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REVIEW ARTICLE

Scholars and scientists in the history of the lymphatic system

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Abstract

The discovery of the lymphatic system has a long and fascinating history. The interest in anatomy and physiology of this system paralleled that of the blood cardiocirculatory system and has been maybe obscured by the latter. Paradoxically, if the closed blood system appeared open in Galen's anatomy and physiology, and took a very long time to be correctly described in terms of pulmonary and general circulation by ibn Al-Nafis/Michael Servetus/Realdo Colombo and William Harvey, respectively, the open lymphatic system was incorrectly described as a closed circuit connected with arteries and veins. In ancient times only macroscopic components of the lymphatic system have been described, although misinterpreted, including lymph nodes and lacteals, the latter being easily identified because of their milk-like content. For about 15 centuries the dogmatic acceptance of Galen's notions did not allow a significant progress in medicine. After Vesalius' revolution in anatomical studies, new knowledge was accumulated, and the 17th century was the golden age for the investigation of the lymphatic system with several discoveries: gut lacteals (Gaspare Aselli), cloacal bursa (Hieronimus Fabricius of Acquapendente), reservoir of the chyle (Jean Pecquet), extra-intestinal lymphatic vessels (Thomas Bartholin and Olaus Rudbeck dispute), hepatic lymph circulation (Francis Glisson). In the Enlightenment century Frederik Ruysch described the function of lymphatic valves, and Paolo Mascagni provided a magnificent iconography of the lymphatic network in humans. In recent times, Leonetto Comparini realized three-dimensional reconstructions of the liver lymphatic vessels, and Kari Alitalo discovered the lymphatic growth factor/receptor system. Far from a complete understanding of its anatomy and function, the lymphatic system still needs to be profoundly examined.

Key words: history of medicine; lacteals; lymphatic growth factors; lymphatic system.

Introduction

The investigation of the lymphatic system has a very long and intriguing history, with several medical figures that brought important contributions (Fig. 1). Along with the better known cardiovascular bloodstream, the lymphatic system consists of an extensive network of vessels deputed to the drainage of extravasated fluids. The contractility of lymphatic vessels and the presence of valves are essential for the generation and regulation of the lymph transport (Gashev & Zawieja, 2001; Aukland, 2005). The fluid circulating in lymphatic vessels appears limpid and clear, and for this reason the word 'lymph' was originally derived from the Greek *Nymph*, indicating a creature associated with clear streams (Shields, 1994), and to the Roman deity *Lympha*, with the meaning of spring clear water (Andrews & Gardiner, 2014).

Apart from fluid and macromolecule transport and homeostasis, it is now well known what the role of the lymphatic system in lipid absorption is, and that it is an essential component of the immune system, with clinical relevance in oncology (Adamczyk et al. 2016). However, its physiological roles have not been fully elucidated.

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In the reference list of this article, only modern papers and books are reported. The classic historical books are not listed, but their full titles and year of publication are mentioned in the text.

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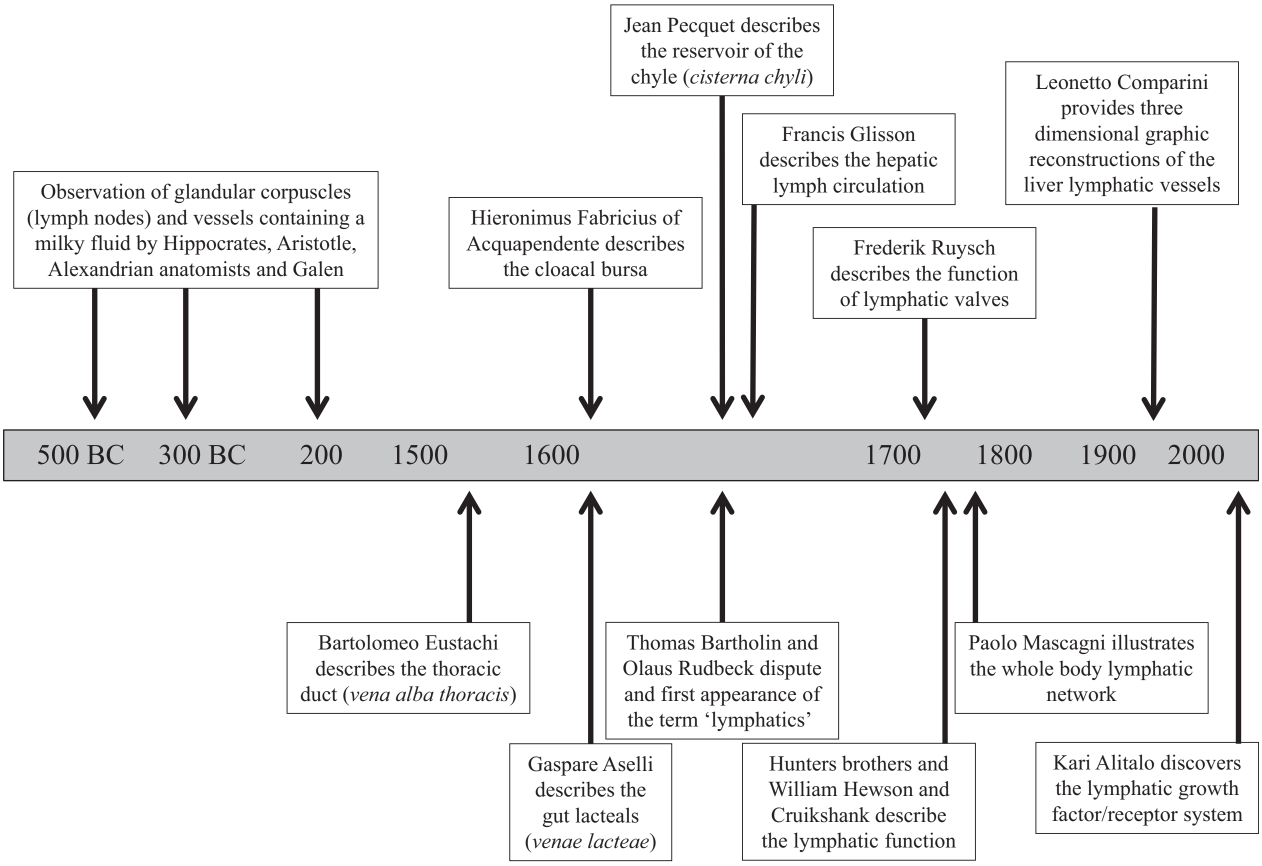

Fig. 1 Schematic diagram showing the principal steps of the scientific progress in the knowledge of lymphatic system anatomo-physiology. Note that the dogmatic Galen's medicine lasted about 15 centuries. The 17th century was the most prolific in terms of anatomical discoveries.

Since the ancient times of the long history of anatomy some components of the lymphatic system were described, although misinterpreted, and several books (Sheldon, 1784; Ceradini, 1875, 1876; Battezzati, 1972) and papers (Dobson & Tompsett, 1968; Sokolowska-Pituchowa, 1969; Eales, 1974; Meyer-Burg, 1974; Fontaine, 1977; Leeds, 1977; Grotte, 1979; Kanter, 1987; Fabian, 1991; Mazana Casanova, 1992; Chikly, 1997; Shields, 2001; Oliver & Detmar, 2002; Ferrandez, 2006; Suami et al. 2009; Ribatti & Crivellato, 2010; Loukas et al. 2011; Suy et al. 2016a,b,c) have been dedicated to the gradual discovery of this important circulatory system, until the most recent and modern concepts of molecular lymphology (Witte, 2008), lymphangiogenesis (Escobedo & Oliver, 2016) and lymphosome indicating the lymphatic skin territories (Suami, 2017).

The present work outlines the history of the discovery of the lymphatic system. Then, past and present investigations meet within a prestigious anatomical tradition.

Early times

The research of the gross anatomy dates back to ancient times, when only macroscopic organs could be examined and described. Hence, all apparatuses have been completely described in terms of microscopic components only in recent times, after the use of the microscope applied to life sciences. Accordingly, the first macroscopic lymphatic structure that could be examined is the lymph node, which appears easily detectable in living beings in specific regions of the subcutaneous tissue (for instance, axillary, inguinal, cervical and submandibular groups), as well as in the vicinity of deep organs during dissection. In this respect, Hippocrates (V century BC), the father of medicine, coined the term *chylos* (chyle), and in his book *Peri Adenon* described lymph nodes in the armpit, around the ears, near the jugular vessels, in the inguinal flexure, scattered in the mesentery, and near the kidneys, containing a fluid absorbed from the tissues named *ichor* (Crivellato et al. 2007; Suami et al. 2009; Choi et al. 2012; Suy et al. 2016a).

The main difficulty in describing the lymphatic vessels comes from the fact that they form a largely invisible, delicate and intricate network. If the more evident sphygmic blood circulation needed centuries to be fully understood – blood vessels confused with nerves, arteries confused with veins, air instead of blood into vessels – not surprisingly the lymphatic system appeared more indefinite, elusive and mysterious.

One of the first descriptions of what could be ascribed to lymphatic vessels can be found in Aristotle. In *Historia Animalium* (book III, chapter VI), some particular fibers endowed with a nature intermediate between nerves and veins have been observed. Some of them would contain a colorless humour, named *sanies*, which flows from nerves to veins and back (Crivellato et al. 2007; Loukas et al. 2011; Suy et al. 2016a).

The Alexandrian School gave a great contribution to the study of the lymphatic system, as derived from Galen's writings, although the question whether or not the structures described were actually lymphatic vessels is still debated. As reported by Galen (II–III century) in the *Anatomicae administrationes* (book VII, end of chapter XVI), in a suckling kid Erasistratus (310–250 BC) showed that, when cut, the abdominal arteries fill with milk. Perhaps, this is the first misinterpreted evidence of mesenteric lacteals (Ambrose, 2006, 2007b; Suy et al. 2016a).

Galen repeated the alexandrian experiment in adult animals, as well, and denied that finding, but he was able to describe mesenteric lymph nodes, pancreas and thymus. Galen's ideas on the lymphatic system are reported mainly in *De usu partium* (book IV, chapter XIX) and *Anatomicis administrationibus* (books VI and XIII). According to the peculiar Galen's physiology, the process of transport of chyle from the gut to the liver via small side branches of the portal vein, and further transformation of the chyle into blood and its transport to tissues – to the gut via the same small side branches of the portal vein – was named *anadosis*. Galen also referred the Erophilus' (335–280 BC) description of special venous vessels that, unlike portal side branches, connect particular glandular organs (pancreas or mesenteric lymph nodes?) and the intestine, endowed with the function to feed the gut. Maybe with these two nutritional pathways addressed to the gut Galen distinguished between the milky and clear limpid content of the lymphatic vessels (Ambrose, 2006, 2007b; Suy et al. 2016a).

Axillary, inguinal and mesenteric nodes and thymus were also described by the Roman physician Rufus of Ephesus (I–II century; Ambrose, 2006).

The Byzantine physician Paul of Aegina (607–690) was a famous surgeon who illustrated the tonsils, and performed the first tonsillectomy, which allowed him to discover and describe infected lymph nodes in the lower cervical region (Loukas et al. 2011).

The Indian and Islamic medicine, especially Avicenna, gave interesting descriptions of lymphedema (elephantiasis), because of the frequent parasitic infections that are more common in oriental regions (Golzari et al. 2012).

In spite of the surgical tradition of the medieval Salernitan School and the foundation of the first universities, for a long time anatomical research did not provide a significant improvement of knowledge. A still confused description of the lymphatic system appeared in the works of some physicians of the Salernitan School, such as Cofone (Medici, 1857, pp. 13–14).

Renaissance and modern anatomy

In the 16th century, Andreas Vesalius (1514–1564), the father of the modern anatomy, advocated the importance to dissect human corpses and, in his illustrated masterpiece, *De Humani corporis fabrica*, published in 1543, more than 200 anatomical mistakes were evidenced and the obsolete Galen's anatomy, mostly derived from animal dissections, was abruptly criticized and rejected. However, Vesalius' description of abdominal organs abode by the Galen's one. A new era began, not only for scientists, but also for artists, such as Leonardo da Vinci and Michelangelo Buonarroti, who could accord art and anatomy. In particular, the knowledge of the cardiocirculatory system made a substantial progress. Based on the first observations made by the medieval Arab anatomist ibn-Al Nafis, the Spanish scientist and theologian Michael Servetus (1511–1553) and the Italian anatomist Realdo Colombo (ca. 1516–1559) shared in the description of the pulmonary circulation, and Andrea Cesalpino (1519–1603) introduced for the first time the term 'circulation' applied to the cardiovascular system. In this context, the anatomical research of the lymphatic system also took advantage.

Indeed, the transition from a liver-centered (hepatocentrism) to a heart-centered model (cardiocentrism) of the blood cardiovascular circulation contributed to better clarify also the lymphatic system.

In the *Liber Introductorius Anatomiae* (1536), Nicola Massa investigated and described something attributable to the renal lymphatic vessels, mentioning *venae chilis* at the beginning of chapter VIII (*De sectione renum, & vasorum urinalium usque ad vesicam*). However, the term *vena chilis*, also used by other anatomists, was synonymous with vena cava. Indeed, according to the Galen's anatomy of blood circulation and to the idea that the milky fluid coming from the gut was erroneously drained by veins, the inferior vena cava addressed to the liver assumed the Greek name of *vena chili*, other than the more correct Latin name of *vena cava* or *concava*. This debated question was elegantly summarized by Morgagni (1728, pp. 90–91, n. 78) in the *Epistolae anatomicae*:

> You say: 'Would Mondino indicate in some way lacteal ducts with *vena chilis*? According to this idea, Eustachius studied his lacteal vein, and Aselli rapidly found easy clues of lacteals, and Pecquet from Aselli'. What is similar? After his accurate description, everybody agrees that Eustachius really observed the thoracic duct and popularized it before Pecquet. On the contrary, what did Mondino mean when writing *vena chili* but the vena cava? Is there somebody who had some doubt on

this topic? All the more so as Mondino began this way in the chapter entitled *The anatomy of the vena chili*: 'Once removed all these organs, two vessels will appear: a great vein which is a descending branch of the vena chili, and a great artery which is a descending branch of aorta; when that vein is at level of kidneys, it generates two branches, named *emulgentes* [efferent renal veins which purified the blood], addressed to right and left kidneys, respectively'. I do not mention other Mondino's passages which all clearly confirm that he identified vena cava with *vena chili*, and that in Mondino's opinion *vena chili* has the same meaning as vena cava in Cicero and almost in all people who treated anatomy in Latin. Not surprisingly, the Greek *koîlos* corresponds to the Latin *cavus*. Fernelio is right when he says: the vein 'named *koílē*, that is cava'. Again Massa: 'Greeks name that vein *chili*, Romans *cava*'. Again Falloppio: 'We named the vena *cava* with the Latin name, according with other anatomists, but the Greek is *koílē*'. I do not mention other Authors, since it is not necessary. Indeed, it is very evident that not only the structure itself, but also the name used by Mondino, *vena chili* or, according to Matteo Curzio's edition, *cyli* or rather *cili*, could lead Eustachius to think of chiliferous veins; unless we do not think that a man who knew very well Greek did not understand what we said, or that he did not know the great difference between *cilos* (*koîlos*), which means hollow as said before, and *chylos* (*khulós*), which means juice, and in particular that named chyle by physicians. And what about the fact that Eustachius did not believe that the thoracic duct was a blood vessel, and that he never suspected that it might be a vessel for the chyle? As far as inexpert he could be in terms of structures and names to such a degree to think the Mondino's vena chili indicated a cheliferous vein, it is evident that when discovering the thoracic duct he did not follow Mondino's opinion.

When discussing the disposition of the azygos vein in horses (*de vena sine pari*) in the *Antigramma XIII* of the *Tractatus de vena* (in *Opuscula Anatomica*), Bartolomeo Eustachi (1500–1574) is credited as being the first to describe the thoracic duct (*vena alba thoracis*) during a horse dissection, but he was unable to identify its termination and its role:

> Ad hanc naturae providentiam quamdam equorum venam alias pertinere credidi, quae cum artificii et admiratione plena sit, nec delectatione ac fructu careat, quamvis minime sit ad thoracem alendum instituta, operae pretium est ut exponatur. Itaque in illis animantibus ab hoc ipso insigni trunco sinistro juguli, qua posterior sedes radicis venae internae jugularis spectat, magna quaedam propago germinat, quae, praeterquam quod in ejus origine ostiolum semicirculare habet, est etiam alba et aquei humoris plena; nec longe ab ortu in duas partes scinditur, paulo post rursus coeuntes in unam, quae nullos ramos diffundens, juxta sinistrum vertebrarum latus, penetrato septo transverso, deorsum ad medium usque lumborum fertur: quo loco latior effecta, magnamque arteriam circumplexa, obscurissimum finem, mihique adhuc non bene perceptum obtinet.
>
> [Several times I believed to this structure of the nature: a certain vein in horses, which is very particular and uncommon. It does not act to feed the thorax. However, since it is pleasant and useful, it deserves to be described. In those animals a great formation arises from this left trunk of the throat, from the posterior part of the root of the internal jugular vein. Other than to have a semicircular hole at its beginning, it is also white and contains aqueous humor; not so far from its beginning, it divides into two parts that early reunite in a unique structure along the left side of the vertebral column, without branches, that crosses the diaphragm to reach the lumbar region, where it becomes larger and envelopes an artery. I did not understand its unknown end.]

In the third observation of *Observationes de venis*, Gabriele Falloppio (1523–1562) described an oily, yellow and bitter fluid coming from liver: *In sima parte hepatis sunt quidam parvi meatus, qui desinunt, ac terminantur in pancreas et in glandulas ibi proximas, qui quidem minimi meatus deferunt quendam succum oleaginosum flavum et tendentem ad aliquam amaritudinem*. [In the flat part of the liver there are little openings that end in the pancreas and in the surrounding glands. These little openings transport an oily, yellow and bitter juice.]

The 17th century: the golden age

After Vesalius' revolution in anatomical studies, new knowledge was accumulated, and the 17th century was the golden age for the investigation of the lymphatic system with several discoveries.

The friend and successor of Gabriele Falloppio, Hieronimus Fabricius of Acquapendente (1533–1619), known as the father of embryology, discovered the cloacal bursa that bears his name in Leghorn chickens and described it in the first chapter of the embryological work *De formatione ovi et pulli* published posthumously in 1621. Fabricius misinterpreted the role of his bursa, thinking it would collect the semen of the rooster, and only in 1956 Bruce Glick and

Timothy Chang found that this structure plays an important role in lymphocyte B commitment and antibody production (Ribatti et al. 2006).

In 1622 Gaspare Aselli (1581–1625; Fig. 2) in a well-fed dog dissected alive observed the presence of 'several thin and beautiful white cords', which at the first glance were interpreted as nerves. Indeed, he was studying recurrent nerves. After dissecting other fed and unfed dogs, he was convinced that he had discovered a fourth type of circulation (apart from arteries and veins, in Aselli's opinion nerves were also hollow structures), described in his famous masterpiece *De lactibus sive lacteis venis*, published posthumously in 1627 with colored illustrations. These new vessels were named *venae albae aut lacteae* (lacteals): *albae* to distinguish them from vessels carrying blood, and *lacteae* for the milk-like fluid they contained (Loukas et al. 2011). Anyway, in line with Galen's physiology, Aselli incorrectly thought that this milky fluid gathered in the pancreas and then was addressed to the liver to contribute to blood formation: *traductio chyli ad hepar* (movement of chyle towards liver; Mazana Casanova, 1992; Ambrose, 2006, 2007b; Suy et al. 2016b).

Once observed in dogs, these new vessels had to be investigated in humans, as well. In this respect, the French scientist Pierre Gassendi (1599–1655) suggested such a research to Fabrice de Peiresc (1580–1637). As a matter of fact, in 1628 de Peiresc described lacteals also in the body of a highly fed malefactor 2 h after his execution. On the other hand, Gassendi conceived that chyle was transported by choledoch duct. So, besides portal vein, also choledoch duct was interpreted as a structure endowed with double-sense trafficking (Sprengel, 1841; Ambrose, 2006).

Fig. 2 Plaster bust of Gaspare Aselli. Gallery of Busts of the Museum of Human Anatomy 'Filippo Civinini' of the University of Pisa.

Surprisingly, the most famous pupil of Fabricius, the English scientist William Harvey (1578–1657), the discoverer of the systemic blood circulation illustrated in the celebrated *Exercitatio anatomica de motu cordis et sanguinis in animalibus* (1628), ignored, at least in part, the progress in lymphatic circulation, and he still admitted the double function of the portal vein (blood and chyle transport, alternatively) and denied the presence of chyle in lacteals and the passage of the chyle from the thoracic duct to the subclavian vein. In particular, in Harvey's opinion the network of lacteals was too extensive to account for the movement of nutrients from the gut to the blood circulation. As in the discovery of the systemic blood circulation – the Galenic daily blood production of the liver would have been unacceptably enormous according to his calculations – a quantitative consideration led Harvey to his conclusion (Ceradini, 1876; Loukas et al. 2011).

In 1647 Johann Vesling (1598–1649) realized detailed drawings of human mesentery lacteals in his *Syntagma anatomicum*, but he ignored the destination of the thoracic duct. He communicated these observations to his pupil Bartholin (Mieli, 1921; Browse, 2003).

Another important step in the lymphatic system research was made by the French physician Jean Pecquet (1624–1674). In his fundamental work *Experimenta nova anatomica, quibus incognitum hactenus chyli receptaculum, et ab eo per thoracem in ramos usque subclavios vasa lactea deteguntur. Eiusdem dissertatio anatomica de circulatione sanguinis, et chyli motu* (1651), he described the thoracic duct with its valves and the reservoir of the chyle, the so-called *cisterna chyli* (*receptaculum chyli*), which was named Pecquet's receptacle after him. More importantly, he also finally established that gut lacteals containing the milk-like lymph empty into the *cisterna chyli* to be conveyed to the thoracic duct and not to the liver, as erroneously assessed by Aselli and other anatomists before him. Nevertheless, Pecquet was unable to describe the invisible network of the lymphatic vessels outside the abdominal and thoracic cavities. The lymphatic valves of the thoracic duct were also described by the Dutch anatomist Jan van Horne (1621–1670) in the *Novus ductus chyliferus*, published in 1652 (IJpma & van Gulik, 2013).

Thanks to anatomical dissections in living animals and human corpses, a Vesling's pupil, the Danish anatomist Thomas Bartholin (1616–1680), extended the topography of lymphatics outside the gut and the liver. In his first book, *De lacteis thoracicis in homine brutisque nuperrime observatis, historia anatomica*, published in 1652, he still conformed to Galen's doctrine. However, further observations in animals and humans were determinant for a more accurate description. Bartholin was then able to distinguish two types of bloodless vessels: the first type is represented by the well-known vessels containing a milk-like fluid coming from the small intestine mesentery; the second type is represented by 'new' vessels containing a water-like fluid coming from liver. Both fluids were shown to reach the thoracic duct independently and ultimately the bloodstream, then indicating that they did not enter the liver. Finally, the chyle originating in mesenteric vessels was distinguished from the clear fluid circulating into systemic vessels, which Bartolin named for the first time 'lymphatics', confirming the correct lymph circulation (*vasa lymphatica in homine nuper inventa*). The new term appeared in the title of a second book, published in 1653: *Vasa lymphatica, nuper Hafniae in animantibus inventa, et hepatis exsequiae*. According to Bartholin's sense of humor, the book title sanctioned the death of the idea that chyle moved to liver with a witty remark, as an epitaph (obsequies of the liver), which was strongly repeated in the title of chapter VIII of his book (*post inventa vasa lymphatica, hepatis exsequiae*). Furthermore, he denied that liver was the seat of blood formation (Ceradini, 1876; Ambrose, 2007b; Fanous et al. 2007; Loukas et al. 2011; Suy et al. 2016c). It should be noted that also George Joyliffe (1621–1658), physician in Cambridge, distinguished between lacteal and non-lacteal vessels, and named the latter lymphatics (Sprengel, 1841).

Although still unpublished, similar observations were made in 1652 by the Swedish scientist Olaus Rudbeck the Elder (1630–1702). In that year Rudbeck published *Disputatio anatomica de circulatione sanguinis*, corroborating Harvey's observations and completing his research on lymphatic circulation (Lindroth, 1957; Fransson, 1997). He carried out his anatomical investigations in the presence of the queen Christina of Sweden. At Uppsala University he dissected small animals and discovered the lymphatic connection between the intestines and the blood circulation. By appropriate ligatures to the lymphatic vessels he could also observe the direction of the flow. In particular, he described the lymphatic network of the liver and showed that lymph, including the hepatic one, is conveyed to the thoracic duct and finally to the subclavian vein (Eriksson, 2004). His data were published in 1653 in the famous *Nova excercitatio anatomica, exhibens ductus hepaticos aquosos, et vasa glandularum serosa, nunc primum inventa, aeneisque figuris delineata*. Then, with different terms, Rudbeck expressed in the title of his work the same concepts reported by Bartholin. The lymphatic vessels containing the clear fluid moving from the liver to the thoracic duct were named *ducti hepatici aquosi* (watery hepatic ducts). In addition, in lungs and kidneys Rudbeck observed also the lymphatic vessels, named *vasa glandularum serosa* (aqueous vessels of glands), draining lymph nodes (conglobate glands). It seems that the publication of his data was slightly anticipated by that of Bartholin. However, Rudbeck strongly claimed the discovery of peripheral lymphatic vessels and wrote also an accusatory letter to explain his considerations. The letter appeared in the *Messis aurea exhibens; Anatomica: novissima et utilissima experimenta: Huic editioni accesserunt de Vasis Lymphaticis tabulae Rudbeckianae*, a book collecting the works by Pequet, Bartholin and Rudbeck, and published in 1659 by Siboldo Hemsterhuis. Then, the priority of this discovery is still debated (Sprengel, 1841; Tigerstedt, 1894; Lindroth, 1957; Ambrose, 2006, 2007b).

The English anatomist Francis Glisson (1597–1677) contributed to a correct description of water-channels (lymphatics) in his book *Anatomia hepatis*, and claimed the priority of the discovery for himself and for his pupil Joyliffe. In the first edition of his work, published in 1654, the anatomo-physiology of lymphatics was reported in the last pages. In subsequent editions, that appeared in 1659 and 1681, the concise title of the book was followed by the explicit subtitle…*et ad calcem operis subjiciuntur nonnulla de lymphae-ductibus nuper repertis*, underlying the novelty of the argument (Ambrose, 2006, 2007a).

One of Bartholin's pupils, the Dutch Niels Stensen (1638–1686), who is famous for the discovery of the parotid duct, also studied lymphatics in collaboration with Jan Swammerdam (1637–1680), describing in the paper *Lymphaticorum varietas* (1675) a large variety of lymphatic branches distribution in dogs. He also discussed the relationship between the thoracic duct and lymphatic vessels in a letter addressed to Bartholin in 1662: *Sudorum origo ex glandulis. De insertione et valvula lactei thoracici et lymphaticorum*. In particular, in this letter Stensen described the valved and concomitant confluence of both thoracic duct and left jugular lymphatic duct at the angle of the left subclavian and internal jugular veins. In another epistolary correspondence with Bartholin, the Dutch scientist Johannes Walaeus (Jan De Wale) in 1641 published the *Epistolae duae de motu chyli et sanguinis*.

On the basis of all these discoveries, the Italian surgeon and anatomist Giovanni Guglielmo Riva (1627–1677) was the first to provide a graphic representation of the lymphatic system in two of his four oil-paintings now preserved in the 'Accademia di Storia dell'Arte Sanitaria' in Rome. In the painting entitled *Microcosmo* he realized a life-sized human body with blood, chyle and lymphatic vessels. In the painting entitled *Hepati* he reproduced the correct blood and lymphatic circulation in the liver, confirming the cardiocentric model. Neither Riva nor his pupil Paolo Manfredi published the observations on lymphatics, and only a manuscript entitled *De latice in animante* witnesses the project to write a book (Mieli, 1921; Riva et al. 2014).

The lymphatic network of the gut was further investigated by the German anatomist Johann Nathanael Lieberkühn (1711–1756) who discovered the origin of lacteals in the intestinal villi by means of microscopic injections and corrosion preparations, as reported in his *Dissertatio anatomico-physiologica de fabrica et actione villorum intestinorum tenuium hominis*, published in 1745. Furthermore, the Swiss Johann Conrad Peyer (1653–1712) in his *Exercitatio anatomico-medica de glandulis intestinorum earumque usu* *et affectionibus* (1677) described the presence of organized lymphatic aggregates in the mucous membrane of the small intestine, named Peyer's patches after him (Loukas et al. 2011).

Not all scientists were open-minded and persuaded into the progress of anatomical studies. The French anatomist Jean Riolan (1580–1657) appeared strongly conservative and bound to ancient anatomists, and disapproved the novel discoveries on the lymphatic system. The Dutch Louis de Bils or Bills (1624–1669), famous at that time for an intriguing technique of corpse preservation (*anatomia incruenta* – bloodless anatomy), in his *Inventa anatomica antiquo nova*, published posthumously in 1692, even proposed a whimsical theory of the lymphatic system characterized by an opposite and centrifugal circulation of the lymph, which was immediately criticized (Sprengel, 1841).

The Enlightenment spirit

Until now, lymphatic vessels were accidentally described, in particular, in the small intestine mesentery, thanks to the natural contrast offered by the white milk-like lipid content of the lymph coming from intestinal villi. On the contrary, in other parts of the body they contain a clear water fluid that renders them nearby invisible and their presence was very difficult to be documented, because of the following technical reasons, as summarized by Sheldon in his *The History of the Absorbent System* (1784, pp. i–ii).

(1) They are in general void of contents in the dead human subject.
(2) Their coats are so pellucid, that it requires the most acute sight of an anatomist, much accustomed to the appearance of the different systems of vessels, to discern and distinguish them from veins or nerves.
(3) There is also much difficulty in opening them in such a manner as to be able to introduce proper instruments for injecting them, which in general (except in the trunk, or thoracic duct) can seldom be effected with any other fluid but quicksilver. The difficulty of injecting even with this subtile fluid is increased, from the minuteness of these vessels in many parts, and from their being crouded with numerous valves, which render it impossible to inject them any otherwise, than from branch to trunk.
(4) Great caution and patience are requisite to dissect and prepare the lymphatics, either for immediate demonstration or future preservation; and the difficulty of dissection is increased, from the necessity of injecting with quicksilver: for if we happen to wound the vessels, our labor will be lost by the escape of that subtile fluid.

We shall still have less occasion to be surprised that the ancient anatomists have not discovered more of the lymphatic system, when we add (to the abovementioned difficulties) that they were not so well acquainted with the art of injecting as the moderns.

As a matter of fact, once lymphatic vessels were pioneering described in different organs, several attempts were made to inject them in order to obtain a more complete and definite knowledge of the lymphatic network in the whole body. The Dutch biologist and microscopist Jan Swammerdam (1637–1680) used suet and wax injections to describe the lymphatic valves (Loukas et al. 2011).

In 1701, the Dutch botanist and anatomist Frederik Ruysch (1638–1731), pupil of Van Horne, described the morphology and the function of the lymphatic valves and corrected the direction of the lymphatic flux. Also known for his innovative techniques for preserving corpses, Ruysch was immortalized in the famous group portrait *The anatomy lesson of Dr. Frederik Ruysch* by the artist Adriaen Backer on 29 March 1670. In that lesson the anatomist was portrayed just performing a dissection of the inguinal lymph nodes on the corpse of Pasquier Joris who was sentenced to death by hanging. Thanks to his preserving techniques, consisting of air or mercury sulfide and glycerol injection, Ruysch was able to demonstrate numerous semilunar lymphatic valves, and published his results in 1744 in the book *Dilucidatio valvularum in vasis lymphaticis et lacteis* (IJpma & van Gulik, 2013).

Another Dutch anatomist, Anton Nück (1650–1692), adopted the mercury injection technique for demonstrating the lymphatic system in his *Adenographia curiosa et uteri foeminei anatome nova*, first published in 1691 with excellent illustrations (Browse, 2003; Loukas et al. 2011).

The Italian histologist and microscopist Marcello Malpighi (1628–1689) gave a great impulse to the development of microanatomy with his pioneering microscope. Apart from the fundamental identification of blood capillaries (*De pulmonibus observationes anatomicae*, published in 1661), he also described the nodes lying along the course of the lymphatic vessels and the white pulp (Malpighian corpuscles) of the spleen.

A new progress in the knowledge of the lymphatic system was provided by the English Hunter brothers. William Hunter (1718–1783) clearly assumed that lymphatics and lacteals were two different features contributing to the formation of the same network of vessels endowed with absorbent function. He noted that a dye injected into the arteries fills the veins but never enters the lymphatic vessels, unless the arterial wall is damaged. This simple observation allowed him to draw important physiological conclusions: unlike veins, lymphatics are not continuous with arteries; lymph is derived from the extravascular and extracellular interstitial fluid; like veins, lymphatics cannot benefit from the sphygmic pulse of the heart and need lateral propulsion; finally, the direction of the lymph flux is dictated by valves. For this research he won the Copley medal in 1769 (Ambrose, 2007a; Loukas et al. 2011).

The famous surgeon John Hunter (1728–1793), who also dealt with the concept of angiogenesis (Lenzi et al. 2016; Natale et al. 2017), collaborated in the research of lymphatics in several animal species with his older brother William, and with other two Williams anatomists: William Hewson and William Cruikshank (Loukas et al. 2011). In particular, in the second part of *Experimental Enquiries* (*Into the lymphatic system*), published in 1774, Hewson reported that lymph nodes are absent in fishes, few in number in birds, and largely present in mammals. He also guessed that thymus and lymph nodes produce lymph rich in globular particles, the forerunner of modern lymphocytes. In 1786, Cruikshank published *The Anatomy of the Absorbing Vessels of the Human Body*, with updated illustrations of the lymphatic networks and a detailed mercury injection tracing of the lymphatic drainage of the human breast (Shields, 1994; Miller & Palmer, 1995; Ribatti & Crivellato, 2010; Loukas et al. 2011).

William Hunter's observations of the morpho-functional properties of the lymphatic system were claimed by Alexander Monro Jr (1733–1817), who marginally considered that item in his school thesis *Dissertatio medica inauguralis de testibus et semine in variis animalibus* in 1755, and more in depth in *De venis lymphaticis valvulosis et de earum in primis origine*, published in 1757. This claim generated a long and articulated priority dispute on scientific journals. The recent discovery of the 1752 notes of a student of Hunter reporting the mention of lymphatic function finally solved the controversy in favor of Hunter. Nevertheless, one century before this dispute, as said before, Glisson gave the right interpretation of lymphatic vessels, but both Hunter and Monro were unaware of his work at the moment of the accusatory controversy (Ambrose, 2007a).

Johann Friedrich Meckel the Elder (1724–1774) also injected the lymphatic vessels with mercury. His observations were published in *Nova experimenta et observationes de finibus venarum ac vasorum lymphaticorum in ductus visceraque excretoria corporis humani, ejusdemque structurae utilitate* and reported in a letter addressed to Haller, *Dissertatio epistolaris de vasis lymphaticis glandulisque conglobates*.

Other authors ventured upon the study of the lymphatic system. Antonio Leprotti in 1731 published a dissertation upon the roots of the human lymphatics. As reported above, John Hunter and Giovanni Sografi made similar studies, and showed that there is no direct communication between blood and lymphatic vessels. Giovanni Battista Bianchi published in 1743 *De lacteorum vasorum positionibus, et fabrica*, and a research on lacteals was also reported in the works by Giovanni Antonio Badariotti in 1743, Giovanni Battista Bologna in 1748, and Giuseppe Prato in 1752. In 1784 Giacomo Rezia wrote *Specimen observationum anatomicarum, et pathologicarum*, containing a history of lymphatic vessels, with personal considerations about lacteals and lymph nodes (De Renzi, 1848), Paul Christian Friederich Werner and Christian Gotthold Feller edited *Vasorum lacteorum atque lymphaticorum anatomico physiologica description* and, as previously said, the British surgeon John Sheldon published an interesting history of the absorbent system, with an accurate human chylography.

The Italian anatomist Paolo Mascagni (1755–1815; Fig. 3) designed a special instrument with glass tubes to inject mercury slowly into lymphatic vessels. After dissecting more than 300 corpses, in 1784 Mascagni published preliminary data in the work *Prodrome d'une ouvrage sur le système des vaisseaux lymphatiques*. The book was written in French, as it was sent to the Academy of Sciences of Paris to participate in a special competition just dedicated to this item, and Mascagni won a cash-prize. In 1787, the complete masterpiece was classically published in Latin with the title *Vasorum lymphaticorum corporis humani historia et ichnographia* (Fig. 4), allowing the author to win fame in all of Europe and to deserve the title of 'prince of anatomists'. The term *ichnographia* just underlines the concept of architectural organization referred to the lymphatic network (Vannozzi, 1996, 2015; Di Matteo et al. 2015).

In the same year 1787, Paolo Assalini published an essay on lymphatics, where the author attempted to demonstrate the existence of air-conducting vessels (De Renzi, 1848, p. 275). In that time Mascagni sent several anatomical specimens (by drying or alcohol process of preservation) illustrating lymphatics to the Museum 'La Specola' in Florence

Fig. 3 Plaster bust of Paolo Mascagni. Mascagni's Gallery of the Museum of Human Anatomy 'Filippo Civinini' of the University of Pisa.

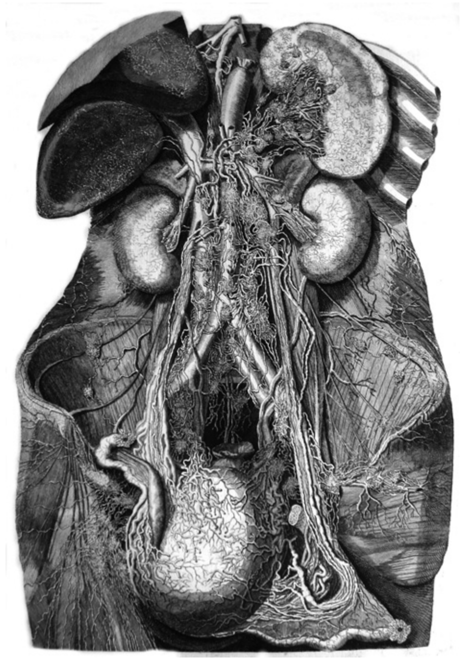

Fig. 4 Original plate XIV from Mascagni's *Vasorum lymphaticorum corporis humani historia et ichnographia* (1787). Romiti's Library, University of Pisa.

directed by Felice Fontana, and also contributed to the preparation of anatomical wax models. Some mercury-injected preparations are also preserved in the Museum of Human Anatomy 'Filippo Civinini' of the University of Pisa.

In his works on lymphatics, Mascagni not only realized for the first time wonderful anatomical plates of the whole lymphatic system, but improved his knowledge with interesting observations. He described the presence of lymphatics in the human dura mater (Bucchieri et al. 2015), and this finding has a great clinical relevance, as outlined in recent papers (Aspelund et al. 2015; Choy & Jandial, 2016). He also found a mistake in the work *Experiments establishing a criterion between mucaginous and purulent matter: and an account of the retrograde motions of the absorbent vessels of animal bodies in some diseases*, the Latin graduating dissertation of Charles Darwin (died at 20), the uncle of the more famous Charles Robert, translated into English by his father Erasmus Darwin and published in 1780. Mascagni just criticized Darwin's idea that lymphatic vessels were also endowed with retrograde transport (Lippi, 1823, p. 19; De Renzi, 1848, p. 463).

After Bartholin's and Rudbeck's intuition of the existence of extra-lacteal lymphatic vessels, and Hunter's and Meckel's observations of lymphatic vessels limited to popliteal and limb regions, respectively, Mascagni had the merit to greatly extend the demonstration of the delicate and fragile lymphatic network throughout the body, including inner organs. Together with Harvey, Mascagni improved the concept of blood circulation with that of lymph circulation. He distinguished deep and superficial lymphatics, and deep lymphatics were described according to their topographical distribution. He also paid attention to the origin of lymphatic vessels and to the structure of lymph nodes. He denied the distinction between arterial and venous lymphatics, and established that arteries and veins are in continuity, and guessed that lymphatics originate from the interstitial spaces as subtle blind vessels provided with microscopic pores. He recognized superficial and deep *conglobate* or *lymphatic glands* (lymph nodes), and observed that sooner or later lymph must meet such an organ, and for this reason he distinguished afferent and efferent lymphatic vessels with respect to the lymph node. For the study of lymph node structure, Mascagni did not inject them with mercury but with glue, wax or plaster to have an appropriate consistence. He found that lymph nodes are vascularised, as other organs, but the mercury injected into the lymphatic pathway does not move into the blood vessels, unless the lymphatic vessel bursts. Then, also in lymph nodes lymphatic and blood vessels are anatomically independent networks. The observation that lymph before and after crossing a lymph node has a different composition led Mascagni to conclude that lymph nodes are deputed to slow the flux of the lymphatic fluid, in order to allow the lymph to undergo composition changes. This finding, together with the fact that lymph can change its composition according to the organ from which it originates and the age of the subject, would account for the different adjectives used to describe its appearance: *cremori similem* (Wharton), *cinereum* (Malpighi), *diaphanum* (Nück), *album* (Morgagni, Haller).

Modern times and the contemporary trends in lymphatic system research

Besides a praise to his master Mascagni (Lippi, 1823), Regolo Lippi (1776–1854) published personal data about the communication between lymphatic and venous vessels in *Illustrazioni fisiologiche e patologiche del sistema linfatico-chilifero mediante la scoperta di un gran numero di comunicazioni di esso col venoso* (1825), where he stated that: (i) some lymphatics can originate from arteries; (ii) venous system originates both from arterial and lymphatic capillaries; (iii) some lacteals reach particular lumbar glands that give origin to lymphatics, named *chilopojetici-oriniferi*, opening into pelvis and urether; (iv) many lymphatics of the lower abdomen open into local veins. For these researches he won the *Prix de physiologie experimentale fondé par M. De Monthyon* of the *Academie Royale des Sciences de Paris* in 1829. However, Lippi's experiments were strongly criticized by Bartolomeo Panizza (1785–1867) in *Osservazioni antropo-zootomico-fisiologiche* published in 1830 (pp. 68–82).

Like Nück and Mascagni, Vincenz Fohmann (1794–1837) used mercury to inject the smallest lymphatic vessels, as reported in his main works: *Anatomische Untersuchungen über die Verbindungen der Saugadern mit den Venen* and *Das Saugadersystem der Wirbelthiere*. His preparations are preserved in museums of Heidelberg and Liège (Dobson & Tompsett, 1968). In 1795 Samuel Thomas von Sömmerring (1755–1830) also treated the pathological features of lymphatics in his work *De morbis vasorum absorbentium corporis humani*.

Another contributor to the understanding of the anatomy of the lymphatic system was the French anatomist Marie Philibert Constant Sappey (1810–1896), who published his work in *Anatomie, physiologie, pathologie des vaisseaux lymphatiques considerées chez l'homme et les vertébres* (1874). He was able to count the valves in the lymphatic vessels (Browse, 2003; Fanous et al. 2007).

In 1858, Carl Friedrich Wilhelm Ludwig (1816–1895) hypothesized that lymph was a blood filtrate deriving from the capillary wall by intracapillary pressure. Rudolf Peter Heinrich Heidenhain (1834–1897) had different opinions with respect to Ludwig on different arguments, and in 1891 proposed that lymph was an active secretion by the lymphatic endothelium. Finally, Ernest Henry Starling (1866–1927) showed that lymph is due to the forces governing fluid movement across the capillary wall (Fanous et al. 2007).

The Romanian anatomist Dimitru Gerota (1867–1939) replaced mercury injection with a mixture of Prussian blue oil paint in turpentine oil and ether using a fine glass tube, but this method had limited applications. In recent times, the use of neoprene latex injections improved the preparation of museum specimens of the lymphatics. Latex is the ideal material for injecting lymphatics as it flows without risk of rupturing the vessels and after a few minutes hardens into a solid elastic material (Dobson & Tompsett, 1968). The British surgeon John Bernard Kinmonth (1916–1982) realized lymphangiography for the radiological demonstration of the lymphatic system, until the more recent indocyanine green fluorescence lymphography and lymphoscintigraphy techniques, and the coinage by Ramon Cabanas in 1977 of the term 'sentinel lymph node', which describes the first lymph node group that receives lymphatic drainage from the tumor (Suami, 2017).

Leonetto Comparini (1924–1999; Fig. 5), who directed the Institute of Human Anatomy of the University of Siena, investigated the structure of human lymphatic collector walls in superficial regions (Comparini, 1958). In 1962, he studied the human limb precollectors and demonstrated that the precollectors arise from the absorbing lymphatic network and drain into prenodal collectors. They follow a tortuous path and vary in diameter. The valves are disposed in an irregular manner, and long portions without valves may alternate with short portions with two or three closely spaced valves. The muscular elements are grouped in columns that encroach on the lumen to different degrees and are disposed in a helical arrangement (Comparini, 1962).

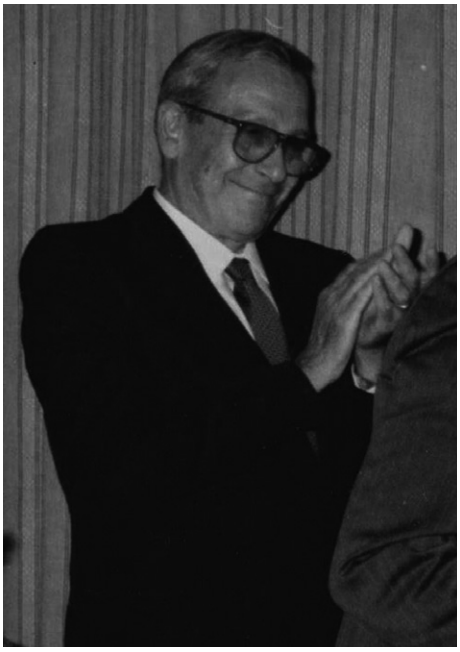

Fig. 5 Prof. Leonetto Comparini. Photo kindly provided by Prof. Elisabetta Weber (University of Siena).

The works contributing to the knowledge on lymphatic vessels of the human liver were extremely original, with particular attention to their three-dimensional graphic reconstructions by means of histological serial sections with the aim of carrying out a detailed study of morphology of lymphatics and their relationships with the arterial and venous vessels, and biliary ducts (Comparini & Bastianini, 1965; Comparini, 1969).

More recently, Comparini and co-workers performed reconstructions of superficial lymphatic precollectors of the lower limb by computer. They demonstrated the morphological and structural variety of these vessels, showing the structural simplicity of the absorbing lymphatics from which they are derived in some places, and the structure of the collectors in others. The lymphatic precollector is characterized by irregularity of calibre due to restricted portions, the singular arrangement of the muscular component, and the irregular, but constant presence of valves, which are not disposed at regular intervals as in a collector. The muscle cells are initially isolated and later confluent, and are disposed in a helix arrangement around the vessel. Brief collecting vessels flow in the precollectors and before their point of entry; these vessels acquire a thin and discontinuous muscle layer. The lumen profile is tortuous and irregular, like that of absorbing lymphatics (Comparini et al. 1996).

Furthermore, Comparini demonstrated that human thigh precollectors are characterized by the alternation of portions with a well-developed muscular coat and portions with an absorbing structure (Sacchi et al. 1997). These morphological features suggest that the precollectors contribute to fluid absorption and lymph propulsion. The frequent myoendothelial contacts suggest that smooth muscle contraction is regulated locally.

As previously stated, the lymphatic vessels have been studied for a long time, but because of the challenges of their visualization and the lack of available molecular tools that specifically recognize them, no precise information had been collected until the late 1990s about the molecular basis of lymphangiogenesis and the development of therapeutic strategies to treat disorders where these vessels are involved. The visualization of lymphatic endothelial cells (LECs) was deeply transformed by the identification of lymphatic-specific biomarkers such as the vascular endothelial growth factor receptor (VEGFR)-3 (Kukk et al. 1996) the prospero homeobox 1 (PROX1) transcription factor (Wigle & Oliver, 1999), the integral membrane glycoprotein podoplanin (Breiteneder-Geleff et al. 1999) and the lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1; Banerji et al. 1999).

One of the key figures of this lymphatic revolution and 'renaissance' is undoubtedly the Finnish researcher Kari Kustaa Alitalo (1952–present time), who made, together with his group and with the collaboration of other international teams, the most significant discoveries concerning the growth factor/receptor system that controls the development of lymphatic vessels and the lymphatic metastasis of tumors. He firstly discovered the lymphatic growth factor receptor, VEGFR-3, and then isolated its first ligand VEGF-C (Aprelikova et al. 1992; Joukov et al. 1997a,b), greatly contributing to the characterization of another ligand called VEGF-D. Moreover, he started studies showing that lymphangiogenic factors in tumors greatly enhance tumor metastasis (Mandriota et al. 2001). Indeed, the development of a VEGFR-3 signaling inhibitor determined the suppression of tumor lymphangiogenesis and metastasis to regional lymph nodes (Karpanen et al. 2001; He et al. 2002). Among the discovered inhibitors of lymphangiogenesis was also found a soluble form of VEGFR-3 that was revealed to be a potent inhibitor of VEGF-C/VEGF-D signaling and, consequently of the new formation of lymphatic vessels (Mäkinen et al. 2001). The role of VEGFR-3 in human diseases was also highlighted by the findings on the heterozygous missense mutations of this receptor that inactivate the tyrosine kinase domain in primary lymphedema (Karkkainen et al. 2000). In 2007, Tammela and colleagues demonstrated, for the first time, that lymphatic vessels can differentiate from lymphatic capillaries in adults and that VEGF-C gene therapy can induce this key process (Tammela et al. 2007).

Although studies of the previous century have considered the lymphatic vasculature as a passive transit system, recently the lymphatic system has been involved also in unexpected pathological processes, such as the transition from benign to highly dysplastic phenotype of colon tumors through the activity of the transcription factor and lymphatic marker PROX1 (Petrova et al. 2008), or in physiological activities, such as the intraocular pressure regulation (Thomson et al. 2014) and the induction of peripheral tolerance via Aire-independent direct antigen presentation by lymph nodes LEC (Cohen et al. 2010).

All these important findings have opened previously unthinkable perspectives on both basic and clinical research on the lymphatic vasculature and its fundamental role in a wide range of human diseases. Furthermore, the research of the last decade paved the way to future therapeutic developments such as the lymphangiogenic therapy that could be beneficial in the treatment of cardiovascular diseases (Vuorio et al. 2017), or the synthesis of new drugs (Heckman et al. 2008; Li et al. 2015) or antibodies (Jian et al. 2015) that inhibit lymphangiogenesis in tumors.

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Author contributions

All the authors contributed to the writing of this review.

Conflict of interest

The authors have no conflict of interest to declare.

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