# Lymphatic Network in Cancer and Some Chemical Observations

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*The lymphatic system, also known as the forgotten circulation has become a topic of high interest in the last years since it was described as a key player in cancer development and metastasis. The aim of the present study was to offer a survey of the literature concerning the anatomy of the lymphatic system in normal conditions, in health, versus the changes undergone in tumors microenvironment. In addition, were discussed the transformations suffered by the lymphatic system molecular markers that form the molecular signature of this system in health and in disease.*

*Keywords: lymphatic system, cancer, molecular markers, lymphangiogenesis.*

The term cancer is used to make mention of a disease entity defined as *an abnormal growth of cells that could be caused by multiple changes in gene expression leading to an errant balance between cell proliferation and cell death, and ultimately evolving into a population of cells that can invade tissues and metastasize to distant sites, causing significant morbidity, and, if untreated, death of the host* [1].

In order to find an effective treatment for the cure of cancer, a considerable number of studies were focused on the elucidation of the mechanism involved in the development of this malady and, also in the discovery and apprehension of all the factors that play key roles in this fatal process.

In the last decade, the interest regarding the lymphatic system has been increased mainly since it was described as an active player in the dissemination of the metastatic cancer cells and the development of metastasis. It has been reported that in the case of breast cancer and melanoma, the two types of cancer with a high incidence worldwide, the metastasis occurs via the lymphatic system.The elucidation of the structure of lymphatic system and its role in the process of tumorigenesis and metastasis is mandatory in order to conceive the mechanisms involved and to develop new effective anticancer treatments.

This review aims to present the survey of the literature regarding the characterization of the lymphatic system in health and disease and it also focuses on the main markers that constitute the molecular signature of the lymphatics.

## *The lymphatic system: a historical and anatomical characterization*

The first reference to lymphatic system was made by Hippocrates who named it the *white blood* [2]. Howbeit, the interest regarding the functions and the roles of this system remained elusive for many centuries due to the lack of knowledge concerning the lymphatic-specific molecular markers involved in the regulation of lymphatic development and function [3].

The period between 1627, which marked the moment of the first discovery oflymphatic vessels, the *milky veins*, by Gasparo Aselli, an Italian anatomist, and 1902 can be associated with the identification of the collecting lymphatics, the thoracic duct, and of the draining function of the lymphatic system, whereas the questions concerning the genesis of the lymphatic system during embryogenesis remained unanswered [3].

A step forward in this direction was noted at the beginning of the twentieth century, when two competing hypotheses: the *centrifugal model* and the *centripetal model* were proposed.

The *centrifugal model* endorsed the blood vascular origin of lymphatics: the lymphatic system is differentiated from the embryonic blood vascular system during early development and the primitive lymphatics afterwards disseminate throughout the body to form the lymphatic networks, whereas the *centripetal model* asserted that lymphatic endothelial cells (LEC) are originated from mesenchymal cell–derived lymphangioblasts. In addition, the lymphatic endothelial cells form the primitive lymphatic plexuses which only later will be connected to the embryonic veins [3, 4].

A pleader of the *centrifugal model* was Florence Rena Sabin (1902), an American anatomist and medical researcher, who demonstrated in ink-injection experiments developed on pigs that the primary lymph sacs are derived from embryonic veins [3-5].

The Huntington's and McClure's findings concerning the origin of the lymphatic system (the first lymphatics vessels arise independently in the mesenchyme) obtained in an experiment realized in domestic cats validated the *centripetal model* [3, 4, 6].

The debate about the possible origin of the lymphatic system lasted another one hundred years until Wiggle and Oliver (1999) obtained a deficient in lymphatic vascular system mouse model by down-regulating the homeo-domain protein Prox1 expression [7, 8], a protein that plays a major role in the migration of the venous endothelial cells

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and in the formation of the primary lymphatic vessels during early embryogenesis [3, 4]. These data support the centrifugal model and offers new insights into the lymphatic research [3, 4].

From an anatomical point of view, the lymphatic system also known as the second vascular system, is a complex open-ended network that comprises lymphatic capillaries, initial lymphatic vessels, collecting lymphatic vessels, the thoracic duct and also the lymphoid organs: lymph nodes, thymus, tonsils, spleen and Peyer's patches [3, 9-15]. It was reported that the lymphatic vessels arise very early both in humans (in utero at week 6-7) and in mice (embryonic day E9.5-10.5) after the development of the cardiovascular system [12, 16]. The origin of the lymphatic system is venous, more specifically it originates from a group of lymphatic endothelial cells (LEC), which are blood-vascular endothelial cells that gained lymphatic identity. These cells have the ability to migrate from the anterior cardinal veins and to form lymph sacs, the units responsible for the formation of the entire lymphatic vascular tree via lymphangiogenesis [12, 16]. These data support the theory of Florence Sabin concerning the venous origin of the lymphatic system, the *centrifugal model.*

**Table 1**

THE LYMPHATIC SYSTEM MOLECULAR MARKERS EXPRESSED IN HEALTH AND IN CANCER

| Molecular Markers | Description | Expresion in health conditions | Expresion in pathological conditions |
|---|---|---|---|
| **Prox1** | - is a homeodomain protein and a transcription factor and was firstly identified as Drosophila protein prospero [19].<br>- it has also been detected in zebrafish, frog, mouse, chicken and humans, and possesses the capacity to maintain the amino acid sequences throughou the species [3, 18]<br>- the capacity of Prox 1 to induce cellular differentiation is cell-specific [7]. | - this marker is expressed in developing liver, nervous system, heart and pancreas and it plays a key role in the cell fate decisions involved to the anchorage of various cell lineages during embryogenesis [20].<br>- the differentiated blood vascular endothelial cells (BEC) expressed Prox 1 what determined the transition of these cells to a lymphatic phenotype [3] | - Prox1 expression is up-regulated in differentiated blood endothelial cells (BEC) infected with Kaposi's sarcoma and herpes virus [3] |
| **VEGFR-2 – KDR or Flk1** | - it is known as a receptor specific for VEGF-A, VEGF-C, and VEGF-D [21]<br>- the function of this receptor in angiogenesis is well characterized, whereas its involvement in lymphangiogenesis in not elucidated at this moment [3, 4] | - the lymphatic endothelial cells and the blood vascular cells in situ and in vitro express this type of marker [3, 21]. | |
| **VEGFR-3 - Flt4** | - is described in the literature as a member of the VEGF receptor family and was the first gene that was specifically expressed in the lymphatic endothelial cells [22]<br>- it is involved in the signaling pathway of the lymphatic-specific growth factors VEGF-C and VEGF-D<br>- it was the first molecular marker with specificity for the | - in normal conditions VEGFR-3 is expressed during embryonic development in the majority of the vascular endothelial cells whereas at later times its expression is resumed only to the lymphatic plexuses [22]<br>- previous studies | - previous studies indicate that cells of benign and malignant vascular tumors possess an up-regulated expression of VEGFR-3 what leads to the idea of a proliferative vascular phenotype<br>- VEGFR-3 is expressed in |

continuated table 1

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| | lymphatics and attracted considerable attention in the field of lymphatic research.<br>- in addition, it was demonstrated that in transgenic mice with skin-specific overexpression of a VEGFR-3–specific mutant of VEGF-C (VEGFC156S) the activation of this marker leads to promotion of lymphangiogenesis [23]. | showed that genetic deletion of VEGFR-3 was associated with a defective blood vessel development characterized by an abnormal vessels structure and fluid accumulation in the pericardial cavity at mouse embryonic day 9.5 (E9.5), which marks the beginning of the lymphatic development [3] | the endothelia of lymphangioma and it represents a useful marker for the diagnostic of this type of cancer [9]<br>- furthermore, it was demonstrated that tumor-associated and wound-associated blood vessels express VEGFR-3 [3, 4] |
| **LYVE-1** | - it is known as a lymphatic endothelium-specific hyaluronan (HA) receptor.<br>- this marker presents some structural similarities with CD44, a specific marker of blood vascular endothelium [3]<br>- it is considered an important lymphatic specific marker together with Prox1 | - it hasn't been detected in the blood vascular endothelial cells<br>- its expression was also observed in other cells including: activated tissue macrophages and sinusoidal endothelium of the liver and the spleen [24]. | |
| **Angiopoietin-2 – Ang2** | - is a ligand for the endothelial cell-specific tyrosine kinase receptor [25]<br>- in a study developed on mice with Ang2 knockout there was noticed damage of the lymphatic system [26] | - this marker hasn't specificity only on lymphatics, being also detected in smooth muscle cells of large arteries, large veins, and venules, [27]. | |
| **Podoplanin** | - is a mucin-type transmembrane glycoprotein and was one of the first lymphatic markers identified in the literature<br>- it was demonstrated | - podoplanin was detected in lymphatic, but not in blood vascular endothelial cells in vivo and in vitro [28]. | |

The lymphatic endothelial cells (LEC) descend from blood progenitor cells and present characteristic molecular markers that differentiate these cells from the blood-vascular endothelial cells, including: vascular endothelial growth receptor-3 (VEGFR-3, also known as Flt-4), the Prospero homeobox-1 fate determining transcription factor, Prox-1, lymphatic vascular endothelial hyaluronan (LYVE-1),podoplanin and other lymphatic-specific chemokines [11, 17].

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|---|---|---|---|
| | that plays an important role in LEC migration, adhesion and tube formation [28]. | - moreover, this marker is expressed in other cell types, like: type I alveolar cells of the lung, cells of the choroid plexus, ciliary epithelial cells of the eye, osteocytes and kidney podocytes [3] | |
| **CCL21 - SLC, Exodus-2, or 6Ckine** | - plays an important role in immunoregulatory and inflammatory processes.<br>-another role of this marker is to promote adhesion and to stimulate the migration of thymocytes, T-lymphocytes, macrophages, and neutrophils [3, 4] | - previous studies indicate its expression in the lymphatic endothelium, but is absent in the blood vascular endothelium, of various organs.<br>- it is also observed in the high endothelial venules and the T-cell areas of lymph nodes and Peyer's patches [3]. | - there are data which sustain the fact that CCR7 is as highly expressed in several breast cancer and malignant melanoma cell lines; metastatic melanoma cell lines that express [4]. |
| **Hepatocyte growth factor (HGF)** | - it is also known as scatter factor and it proved to be a potent lymphangiogenesis factor [29]<br>- the main roles of this factor are: to induce proliferation, migration, and tube formation of LECs via its receptor HGF-R [10] | | |

In table 1 are presented the main molecular markers expressed in lymphatics described in the literature.

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