propensity to metastasize. We focused our attention on lncRNAs that are downregulated in the signature. The lncRNA TINCR is downregulated in metastatic melanomas as compared to nevi and primary melanomas. TINCR silencing by shRNA promotes melanoma cell migration in vitro and tumor formation in vivo, by inducing a transcriptional reprogramming of the cells to an invasive state. Furthermore, ectopic expression of TINCR in metastatic melanoma patient-derived xenografts (PDX) dramatically reduces tumor growth and metastasis formation in NSG mice. Notably, TINCR overexpression in PDX cells reverts the resistance to the MEK inhibitor Trametinib. These findings support a key role for TINCR in melanoma progression, and suggest that TINCR can be a novel therapeutic target in melanoma.

A transcriptionally inactive ATF2 variant drives melanomagenesis

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Melanoma is one of the most lethal cutaneous malignancies, characterized by chemoresistance and a striking propensity to metastasize. The transcription factor ATF2 elicits oncogenic activities in melanoma, and its inhibition attenuates melanoma development. We found that expression of a transcriptionally inactive form of *Atf2* (*Atf2*$^{\Delta 8,9}$) induce nevi formation in *Braf*^{WT} mice; promote melanoma formation in *Braf*$^{WT/V600E}$ mice; and augment pigmentation, tumorigenicity, and metastasis in *Braf*$^{V600E/V600E}$;*Pten*$^{-/-}$ mice. The expression of genes associated with enhanced pigmentation, immune infiltration, and metastatic propensity is elevated in *Atf2*$^{\Delta 8,9}$-driven tumors. The transcriptionally inactive human *ATF2* splice variant 5 (*ATF2*^{SV5}) enhances the growth and migration capacity of cultured melanoma cells and immortalized melanocytes, as seen for the mouse *Atf2*$^{\Delta 8,9}$. Analysis of human melanoma specimens identified that elevated expression of *ATF2*^{SV5} coincides with poor prognosis. The gain-of-function activity elicited by the transcriptionally inactive *ATF2* forms provides unexpected insight into the mechanisms underlying melanoma development and progression.

Poster Session

P-01

WDR5 silencing inhibits tumor growth and cell migration in a PDX model of metastatic melanoma

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We have previously identified a series of epigenetic regulators involved in the tumorigenesis of metastatic melanomas (MMs) by means of in vivo shRNA screenings in patient-derived xenografts (PDXs). Here we show that WDR5, a core subunit of the COMPASS complex, promotes tumor growth in vivo and cell migration in vitro in MM PDXs.
Analysis on WDR5 expression on a TCGA data set of skin cutaneous melanomas showed that high WDR5 expression exhibited an inverse correlation with melanoma overall survival ($P = 0.0038$), suggesting that WDR5 could be a prognostic factor in melanoma. To validate this finding, we knocked down WDR5 expression by shRNA interference and we observed that WDR5 silencing inhibited in vivo growth (80–90%) and in vitro cell migration (50–70%), both in NRAS and BRAF mutated MM PDXs. To deepen the study on the determining role of WDR5 in high aggressive carcinomas, we evaluated the response to WDR5 silencing in breast cancer patients using a PDX model of metastatic breast cancer (MBC) and we observed that WDR5 silencing induced a significative reduction of in vivo tumor growth of MBC PDXs independently by the subtype considered. These results suggest WDR5 as a potential and promising target in the treatment of metastatic malignancies.

P-02

Clonal tracking analysis of melanoma cells in vivo

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Metastatic melanoma is one of the most aggressive cancers. Virtually every patient is refractory to current therapies, because of the emergence of drug-resistant tumor cell clones. A definitive understanding of in vivo dynamics of melanoma cells is fundamental to discriminate for cell subpopulations relevant for tumor development and dissemination, and the design of specific anti-cancer therapies. With our in vivo loss-of-function shRNA screens in patient-derived xenografts of metastatic melanoma, we identified novel druggable epigenetic genes that are not mutated in the tumor. We showed high numerosity of these genes and surprisingly, high heterogeneity among patients.
To understand the clonal distribution of melanoma in vivo, patient-derived melanoma cells are individually and genetically barcoded and then tracked in vivo after transplantation into mice. The clonal tracking analysis suggests that only a fraction of cells contribute to the in vivo formation of the tumor and show a tumor-initiating cell frequency lower than the one observed by single-cell-transplantation experiments. These results suggest that the in vivo growth of tumor cells in pools and not as single cells can be different, and that the clonal tracking analysis can be a useful tool to better predict the shRNA screens feasibility.

P-03

Role of MARCH5 mitochondrial E3 ligase in the response to BH3 mimetics

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BH3 mimetic compounds induce tumor cell death through targeted inhibition of anti-apoptotic BCL2 proteins. Resistance to one such compound, ABT-737, is due to increased levels of anti-apoptotic MCL1. Using chemical and genetic approaches, we show that resistance to ABT-737 is abrogated by inhibition of the mitochondrial RING E3 ligase, MARCH5. Mechanistically, this is due to increased expression of pro-apoptotic BCL2 family member, NOXA, and is associated with MARCH5 regulation of MCL1 ubiquitylation and stability in a NOXA-dependent manner. MARCH5 expression contributed to an 8-gene signature that correlates with sensitivity to the preclinical BH3 mimetic, navitoclax. Furthermore, we observed a synthetic lethal interaction between MCL1 and MARCH5 in MCL1-dependent breast cancer cells. Our data uncover a novel level at which the BCL2 family is regulated; furthermore, they suggest targeting MARCH5-dependent signaling will be an effective strategy for treatment of BH3 mimetic-resistant tumors, even in the presence of high MCL1.

P-04

Melanoma heterogeneity and resistance to BRAF targeted therapy defined by microRNA profiling

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Advanced cutaneous melanoma is an aggressive disease characterized by poor prognosis. Two classes of drugs, inhibitors of BRAF signaling and checkpoint inhibitors, have recently achieved substantial survival advantage in metastatic melanoma patients. Despite this breakthrough, most patients develop resistance and some are intrinsically unresponsive. We recently reported that melanoma lines and clinical samples could be classified into three different subtypes. In particular, one subtype, displaying the highest and lowest expression of EGFR- and ERBB3-encoding genes (EGFRHIGH/ERBB3LOW subtype), includes BRAF-mutant tumors all intrinsically resistant to BRAF inhibitors (BRAFi). Here we investigated whether a set of microRNAs that we recently reported as differentially expressed in a cell line with acquired resistance to BRAFi, compared to its sensitive counterparts, could play a role also in intrinsic resistance to BRAFi. Therefore we classified melanoma cell lines from which both gene expression and microRNA profiles were publicly available in the three different subtypes. Among microRNAs differentially expressed between EGFRHIGH/ERBB3LOW subtype and the other two, twenty-three overlapped with those identified in the model of BRAFi acquired resistance. Several of them were involved in the control of cell proliferation and apoptosis. Validation of their role in the clinical setting of BRAFi melanoma resistance is ongoing.

P-05

Characterization of MITF CRISPR knock out melanoma cell lines

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Microphthalmia-associated transcription factor (MITF) acts as the master regulator of melanocytes and is considered a melanoma oncogene. In order to better understand the role of MITF in melanoma cells, we knocked the gene out in Skmel28 cells using CRISPR technology, targeting exons 2 and 6 separately. Both cell lines exhibit decreased proliferation rate compared to the parental line. This is consistent with the MITF rheostat model. Unexpectedly, the cells did not show signs of senescence. In addition, propidium iodide stainings showed no cell cycle arrest and no induction of apoptosis in the MITF knock out cells. Scratch assays revealed a slower migration rate when compared to wild type cells. Finally, MITF knock out cells exhibited a more granular morphology and were larger in size than the wild type control cells.

To study the role of MITF at the transcriptional level, RNA-seq experiments will be performed using the MITF knock out cells. By doing this, we want to identify a set of differentially expressed genes in response to loss of MITF in melanoma cells. This in turn will improve our understanding of the role of MITF in melanoma with the final aim to elucidate how MITF levels affect disease progression.

P-06

Regulation of MC1R-mediated pigmentation by the GRD domain of neurofibromin in human melanocytes

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We studied the role of the GRD of Nf1 in the melanogenesis pathway and showed that GAP activity of Nf1 controls melanogenic enzyme expression and melanin production. To further investigate the mechanism, we demonstrated, using co-immunoprecipitation and Bioluminescence Resonance Energy Transfer experiments, a physical interaction between Nf1 and MC1R and more importantly that the GRD of Nf1 interacts with MC1R. Furthermore, in Nf1 deficient cells, α-MSH was shown to enhance cAMP accumulation with a stronger efficacy compared to control cells, suggesting that Nf1 negatively regulates MC1R signaling.

This study demonstrates a new degree in the regulation of MC1R-operated signaling which can be of interest in understanding the mechanisms involved in the regulation of skin pigmentation and can bring new strategies for correcting skin pigmentation disorders.

P-07

Reactivation of p53 by prima-1met is not affected by intracellular glutathione levels

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PRIMA-1Met, a novel drug that restores the activity of mutated and unfolded TP53, is in clinical development. PRIMA-1Met is converted to the methylene quinuclidinone (MQ) which targets also the cellular redox balance by inhibiting thioredoxin reductase and depleting glutathione. We recently reported that PRIMA-1Met sensitizes V600E/KBRAF melanoma to vemurafenib (Krayem EJC 2016). However, the underlaying mechanisms of action and potential off-target effects due to thiol group recognition by the drug are not fully understood yet. First, to investigate the effect of PRIMA-1Met on GSH/ROS balance, we measured total GSH in a panel of melanoma cell and found that PRIMA-1Met impaired the total GSH content in a dose-dependent manner. Then, we showed that GSH depletion mediated by BSO increased the cytotoxicity effect of PRIMA-1Met alone or in combination with vemurafenib in both wild-type and mutated p53 cells. Furthermore, total intracellular GSH concentration correlated with the PRIMA-1Met IC50 values in melanoma cell lines ($N = 27$, $P = 0.011$), indicating that a certain amount of the drug is bound and deactivated by GSH. However, depletion of the latter did not alter both p53 activity nor cell sensitivity to vemurafenib, further supporting that melanoma cell

sensitization to vemurafenib by PRIMA-1Met is primarily associated with p53 reactivation.

P-08

Canine melanoma types ss models for genetics and therapies of human mucosal, uveal and cutaneous melanoma

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Specific dog breeds are affected by given melanoma types, which closely resemble human types (mucosal, uveal and cutaneous), making dogs unique spontaneous models to study genetics and to envisage clinical trials « first in dogs » to improve therapies. The epidemiological, clinical and histopathological analysis of 400 canine melanoma with a 3 years follow-up allowed refining the human/dog homologies. To describe the somatic mutational landscape, we performed exome sequencing of 75-paired samples of oral melanoma cases (tumour DNA/matched blood DNA) in 25 dogs from three breeds. We identified 50 mutated genes including known tumour suppressor genes, as TP53, PTEN and oncogenes, as NRAS, but no BRAF mutations; as well as new mutated candidate genes and SNVs. A clear non-UV mutations signature as well as patterns of exclusive mutations are observed. In addition, a retrospective and prospective collection of uveal melanoma (100 FFPE and 20 fresh tissue and blood samples; respectively) are used to perform a comparative genetic and histopathological analysis. From those, six canine uveal tumor cell lines have been developed. These results demonstrate the strengths of the dog model to identify pathways, leading to advances in translational medicine both benefitting to dogs and humans.

P-09

Septins play a role in melanoma cell migration through store-operated Ca2+ Entry (SOCE)

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Septins, considered to be the fourth components of the cytoskeleton, are GTP-binding proteins that form hetero-oligomeric complexes, filaments and higher-order structures in the cell cortex. Septins assemble at plasma membrane domains close to puncta of the endoplasmic reticulum suggesting that they may play role in STIM1-Orai1 interaction. Silencing certain septin types or inhibition of septin assembly can block store-operated Ca2 + entry (SOCE) and downstream signalling. We hypothesized that septins can effect melanoma migration and metastasis formation through SOCE.

Presence of septins 2, 4, 5, Orai1, 2, 3 and STIM1, 2 were proved at mRNA and protein level in the two investigated melanoma cell lines (HT199, WM35). Inhibition of septin assembly with forchlorfenuron (FCF) resulted in morphological changes of cells, without any detectable reorganization of actin cytoskeleton. FCF induced neither apoptosis, nor necrosis, but we found a striking ten-fold reduction of fibronectin guided migration in Boyden chamber. PTI measurements recorded SOCE in melanoma cells under control circumstances, but FCF significantly reduced the amplitude of these Ca2 + -events. Determination of proliferation and invasion is still in process.

Our results suggest that septin assembly is essential for proper SOCE and both septins and Ca2 + -events play role in the chemoattraction guided migration of melanoma cells.

P-10

SCD5 reverts the EMT-like program associated with melanoma dissemination

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The Epithelial-Mesenchymal Transition (EMT) is a reversible embryonic program, which in carcinoma cells might eventually determine metastatic dissemination. Although non canonical, an EMT driven by aberrant expressions of transcription factors (EMT-TF) was reported in melanoma.

We previously demonstrated in human A375M melanoma the antimetastatic role of Stearoyl-CoA desaturase 5 (SCD5) associated with decreased stromal deposition consequent to SPARC reduced secretion. As in melanoma the EMT-like program is controlled by SPARC, we looked for the involvement of SCD5 in reverting this process. In view of the reduced malignancy of SCD5 overexpressing cells, results showed a significant reduction of ZEB1, SLUG and SNAI1 paralleled by ZEB2 upregulation. In line with these findings was the SCD5-associated increase of Microphtalmia associated Transcription Factor (MITF), a master regulator of melanoma phenotype, which plays either a role in regulating EMT-TF expression or Tyrosinase and melanin synthesis, indicating a more differentiated phenotype. Indeed, SCD5 transduction in the murine 4T1 mammary cell line evidenced a more typical switch from EMT to MET phenotype characterized by E-cadherin reexpression at membrane levels.

Overall, these phenotypic changes, although partial in melanoma, indicate that SCD5 antimetastatic properties at least in part go through an EMT reversion toward a more epithelial phenotype.

P-11

Histone chaperone HIRA is required for melanocyte stem cell maintenance and suppression of hair graying during ageing

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Hair follicle pigmentation is due to a population of melanin producing cells, the melanocytes, which are maintained throughout the hair cycle via melanocyte stem cells (MSCs). Any defect in melanocyte function and/or maintenance or differentiation of MSCs can lead to pigment defects including premature hair graying, a hallmark of ageing. Here we describe a new conditional knockout mouse model (Tyr-Cre:Hirafl/fl) in which melanocytes are specifically deficient for Hira, a histone chaperone that deposits H3.3 histone variant in non-dividing cells at expressed genes and gene regulatory regions. Tyr::Cre HIRAfl/fl mice are born with a mildly hypopigmented coat with a decrease in abundance of histone H3.3. Their coat colour becomes progressively white with age, a marked premature hair graying phenotype. While little effect is observed on melanocyte and MSC numbers in young Hira-deficient mice, aged mice are profoundly depleted of these cells suggesting a defect in stem cell maintenance. In addition, Hira-deficient melanocytes fail to proliferate and survive in vitro. These data suggest an important role of HIRA in the maintenance of melanocyte stem cells and a healthy pigmentary system. More generally, we anticipate that this model can inform on the role of proper epigenetic control and epigenetic maintenance in healthy aging.

P-12

Role of ENPP1 in pigmentation

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Cole disease is a rare autosomal dominant disorder characterized by hypopigmented macules and hyperkeratosis. In hypopigmented macules, a normal number of pigmented melanocytes but a decreased melanin content in keratinocytes suggesting an impairment of melanosome transfer have been reported. Five different mutations in somatomedin-B-like domains of EctoNucleotide Pyrophospahatase /Phosphodiesterase 1 (ENPP1) have been identified in five families with Cole disease. Since it is difficult to have cells from patients, we constructed overexpressing lentivectors coding for wild-type ENPP1 and for the first three mutations identified in ENPP1 and then transduced melanocytes and keratinocytes. In non transduced cells, enzymatic activity of ENPP1 seemed greater in melanocytes than in keratinocytes and mutations did not inhibit enzymatic activity. To better understand influence of ENPP1 mutations in pigmentation we analyzed expression of MITF, tyrosinase, TRP-1 and TRP-2 by qPCR, western-blot and immunocytochemistry on cells or immunofluorescence on genetically modified reconstructed epidermis. In melanocytes, the level of expression of TRP-1 and tyrosinase seemed inversely correlated to the level of expression of mutated ENPP1. TRP-1 was also decreased in reconstructed epidermis made with genetically modified epidermis. Thus mutations of ENPP1 seemed directly implicated in establishing and sustaining hypopigmentation in Cole Disease.

P-13

Melanoma cell lines express stem cell markers by influence of melanoma-associated fibroblasts and in non-adhesive condition

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Introduction Similarly to other tumors, the intercellular interactions are crucial for cancer stem cells niche and for biological properties of melanoma. Cancer stem cells can contribute formation of resistance to treatment and can be responsible for tumor recurrence. We used culture with adhesive and non-adhesive condition and influence of melanoma-associated fibroblasts (MAF) for the study of stem cells markers in melanoma cell lines.

Methods Melanoma cell lines (BLM, G361) were exposed to non-adhesive condition alone and under the direct and indirect influence of MAF. We studied expression of stem cells markers (Oct4, Nanog, CD271), melanocytic markers (HMB45) and cytokeratins in suspension regiment and after repeated adhesion.

Results Expression of stem cells markers Oct4, Nanog, and CD271 was detected using a non-adhesive condition and the influence of MAF in melanoma cell lines. In contrast, the expression of other markers (Nestin and HMB45) was not affected by changing conditions.

Conclusion This study showed plasticity of melanoma cells depending to different condition in in vitro modeling of tumor microenvironment. This ability of melanoma cells to change phenotype can assist in the formation of metastases and resistance to the therapy. The results indicate that tumor microenvironment can play a key role in this process.

P-14

Investigation of heat shock protein expression in skin after heat and UV irradiation

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When you are sunbathing the skin is not only exposed to UV irradiation but will also become heated. The UV generates damage to biomolecules such as DNA, which later might lead to malignant transformation. The thermal stress causes protein misfolding which induce the heat shock response, which consists of

protein members of the molecular chaperon family, called heat shock proteins (HSP).
Their function is to stabilize and refold mis-folded proteins in order to restore protein function and protect the cell towards damage. With the goal to characterize the expression of HSPs in the skin, we recruited volunteers and exposed their forearm to heat and UV irradiation separately and in combination. Biopsies were obtained and expression of HSP-27, -70 and -105 was analyzed.
HSP-27 and -70 were significantly increased 5 h after heat exposure (41 °C, 45 min), while no significant alterations could be found after UVA irradiation (0.5 SED; standard erythema dose). The combination of UVA and heat up-regulated HSP-27. The results highlight the important balance between apoptosis to elimination of photo-damaged cells and cell survival to maintain homeostasis in the skin.

P-15

Mahogunin RING finger 1 regulates the melanosomal Ph and decreases pigmentation by acidification of the melanosome

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Signaling from MC1R, a major determinant of pigmentation, is modulated by MGRN1, an E3-ubiquitin ligase mutated in dark-furred mahoganoid mice. We investigated the mechanisms of hyperpigmentation in Mgrn1-null melan-md1 melanocytes, compared with control melan-a6. Whereas tyrosinase activity (Tyr) measured in cell-free extracts, Tyr protein and mRNA levels were comparable in both cell lines, Tyr activity measured in live cells was 10-fold higher in melan-md1 cells. Therefore, Mgrn1 acts to modify other melanosomal biochemical and/or physicochemical characteristics rather than Tyr expression, resulting in post-transcriptional activation of Tyr. Since melanosomes are acidic organelles and Tyr activity is maximal at nearly neutral pH in vitro, we analyzed the melanosomal pH in melan-md1 cells. Treatment with lysosomotropic neutralizing agents (NH4Cl and chloroquine) increased Tyr activity in vivo, melanin content and organelle pH in melan-a6 cells, with minor effects on melan-md1 cells, suggesting that Mgrn1 mediates melanosome acidification. To identify possible genes/proteins mediating the effect of Mgrn1 on organelle pH, we compared gene expression profiles in melan-md1 and melan-a6 melanocytes. This confirmed similar expression of melanogenic enzymes, but significant changes in genes involved in organelle acidification and biogenesis. Accordingly, a Mgrn1-mediated regulation of melanosome pH seems a major cause of hyperpigmentation in mahoganoid melanocytes.

P-16

cAMP-independent non-pigmentary actions of melanocortin 1 receptor signaling in melanocytes: regulation of defensive responses to oxidative damage

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Ultraviolet radiation (UVR) causes DNA lesions either directly or indirectly, via generation of reactive oxygen species. Cutaneous pigmentation is the main photoprotective mechanism against UVR-induced DNA damage. Melanocytes also take advantage of other photoprotective processes, including melanocortin 1 receptor (MC1R)-dependent activation of DNA repair mechanisms and antioxidant defenses. We analyzed the signaling pathways downstream of MC1R activation by α-melanocyte-stimulating hormone (αMSH) responsible for protection against oxidative damage in human melanoma cells (HMCs) and epidermal melanocytes (HEMs). αMSH pretreatment before a short pulse of H2O2, to mimic UVR-induced transient oxidative stress, decreased i) the generation of 8-oxo-7,8-dihydro-2′deoxyguanine, a major product of oxidative DNA damage ii) the phosphorylation of histone H2A variant H2AX and the number of DNA repair foci, and iii) the formation of DNA breaks. These responses were mainly, but not exclusively, cAMP-dependent in HMCs wild-type for MC1R, since significant protection was afforded by αMSH under conditions of complete adenylyl cyclase inhibition. Moreover, αMSH significantly decreased oxidative DNA damage in HMCs and HEMs harboring hypomorphic MC1R allelic variants lacking detectable cAMP signaling, most likely through cAMP-independent PI3K/AKT activation. These results demonstrate occurrence of cAMP-independent protective roles of αMSH that should be considered for the design of improved photoprotective pharmacological strategies.

P-17

Common delayed senescence of melanocytes from multiple primary melanoma patients

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Approximately 5% of melanoma patients develop at least one additional independent melanoma; otherwise known as multiple primary melanoma (MPM). Reasons for developing MPM are not fully understood but genetic factors are implicated as family history is the strongest risk factor. Germline mutations common in MPM patients are known or predicted to result in a delay in replicative senescence (a state of irreversible proliferative arrest following extensive telomere attrition). These include CDKN2A coding for p16, a tumour suppressor involved in inducing melanocyte senescence and components of the telomere cap

shelterin, responsible for maintenance of telomere integrity. We hypothesised that MPM arises owing to a delay in melanocyte senescence and tested this by analysing the lifespan of 'normal' melanocyte cultures from MPM or single primary melanoma (SPM) patients, wild-type for known familial melanoma genes. Melanocytes from MPM patients did indeed undergo significantly more population doublings, independent of donor age. A significant inverse relationship between donor age and culture lifespan was observed for cells only from MPM patients, not controls, giving further evidence for genetic factors being involved in MPM susceptibility. Finally, we showed that mechanisms of senescence induction were heterogeneous in MPM lines, which we suggest possibly arises from common CDKN2A polymorphisms.

P-18

Three-dimensional structures of melanosomes from melanoma B16 cells and melanin granules from human hair

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Melanosomes are specialized organelles in pigment cells controlling melanin biosynthesis and storing. The matured melanin is transferred from a pigment cell to neighboring cells such as keratinocytes. Melanin contributes to the determination of hair and skin color, the biological defense to ultraviolet rays, etc. in humans. These structures have not been determined in detail, yet. We purified melanosomes from culture broth of melanoma B16 cells and melanin granules from Japanese human hair, successively examined their ultrastructure by scanning electron microscopy imaging and transmission electron microscopy imaging. Furthermore, as we had demonstrated the three-dimensional structure of human hair in J Cosmet Sci 2004;55S S25-7, we tried to analyze and compare both melanosomes. We successfully demonstrate results about the three-dimensional structural imaging of isolated melanosomes.

P-19

Direct and indirect effect of MC1R gene on melanoma

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The Melanocortin-1-receptor (MC1R) gene is highly polymorphic. Some of its variants are associated both with melanoma and with the "red hair color" (RHC) phenotype, characterized by fair skin, red hair, freckles and sun sensitivity. The aim of this study is to decompose the total risk estimate of MC1R on melanoma into two different effects: the one due to non-pigmentary pathway (direct effect) and the one due to pigmentary pathway (indirect effect).

Data from 7 studies with complete gene sequencing and information on RHC phenotype were assembled by the MC1R gene, SKIn cancer and Phenotypic characteristics (M-SKIP) study. We performed a mediation analysis on each study and then pooled risk estimates with random-effects models. Analyses were possibly adjusted by age, sex, melanoma familiarity, sun exposure, sunburns, common and atypical naevi. The OR(95%CI) for the total effect of MC1R variants on melanoma risk was: 2.11(1.44–3.09). It was decomposed into the significant direct effect (OR, 95% CI:1.93, 1.32–2.83) and the not-significant indirect effect (OR,95%CI:1.08, 0.99–1.18).

In conclusion, we found a direct role of MC1R on melanoma risk, independent of RHC phenotype. This finding is particularly important for developing prevention strategies, which may be directed to darker-pigmented subjects with MC1R variants as well as to lightly-pigmented subjects.

P-20

A variegated medaka mutant Va: a model of human carney complex

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Medaka Va is a variegated body color mutant having irregular black patches of melanophores in the skin. In the patched regions melanophores are observed more densely than in the non-patched regions. At least two loci are responsible for the Va phenotype, one of which is recessive and the other is dominant. As a result of positional cloning, the recessive locus of Va turned out to be prkar1b, which encodes a type I regulatory subunit of the cAMP-dependent protein kinase (PKA). We found that a large insertion in intron 8 of prkar1b caused an aberrant splicing of the mRNA and subsequent production of a truncated protein lacking a C-terminal cAMP binding domain, which in consequence resulted in elevated PKA activity in Va mutant.

According to previous studies of intractable human disease, mutations in PRKAR1A, a paralog of PRKAR1B, cause Carney complex (CNC). The clinical manifestations of CNC are quite variable but most characterized by spotty skin pigmentation, thus we postulated that medaka Va mutant can be a model of the human CNC.

We hope our future identification and characterization of the dominant locus of Va will promote better understanding of pathogenesis of the human CNC disorder.

P-21

Cellular response to UV-induced cell damage is controlled by lysosomal membrane permeabilization and lysosomal exocytosis

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UV radiation constitutes an important risk factor for skin damage and malignant transformation. Apoptotic cell death is regulated by lysosomal membrane permeabilization, which cause release of lysosomal proteases, cathepsins, to the cytosol with ensuing activation of the caspase cascade. The importance of lysosomal participation is illustrated by the fact that UVA and UVB-induced apoptosis is prevented by pretreatment with inhibitors of either cysteine or aspartic cathepsins.

Besides initiation of cell death, lysosomes are also important organelles for prevention of cell lysis upon plasma membrane damage. In order to rescue a cell with ruptured plasma membrane, lysosomes are transported to the damaged area and by donation of their membrane prevent lysis of the cell.

Recently, we found that UVA radiation causes plasma membrane damage that is rapidly repaired by lysosomal exocytosis. Lysosomal exocytosis is accompanied by release of cathepsins, acidic sphingomyelinase and formation of lipid rafts harboring signaling platforms that mediate both endocytosis and shedding of the plasma membrane. Interestingly, no lysosomal exocytosis is detected after UVB. Our investigation sheds new light on the differences in damaging mechanisms between UVA and UVB. The possible application of the results for the understanding of UV-induced affects in the skin will be discussed.

P-22

The Eumelanin carboxyl conundrum: unexpected impact of esterification on 5,6-dihydroxyindole-2-carboxlic acid antioxidant activity and oxidative polymerization

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Distal to tyrosinase activity, eumelanin synthesis is governed by the rearrangement of dopachrome which may proceed with or without decarboxylation giving 5,6-dihydroxyindole (DHI) and/or 5,6-dihydroxyindole-2-carboxylic acid (DHICA), that exhibit different chemical and biological properties. In melanocytes the reaction occurs under strict enzymatic assistance directing the course of melanogenesis toward DHICA and preventing the spontaneous decarboxylation to DHI. To inquire into the role of the carboxyl group as determinant of the chemical properties of the 5,6-dihydroxyindole system, DHICA and its methyl ester were compared for both their antioxidant activity and their conversion to eumelanin chromophores via oxidative polymerization. The results revealed that DHICA ester oligomers are stronger antioxidant compared to the free acid ones. Moreover, whereas the spectrophotometric course of DHICA oxidation proceeds via a purple chromophoric phase followed by band broadening to give a dark brown eumelanin, DHICA ester is converted to light brown species devoid of the typical euemelanin properties. These results suggest that the carboxyl group plays a more complex role than simply blocking the 2-position of the indole ring. The actual biological significance of these observations and their possible exploitation for dermocosmetic applications are currently under assessment in our laboratory.

P-23

Melanoma invasion is facilitated by UV-induced fibroblast activation protein-A (FAP-A)

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Fibroblast activation protein-α (FAP-α) is expressed at high levels on the surface of activated fibroblasts promoting tumor growth and cell invasiveness through extracellular matrix degradation (ECM). Our aim was to assess whether ultraviolet radiation (UVR), the major risk factor for malignant melanoma, influences the expression of FAP-α in skin and contribute to melanoma progression. Using ex vivo skin we show that UVR induces FAP-α expression in melanocytes only leaving keratinocytes unaffected. The invasion of primary melanoma cells in a xenograft tumor model using zebrafish

embryos (Danio Rerio) is stimulated by UVR and inhibition of FAP-α reduces invasion. Conditioned cell culture media from UV radiated melanocytes or melanoma cells induced TGF-β1 dependent increase of FAP-α expression in dermal fibroblasts. We have previously shown that melanoma is associated with altered lysosomal function and described cathepsin dependent invasion and proliferation of melanoma cells. Herein, when inhibiting lysosomal cathepsins, a decreased release of TGF-β1 and subsequent reduction in FAP-α expression was seen in primary melanoma cells. In conclusion, our results demonstrate UV induced FAP-α dependent ECM degradation enabling migration and invasion. FAP expression was initiated by cathepsin-induced intercellular crosstalk through activation of TGF-β1 implying the lysosome as an important key player in melanoma invasion.

P-24

Protein nanocages for cutaneous drug delivery

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The skin protects the body from UV-induced DNA damage by the sun exposure through the pigment, melanin produced by the melanocytes. This pigment is sometimes over-expressed leading to pigmentation disorders such as melasma. Current treatment involves using tyrosinase inhibitors and lasers, leads to complications such as depigmentation, irritation, and dermatitis, with only 50% patient response. This is mainly due the inability of the delivery system to penetrate the stratum corneum layer of the skin and its non-specificity to the melanocytes. This project is aimed at engineering E2 protein nanocage for enhanced penetration into the stratum corneum layer of the epidermis and targeting/penetrating the melanocytes for the delivery of

therapeutics. Genetic fusion of SPACE (Skin Penetrating And Cell Entering) peptide to the E2 nanocage helps its transduction through the stratum corneum layer, in vivo and to the interior of the melanocytes in vitro. Further modification of the E2 protein cage with targeting ligands can facilitate its uptake in melanocytes through the corresponding cell membrane receptors. Successful delivery of the engineered protein can aid the formulation of novel protein-based drug releasing molecules to be applied to the skin, which can be biocompatible with efficient pharmacokinetics.

P-25

TFEB in the pathogenesis of ocular albinism type 1

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Melanosomes are specialized organelles present only in pigment cells and devoted to the synthesis, storage and transport of melanin. They are considered lysosome related organelles (LROs), as they are related to lysosomes. Among the many morphological and structural features in common with lysosomes, the two organelles are also regulated by similar transcriptional mechanisms. The master gene of melanosomes is, indeed, MITF, a transcription factor belonging to the same family of TFEB, the master gene of lysosomes. Melanosome biogenesis is altered in Ocular Albinism type 1 (OA1), a disease due to the loss of function of OA1, a G protein coupled receptor localized on the membrane of melanosomes and lysosomes. It has been shown that OA1 regulates MITF expression, as the transcription factor is downregulated in absence of OA1. We found that also TFEB expression and activity are altered in OA1-KO melanocytes, i.e. TFEB is upregulated and abnormally recruited to the nucleus. In order to establish whether TFEB is implicated in melanosome biogenesis and in the pathogenesis of albinism, we are dissecting both TFEB upstream and downstream signaling pathways. Preliminary results indicate that OA1-KO melanocytes display a misregulation of mTOR activity and alteration of several related pathways in the transcription profile.

P-26

Loratadine, a H 1 antihistamine inhibits melanogenesis in human melanocytes

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It has long been believed that histamine is associated with cutaneous melanogenesis.

Specifically, H 2 antihistamines reportedly inhibit melanogenesis, but H 1 antihistamines, which are some of the most commonly prescribed medicines in dermatology, have not been studied to determine whether and how they regulate melanogenesis. Therefore, we screened H 1 antihistamines for anti-melanogenesis, and found that loratadine was particularly effective, in this regard without compromising cellular viability of normal human melanocytes.

Loratadine downregulated the mRNA and protein level of microphthalmia-associated transcription factor (MITF) and tyrosinase in melanocytes. Loratadine reduced activity of PKC-βII, while it activated Erk. As expected, histamine treatment reversed the reduction in melanogenesis induced by loratadine. Taken together, our data indicate that loratadine downregulates melanogenesis via Erk/MITF and PKC-βII signaling. The anti-melanogenic effects of loratadine have potentially significant and useful roles in dermatologic practice.

P-27

Belted cattle- A spontaneous coat color mutant with a structural variant in the 5-flanking region of the TWIST2 gene

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Belted cattle have a circular belt of unpigmented hair and skin around their midsection. The belt is inherited as a monogenic autosomal dominant trait. We mapped the causative variant to a 54 kb segment on bovine chromosome 3. We sequenced the genome of 2 belted cattle, called variants with respect to the cattle reference genome, and compared the variants to the genomes of 132 control cattle. This analysis yielded only a single genetic variant in the critical interval, which was private to the two belted animals. The belt-associated variant was a copy number variant (CNV) involving the quadruplication of a 6 kb non-coding sequence located approximately 20 kb upstream of the TWIST2 gene, which was strongly associated with the belt phenotype in a cohort of 239 cases and 1303 controls (P = 1exp-265). Given the nature of the belted phenotype, we hypothesized that overexpression and/or aberrant ectopic expression of TWIST2 caused by the CNV might result in reduced numbers of melanocytes in belted animals, e.g. through suppression of functional MITF. Consistent with such a model, functional studies in transgenic zebrafish showed that overexpression of bovine TWIST2 in neural crest cells led to a decrease in melanocytes.

P-28

Signals determining the subcellular localization of MITF-M

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Microphthalmia-associated transcription factor (MITF) is a central player in melanoma biology. MITF-M is the predominant isoform expressed in melanoma cells and unlike MITF-A or its relatives TFEB and TFE3, MITF-M is found constitutively nuclear. Nuclear localization of MITF-M is determined by four arginines in the basic domain, comprising a nuclear localization signal (NLS). Consequently, amino acid substitution leads to cytoplasmic distribution of the mutant protein. The molecular mechanisms

that link signaling and MITF-M localization remain, however, poorly understood. We thus aim to identify kinases that target this multifaceted transcription factor and to assess how phosphorylation events govern its nuclear import and/or export. Our findings in human 501mel cells demonstrate that MITF-M is phosphorylated by multiple kinases, including MAPKs, mTOR and GSK3. Interestingly, kinase inhibition experiments revealed differences in the phosphorylation pattern of the cytoplasmic NLS mutant MITF-M and its wild type counterpart. Alanine mutants mimicking the dephosphorylated state of MITF-M at specific serine residues show a significant effect on its subcellular localization when compared to the wild type protein. Unraveling the phosphorylation pattern of MITF-M at specific serine residues in response to pathway inhibitors will help us to better understand the interplay of signaling networks and MITF in melanoma.

P-29

cKIT mutation or amplification predicts high sensitivity to the tyrosine kinase inhibitor dasatinib in melanoma cells

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There is an urgent need to evaluate the cytotoxicity of new targeted drugs and to understand the associated mechanisms of resistance in melanoma. We assessed the effect of dasatinib on melanoma cell survival. We examined 17 melanoma cells and found that 2 lines were highly sensitive to dasatinib 5 were moderately sensitive and 10 were resistant. Highly sensitive lines express high cKIT levels.

Importantly, the highly sensitive lines had no mutation on BRAF or NRAS, while 12/15 others lines harbour one of these two activating mutations. Surprisingly, 10-6 M dasatinib was less effective than lower concentrations. Such resistance was associated with an increase in the expression of MITF and BCL-2. Also, 10–5 M forskolin completely inhibited the cytotoxic effect of dasatinib through the stimulation of MITF synthesis and induction of BCL-2. In conclusion, we found that very low dasatinib concentrations were highly effective to induce cytotoxicity in a subgroup of melanoma lines characterized by cKIT mutation or amplification, and that MITF/BCL-2 may modulate such sensitivity. Consequently, some metastatic melanoma patients would benefit from dasatinib treatment considering the expected wide therapeutic window of the drug.

P-30

A large-scale RNAi screen identifies LCMR1 as a critical regulator of Tspan8-mediated melanoma invasion

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Melanoma is the deadliest skin cancer due to its proclivity to metastasize. The recently developed therapies have not yielded the expected results, since almost all patients relapse. Therefore, understanding the molecular mechanisms of early melanoma cell invasion is crucial to improving patient survival. We previously showed that, whereas the Tetraspanin 8 protein (Tspan8) is undetectable in normal skin and benign lesions, its expression arises with the progression of melanoma and increases cell invasiveness. Therefore, to identify Tspan8 transcriptional regulators that could explain Tspan8 expression onset, thereby conferring an invasive phenotype, we performed a RNA-interference-based screen. We identified several Tspan8 regulators and in particular LCMR1, recently identified as overexpressed in numerous carcinomas. Our study identified Tspan8 as the first LCMR1 target that could explain its function in melanoma development. LCMR1 modulation was sufficient to activate Tspan8 expression, with concomitant loss of cell-matrix adherence and increase in invasion. Moreover, LCMR1 and Tspan8 overexpression were shown to correlate in melanoma lesions, and both proteins were downregulated in vitro by vemurafenib. So, this study highlights the importance of Tspan8 and its regulators in the control of melanoma invasion and suggests that they may be promising therapeutic targets downstream of the RAF-MEK-ERK signalling pathway.

*Equal contributions.

Flash Talks

FT-01

Melanoma targeted radionuclide therapy with melanin–ligands: molecular mechanisms and potentialisation

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Our group develops radiolabelled melanin-ligands for imaging and targeted radionuclide therapy (TRT) of metastatic melanoma (Cachin et al, 2014). Systemic injection of [131I]ICF01012 lowers tumor growth in syngeneic (B16Bl6) and xenograft (SKMel-3) preclinical melanoma models (Degoul et al, 2013, Viallard et al, 2015). It reduces spontaneous metastases and number of lung colonies after cell i.v. injection in C57Bl6/B16Bl6 model. [131I]ICF01012 TRT activates DNA double-strand break induced pathways in B16Bl6 tumors. We combined [131I]ICF01012 with a DNA repair inhibitor, the coDbait, which demonstrated radiosensitization activity in external beam radiotherapy (Le Tourneau et al, 2016). This treatment combination shows radiosensitization additive effect in the B16/BL6 model and synergistic effect in the SKMel-3 model. We used in-vitro SKMel-3 spheroids to study mechanisms induced by [131I]ICF01012 TRT on genes involved in pseudo epithelial mesenchymal transition or in tumor-initiating cells. Relevance of the model was confirmed by colony forming tests, showing significant reduction of colony number after [131I]ICF01012 treatment. Preliminary data on this 3D model showed a transient decrease in expression of two main transcriptional factors implicated in melanoma (MITF and ZEB2) following [131I]ICF01012 TRT. These mechanistical studies are ongoing to assess the interest of [131I]ICF01012 TRT and to document potential combination with innovative therapies.

FT-02

Opposite roles for MITF and p53 in the resistance of mutant NRAS melanoma to MEK inhibition

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Activating mutations in NRAS are found in 15–30% of melanomas and are associated with a poor prognosis. The MEK inhibitor pimasertib showed clinical benefits in melanoma patients but had limited efficacy as single agent. We aimed to identify the roles of MITF and p53 in the resistance of Q61K/L/RNRAS melanoma cells to pimasertib. Indeed, activation of MITF or alteration of p53 signaling could be involved in the restraint effects of MEK inhibitors. First, we showed that pimasertib inhibited cell proliferation with IC50 ranging from 0.01 to 0.05 μM in four Q61K/L/RNRAS melanoma cell lines. Direct p53 reactivation using PRIMA-1Met synergized with pimasertib to induce apoptosis. Further, we found that cAMP induction conferred resistance to pimasertib through MITF-mediated upregulation of Bcl-2. This resistance is reversed by the selective Bcl-2 inhibitor ABT-199 or p53 activation by PRIMA-1Met. Furthermore, we developed a line with acquired resistance to pimasertib and found that such resistance was associated with the activation of MITF-Bcl-2 pathway, thus supporting our previous findings. This particular anti-apoptotic mechanism in mutant NRAS melanoma warrants further investigation to further evaluate the benefit of combining MEK inhibition to p53 reactivation or BcL-2 inhibition as a promising therapeutic strategy.

FT-03

A systematic analysis of melanosome structure and distribution in different skin color phenotypes: identification of melanocore clusters as lysosome related organelles

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The distribution of melanosomes in keratinocytes was systematically investigated, from highly, moderately and lightly pigmented human skins, classified according to their Individual Typological Angle (ITA), a representative parameter of skin color phenotype. Electron microscopy of skin samples revealed qualitatively and quantitatively that in highly pigmented skins, although melanosomes are mostly isolated and distributed throughout the entire epidermis, clusters can also be observed. In moderately and lightly pigmented skins, melanosomes are mostly present in the first layer of epidermis where they can appear isolated but for most of them, are grouped as clusters of melanocores (melanosomes devoid of membrane), embedded in an electron dense matrix and surrounded by a single limiting membrane. Electron tomography resolving the 3D organization of organelles reveals that clustered melanocores depict contacts with other organelles such as endoplasmic reticulum and mitochondria. Immunogold labeling followed by statistical analysis highlighted that clusters of melanocores do not correspond to autophagosomes or melanophagosomes but rather to non-acidic lysosome related organelles, similar to isolated melanosomes found in melanocytes. These observations enlighten the melanosome structure and fate in keratinocytes and open new avenues to understand the basis of the skin pigmentation in the different skin color phenotypes.

FT-04

A novel ion channel activator, CyPPA inhibits melanogenesis via the gsk3β/β-catenin pathway

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The research of anti-melanogenesis materials for skin diseases has become more popular and expanded into the area of ion channel modulators. In the course of screening of ion channel modulators exhibiting anti-melanogenesis function in B16F10 cells, we found that CyPPA, a positive modulator of small-conductance Ca2 + activated K+channel strongly inhibited melanogenesis. Non-cytotoxic cocentrations of CyPPA decreased melanin content in a concentration-dependent

manner in normal human melanocytes. Tyrosinase activity in CyPPA-treated cells was reduced with dose-, and time-dependent manner. Treatment with CyPPA decreased transcription level of MITF and GSK3β activity was modulated by CyPPA, causing decrease of β-catenin/MITF.In addition, MelanoDerm™ visual evaluations also revealed a significantly lower melanin content in the CyPPA-treated human skin model than in the untreated control. Taken together, we identified a novel anti-melanogenesis role of CyPPA which modulates K+ion channels.

FT-05

Oncogenic MITFE318K promotes senescence delay and melanoma progression

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MITF encodes an oncogenic lineage specific transcription factor of which, a germline mutation (MITFE318K) was identified in human patients predisposed to both nevus formation and, among other tumor types, melanoma. The cellular mechanisms underlying the oncogenic activity of MITFE318K remained unknown. To determine the role of the mutation in vivo we have generated a MitfE318K knock-in (KI) mouse model. We provide genetic evidence that MITFE318K enhances BRAFV600E-induced nevus formation in vivo. Importantly, while MitfE318K was not sufficient to cooperate with BRafV600E alone in promoting metastatic melanoma, it accelerated tumor formation on a BRafV600E; Pten-deficient background. Moreover, our findings show that MitfE318K impairs the ability of human melanocytes to undergo BRAFV600E-induced senescence in vitro.
Here, we characterize the functions of the melanoma-associated MITFE318K mutations. Our results show how MITFE318K by lowering the program of senescence might favor melanoma progression in vivo.

FT-06

MITF, TFEB and TFE3 in melanoma – regulation and interaction

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The transcription factor MITF is crucial for melanocyte development and survival and a lineage specific oncogene in melanoma. The closely related TFEB and TFE3 proteins are involved in the biogenesis and function of lysosomes and autophagosomes, regulating the cellular clearance pathways.
We have investigated the interaction, cross-regulatory relationship and nuclear localization of MITF, TFE3 and TFEB in melanoma cells. Like MITF, TFEB and TFE3 are expressed in melanoma cells and they regulate each other's expression. Using co-immunoprecipitation studies, we demonstrate that MITF, TFEB and TFE3 interact in melanoma cells. Transactivation assays show an overlap in the ability to activate expression of autophagy, lysosomal and melanosomal genes but interestingly, some genes are exclusively regulated by one of the factors. Using RACE studies we identified a shorter melanocyte-specific isoform of TFEB. MITF-M is mostly nuclear, whereas TFEB and TFE3 are located in the cytoplasm. Nutrients, mTOR and GSK3β signaling impact the subcellular localization of all the factors in melanoma cells.
The relationship between MITF, TFEB and TFE3 is complex and involves gene expression, interaction and signaling. It is important to unravel this relationship in melanoma since these factors and autophagy are considered therapeutic targets in cancer.

FT-07

New mouse models of albinism generated with CRISPR-Cas9 technology

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Animal models have been generated and used since many years for the study of numerous human diseases, including human rare diseases such as albinism. Our laboratory has been contributing to this field with diverse genetically-modified mice that have been instrumental for our current understanding of some types of albinism, in particular oculocutaneous albinism type I (OCA1), associated with mutations in the TYR locus. We have used transgenic mice, to define the entire Tyr expression domain, its crucial DNA regulatory elements responsible for the faithful expression of the gene and, also, to investigate the visual abnormalities that are diagnostic for all types of albinism. With the appearance of the CRISPR-Cas9 technology, we are now also able to reproduce in mice mutations observed in the human population. We can easily edit the mouse genome or to generate targeted alterations. We have applied successfully CRISPR-Cas9 strategies for preparing new and informative mouse models of different types of albinism. Particularly, we have used CRISPR-Cas approaches to inactivate and functionally assess the relevance of non-coding genomic sequences in the Tyr locus. This presentation will review our efforts using CRISPR-Cas9 technologies for the creation and analysis of genome-edited mice, for progressing into our understanding of albinism.

Interactive Session with Researchers and Clinicians

Melanoma models: what is needed to move from bench to bedside? relevance of in vivo and in vitro models for melanoma

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e incidence of cutaneous melanoma is increasing regularly in France and western countries. The rate of mortality remains high despite the availability of innovative therapies on the market. The failure of these new therapies is due in part to insufficient basic knowledge, inappropriate cell systems and in vivo melanoma models. Malignant melanoma is an aggressive human tumour and both genetics and epigenetics events contribute to regulate its initiation and progression. The laboratory aims to improve understanding, in an integrative manner, of the molecular and cellular mechanisms associated with the normal and pathological development of melanocytes and melanoma. Consequently, we

generate crucial information to the understanding of the establishment of melanocytes from melanoblasts during development and the renewal of melanocytes from melanocyte stem cells. We decipher, in vitro and in vivo, various signalling pathways. In particular, we study proteins engaged in melanoma initiation: BRAF/NRAS, involved in proliferation and CDKN2A/PTEN/CTNNB1 involved in immortalisation. Generating appropriate models and approaches are essential for advances in understanding melanoma initiation and progression. We are also developing a coherent preclinical in vitro and in vivo pipeline using human and mouse cell lines (2D and 3D) and associated murine models to allow the prioritisation of new therapies.

Session 7: Cell Metabolism Dysregulation in Melanoma

An evolutionarily conserved driver of migration and invasiveness

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The intra-tumor microenvironment generates phenotypically distinct but inter-convertible malignant cell subpopulations that fuel metastatic spread and drug-resistance. Whether different microenvironmental cues impose a proliferative-to-invasive or drug-resistant phenotype-switch via a common mechanism is poorly understood. Here we show that in melanoma nutrient limitation and inflammation converge to reprogram translation, a hallmark of starvation. Translation reprogramming initiates the first steps in metastatic colonization by imposing a slow-cycling, invasive, tumour-initiating phenotype, and couples the integrated stress response to melanoma de-differentiation. Repression of the MITF lineage-survival oncogene in response to translation reprogramming also generates an MITF-low/AXL-high drug-resistant state observed in human tumors. Since we also show that translation reprogramming drives neural crest migration and invasion by yeast, our results suggest that migration/invasiveness is an evolutionarily conserved response to starvation that has been hijacked by microenvironmental 'get-out-of-here' stress signals in cancer.

O-17

Metabolic reprogramming in melanoma: the role of peIF2alpha

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The recent literature has focused on the role of metabolic reprogramming in melanoma. The existence of a BRAF-MITF-PGC-1α axis has been described, with V600BRAF switching on this metabolic reprogramming. Nevertheless, several mechanisms seem to be involved in this process. The aim of this study is exploring and comparing metabolic and functional changes occurring in primary and metastatic V600BRAF melanoma cell lines. In particular, in V600BRAF cell lines we found a downregulation of PGC-1α/β and MITF and a decrease of OXPHOS activity with an increase of glycolytic ATP, lactate, HIF-1α and MCT4 levels. Furthermore, the induction of autophagy and the presence of ER stress markers in V600BRAF metastatic melanoma (MM) cells suggest a metabolic adaptation of these cells as compensatory survival mechanisms. In our study we highlight an increase of peIF2α in MM but not in primary V600BRAF cells, thus delineating autophagy activation by ER stress as a pro-survival mechanism in peIF2α-proficient cells (MM V600BRAF cells). To our knowledge, this is the first report of peIF2α as a marker of a more aggressive phenotype in melanoma.

O-18

New compounds triggering endoplasmic reticulum stress exert anti-melanoma effects and overcome braf inhibitor resistance

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Despite the spectacular progresses recently made in the treatment of melanoma by using targeted therapies or immuno-therapies, most patients with melanoma will need additional treatments. Using structure/activity relationship studies, we identified a new series of molecules, the thiazole benzenesulfonamides, that exhibits strong lethal effects on melanoma cells (cancer cell, 2016, in press). We show that the lead compound, HA15, displays anti-cancerous activity on melanoma cell lines, primary cells isolated from patients and melanoma cells resistant to BRAF inhibitors. HA15 efficiently inhibited the tumor growth of BRAF inhibitors-sensitive or -resistant melanoma cells in mice. Transcriptomic, proteomic and biochemical studies identified the endoplasmic reticulum protein BiP/GRP78/HSPA5 as the specific target of HA15. The specific interaction between HA15 and BiP increased endoplasmic reticulum (ER) stress and melanoma cell death through a concomitant induction of autophagic and apoptotic mechanisms. Interestingly, other liquid and solid tumors were also highly sensitive to HA15. Taken together, our data suggest that HA15 displays anti-melanoma effects by targeting the ER stress axis, highlighting the key role for this specific pathway in melanoma malignancy and in cancer. These findings reveal that this series of compounds, of which HA15 is the lead, may represent an attractive drug candidate for melanoma treatment.

Integrative and Comparative Genomics Identifies New Melanoma Tumor Suppressor Genes

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Malignant melanoma is the deadliest form of skin cancer with few known targetable (epi)genetic alterations. Using genome sequencing, the landscape of genomic alterations of spontaneously acquired BRafv600E- and NRasQ61K-driven mouse melanomas was established and used to prioritize relevant lesions from the complex human melanoma genome. This analysis confirmed the importance of several genes/pathways previously implicated in human melanoma, and thereby the relevance of these mouse models, and identified new putative tumor suppressor genes with prognostic and therapeutic relevance. Engineered deletion of several of these genes accelerated mouse melanomagenesis. This integrated effort offers a framework for future functional genomic studies and key melanoma TSGs.

September 15, 2016
Session 8: Melanoma Microenvironment and the Role of Inflammation

O-19
Varying proliferative and clonogenic potential in NRAS-mutated congenital melanocytic nevi according to size

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Congenital melanocytic nevi (CMN) are benign proliferations that may be associated with various consequences depending on their size. They are characterized by a specific molecular signature, namely a post-zygotic somatic NRAS or BRAF mutation. We have recently reported that large CMN (lCMN), which are classically associated with an increased melanoma risk, harbor cell subpopulations with specific clonogenic and tumorigenic potential. We wished to ascertain whether cells displaying similar properties persisted postnatally in medium CMN (mCMN). Eighteen medium M1, nine large and one giant NRAS-mutated CMN were prospectively included in the study. Subpopulations of mCMN cells expressed stem cell/progenitor lineage markers such as Sox10, Nestin, and Oct4, as was the case in lCMN. Nevertheless, conversely to lCMN, mCMN cells with clonogenic properties were rarer. In vitro, approximatively 1 in 1500 cells isolated from fresh mCMN formed colonies that could be passaged. In vivo, mCMN seemed to harbor cells with less proliferative potential than the larger lesions as lCMN biopsies displayed a three-fold expansion compared to mCMN when xenografted in Rag2-/- mice. Thus, our data revealed variations in clonogenicity and tumorigenic properties in NRAS-mutated CMN according to size.

[†]Both authors contributed equally to this work as senior co-authors.

O-20
The relationship between UV induced lysosomal exocytosis and melanosome transfer in melanocytes

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The lysosomal function goes far beyond the degradation mission. Lysosomes are identified as important regulators of cell homeostasis and are able to exocytose and donate their membranes to repair cells. In melanocytes, the photoprotective melanin is produced in specialized lysosome related organelles, melanosomes. Although melanosomes and lysosomes share constituents and common origin, their response during UV-exposure requires further elucidation.

Herein, we provide novel insight into UVA initiated communication between melanocytes and keratinocytes. We show an immediate shedding of extracellular vesicles (EVs) of lysosomal origin triggered by UVA-induced plasma membrane damage and subsequent lysosomal exocytosis. In co-cultures, the melanocytic derived EVs enhanced keratinocyte proliferation. In contrast, UVB did not affect membrane integrity and no lysosomal exocytosis was detected. Spontaneous transfer of melanosomes occurred after 24 h, the transfer was enhanced after UVA and UVB and was mechanistically unrelated to the EVs shedding.

While melanosomes contribute to protection against UV damage, EVs alter proliferation in the recipient cells. Thus, EVs might affect epidermal homeostasis in vivo, by stimulating renewal of keratinocytes and promote sun-induced thickening of epidermis.

The study discerns the melanocytes as important player in the protection against UV, not only by distribution of melanin but through rapid generation of EVs.

Fritz Anders Memorial Lecture

Precision medicine for melanoma

Richard Marais

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The discovery of new targeted and immunotherapies has heralded a new era in cancer treatment, resulting in substantial improvements in patient survival and in some cases leading to cures. However, many patients do not respond to these revolutionary treatments, or they derive only short-lived benefit because after a brief period of clinical response they develop resistance. Moreover, once first line treatments fail, it is often difficult to select effective second line treatments. Advances in next generation sequencing (NGS) approaches gives the capacity to reveal nucleotide-level genetic resolution of an individual patient's tumour. This can lead to identification of tractable therapeutic targets and provide hypothesis-driven second-line treatment options. However, the implementation of these approaches is technically difficult and ethically complex; the approaches are also expensive and can be slow for patients who to not have the luxury of time. Thus, we still face substantial hurdles in patient stratification for first and subsequent lines of treatment, and this is even more difficult when combinations of drug are indicated and their cumulative toxicity has not been assessed. Clearly, the future of cancer medicine holds great promise, but we will have to overcome substantial challenges if we are to realise the full promise of precision medicine. The key question for our patients is how long will it take?

Session 9: Highlights from the Other Pigment Cell Societies

O-21

New aspects of melasma treatment in Asians

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Melasma is characterized by epidermal pigmentation. But it is not only a disease of melanocyte but also a disease of surrounding environments. Recently, there have been reports of extra-melanocyte abnormalities which are associated with increased pigmentation. Furthermore, there is a complex signaling mechanism in the melanogenesis. To manage a complex mechanism, multi-target approach will be inevitable. In this presentation, we are going to present heterogeneous pathology of melasma and biologic mechanism which can affect melanogenesis and possible strateges for the treatment of melasma. First of all, tyrosinase is 1st and most important target in depigmenting agents. Topical hydroquinone is the drug of choice for the treatment of melasma in dermatology practice. However, it is not clear whether hydroquinone is really a good tyrosinase inhibitor. Secondly, intracellular mechanism which includes transcriptional and translational process of tyrosinase, is important. We also present some example of signal regulators for depigmenting agents. More importantly, melanocytes are under the influences of extra-melanocytes factors. We are also going to present heterogeneous pathology of melasma and some examples of approach to control these problems.

O-22

The Metabolic Fate of Ortho-Quinones Derived from Catecholamine Metabolites

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Neuromelanin, a dark pigment present in the substantia nigra and locus coeruleus of the brain, is produced from dopamine and norepinephrine via an interaction with cysteine, but it also incorporates their alcoholic and acidic metabolites. We examined the metabolic fate of ortho-quinones derived from the catecholamine metabolites, 3,4-dihydroxyphenylethanol (DOPE), 3,4-dihydroxyphenylethylene glycol (DOPEG), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylmandelic acid (DOMA). The oxidation of catecholic substrates by mushroom tyrosinase was followed by UV-visible spectrophotometry. HPLC analysis after reduction with NaBH4 or ascorbic acid enabled the measurement of the half-lives of ortho-quinones and the identification of their reaction products. The major product from DOPE-quinone was DOPEG that was produced through the addition of a water molecule to the quinone methide intermediate. DOPEG-quinone yielded a ketone, 2-oxo-DOPE, through the quinone methide intermediate. DOPAC-quinone and DOMA-quinone degraded immediately with decarboxylation of the ortho-quinone intermediates to form 3,4-dihydroxybenzylalcohol (DHBAlc) and 3,4-dihydroxybenzaldehyde (DHBAld), respectively. DHBAlc-quinone was converted to DHBAld with a half-life of 9 min, while DHBAld-quinone degraded rapidly with a half-life of 3 min. This study confirmed the fact that ortho-quinones from DOPE, DOPEG, DOPAC and DOMA are converted to quinone methide tautomers as common intermediates, through proton rearrangement or decarboxylation.

O-23

Testing HSP70iQ_{435A} treatment for vitiligo in large animals

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Inducible heat shock protein 70 (HSP70i) is central to vitiligo development. Meanwhile, modified HSP70i$_{Q435A}$ could tolerize dendritic cells and overcome depigmentation, even supporting repigmentation in vitiligo mouse models. Here we applied HSP70i$_{Q435A}$-encoding DNA to Sinclair swine that develop vitiligo as melanomas regress. Thus far, 6 vitiligo lesions of 10 cm^2 on average were treated by jet injection weekly for 4 weeks. Treated and untreated lesions were followed for 25 weeks. We measured lesional size and evaluated T cell infiltration in perilesional skin and anti-HSP70i titers in serum. Depigmentation was significantly reduced from +2.8 cm^2 in untreated, to −6.2 cm^2 in DNA treated lesions. This >60%

repigmentation occurred in perifollicular and perilesional patterns. In pilot experiments (n = 2) T cell accumulation was markedly reduced in DNA treated skin for >10 weeks whereas 1 treated animal showed a 3-fold increase in anti-HSP70 titers at week 4. Tumor measurements were unaffected, and treatment was well tolerated. One untreated animal exhibited tumors at euthanasia, while a treated animal developed an ulcerating tumor that subsequently regressed. No further adverse events were found and no inflammation or other ocular changes were observed. Thus DNA encoding $HSP70i_{Q435A}$ might likewise be used to treat vitiligo without suppressing anti-tumor responses.

Authors Index