

Role of lymphangiogenesis in lung cancer

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Abstract: Lung cancer represents one of the most frequent causes of death due to neoplastic disease in Poland and around the world. The high mortality which accompany neoplastic diseases used to be ascribed mainly to dissemination of cancerous cells. Studies on animal models suggest that tumour lymphangiogenesis represents the principal factor in the process of metastases formation. Lymphangiogenesis involves a process of formation of new lymphatic vessels from already existing lymphatic capillaries. Lymphangiogenesis is stimulated by vascular endothelial growth factors (VEGF) and other, recently reported factors, such as, *e.g.*, cyclooxygenase 2, fibroblast growth factor 2, angiopoietin-1 and the insulin-resembling growth factor. In lymphangiogenesis a key role is played by neuropilin 2 or podoplanin and this promoted development of studies on lymphangiogenesis. Activation of VEGF-C/VEGF-D/VEGFR-3 axis increases motility and invasiveness of neoplastic cells, promotes development of metastases in several types of tumours such as, *e.g.*, lung cancer, mammary carcinoma, cancers of the neck, prostate and large intestine. In recent years lymphangiogenesis provided topic of many studies. A positive correlation was detected between expressions of VEGF-C/D and VEGFR-3 in non-small cell lung cancer. In patients with lung cancer with high expression of VEGF-C a markedly abbreviated survival was noted. Positive correlation was detected between expression of VEGF-C and VEGF-D on one hand and expression of LYVE-1 on the other in sentinel lymph nodes with metastases of neoplastic cells in patients with non-small cell lung cancer. Also, high density of lymphatic vessels and high density of intraneoplastic microvessels proved to be independent poor prognostic indices in patients with non-small cell lung cancer. Extensive hope is linked to studies on inhibitors of lymphangiogenesis, which may improve results of treatment also in tumour patients.

Key words: lung cancer, lymphangiogenesis, vascular endothelial growth factors, markers of lymphatic vessels

Introduction

Lung cancer represents the most frequent cause of death due to a neoplastic disease, the most frequent malignant tumour in the world and in Poland the most frequent malignant tumour in males [1-3]. The high mortality which accompanies neoplastic disease is ascribed mainly to the spread of cancerous cells [4,5]. Role of angiogenesis in development of neoplastic metastases has been relatively well recognised while the role of lymphangiogenesis in the process remains incompletely clarified [6,7]. Studies on animal models suggest that tumour lymphangiogenesis plays leading role in the metastatic process [8].

Structure of lymphatic vessels promotes dissemination of tumour cells. They represent thin-walled, low pressure vessels, which transport intercellular fluid, proteins, lymphocytes and antigen-presenting cells to lymph nodes and to circulation. On the other hand, blood vessels work under higher internal pressures and their wall is thicker [9]. Neoplastic cells which penetrate lymphatic vessels may be passed to the circulation by the thoracic duct or by side anastomoses (leaks), probably existing in the tumour structure between blood and lymphatic systems [10].

Role of lymphangiogenesis, probably responsible also for metastases in a neoplastic disease has provided topic of few reports in the recent decade. This reflected, among other, the absence of specific markers which might specifically identify lymphatic vessels.

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Structure and function of lymphatic system

The lymphatic system was described for the first time by Gasparo Aselli in 1627. In 1902, Florence Sabin advanced a theory related to formation of lymphatic system during embryogenesis. According to the theory, peripheral lymphatic system developed from primitive lymphatic vesicles, arising due to budding of venous endothelial cells. The report of 2002, published by Jussila and Alitalo, confirmed the theory of Sabin [11]. The lymphatic system consists of thin-walled, low pressure vessels, lymph nodes localised along the vessels, lymphoid organs such as the spleen and the thymus and of circulating lymphocytes [12]. Due to the control of intercellular fluid transport, the lymphatic system maintains an appropriate plasma volume, prevents against pressure build-up in tissues and plays a significant role in the immune defence system of the body [13].

Lymphatic vessels differ in their function and structure from those of blood vessels. Walls of lymphatic vessels are thin, cytoplasm of endothelial cells is less dense, basement membrane is discontinuous, which increases permeability of the vessels. Lumen of lymphatic vessels shows a three-fold higher diameter than lumen of small blood vessels, shape of lymphatic vessels is less regular and the vessels may be occluded (frequently collapsed) [14]. The wall of lymphatic vessels is joined to the extracellular matrix by reticular and collagen fibres. With an increasing inflow of intercellular fluid and in conditions of an increased pressure the connective tissue fibres stretch to open vascular lumen [15]. Lymph flow is facilitated by contractions of smooth muscles which surround the vessels and a direction of flow is enforced by the system of valves in lymphatic vessels [14]. Destruction or a disturbed continuity of lymphatic vessels by infectious, mechanical agents, obliteration, irradiation, or surgical procedures lead to oedema in the lymphatic system and to increased pressure in the surrounding tissues [16].

Markers of lymphatic vessels

Lymphangiogenesis involves a process of new lymphatic vessel formation from the already existing lymphatic capillaries [17]. Progress in studies on lymphangiogenesis was hampered due to the lack of specific markers of lymphatic system. In the past, imaging of lymphatic vessels was restricted to techniques linked to administration of specific dyes to the lymphatic system. The dyes used at present, *e.g.* Evans blue or trypan blue are much better tolerated than those used in the past [14]. The dyes and rhodamine dextran are routinely applied in studies on animals [18]. Until now, immunohistochemical identification of lymphatic vessels was possible due to comparison of expression of endothelial markers with those of basement

membrane. For example, CD31, the expression of which is observed in blood and lymphatic vessels was used in combination with markers of basement membrane and collagen type IV [19]. The vessels which were labelled with CD31-specific antibodies but without expression of basement membrane proteins and containing no erythrocytes in their lumen, were taken as representing lymphatic vessels [20]. Use of a specific marker of blood vessels, PAL-E, in combination with PECAM-1 (platelet/endothelial cell adhesion molecule-1), was also helpful in identification of lymphatic vessels [21].

It was not until the recent few years that more numerous reports on lymphangiogenesis appeared. This was linked to distinguishing specific proteins which manifest expression in lymphatic vessels and to discovery of factors which control and stimulate growth of lymphatic vessels [22].

The recently discovered receptor for hyaluronic acid, located in endothelium of lymphatic vessels, LYVE-1 (lymphatic endothelium hyaluronate receptor) represents an important marker of lymphatic vessels [23] (Fig. 1). LYVE-1 plays a key role not only in metabolism, binding and transport of hyaluronic acid from tissues to lymphatic vessels but also in transplacement of leukocytes in lymphatic vessels and lymph nodes [24]. LYVE-1 in 41% manifests a structure homologous to CD44 molecule, the expression of which is accompanied by an elevated metastatic potential of tumour cells [25]. Expression of LYVE-1 is observed also in hepatic sinuses and in splenic endothelium [24,26,27]. Until now, LYVE-1 has not been detected in any non-sinusoidal vascular endothelium except of blood vessels in lungs [28] and in lymph nodes [27]. Expression of LYVE-1 in lymph nodes is not restricted to lymphatic vessels and sinuses but it is also present in high endothelium venules (HEV) [27]. Cells of the endothelium exhibit a cuboid or cylindrical shape, they are high and typically protrude to the lumen of the vessel. In this respect they are basically different from endothelial cells in other blood vessels [29,30]. Moreover, HEV represent the site in which lymphocytes emigrate from peripheral blood [31].

Another marker of lymphatic vessels involves the human transcription factor, Prox-1. It was identified for the first time in 1999 as a molecule which undergoes expression in cells of lymphatic endothelium. Prox-1 is involved in growth and elongation of lymphatic capillaries [32].

Another protein which is expressed in a mature lymphatic endothelium is neuropilin 2 (NRP2). The protein is a receptor which binds VEGF-C (vascular endothelial growth factor) and also plays role of a co-receptor for VEGFR-3 (the receptor for VEGF) [33].

An additional protein which is typical for lymphatic endothelium involves podoplanin, belonging

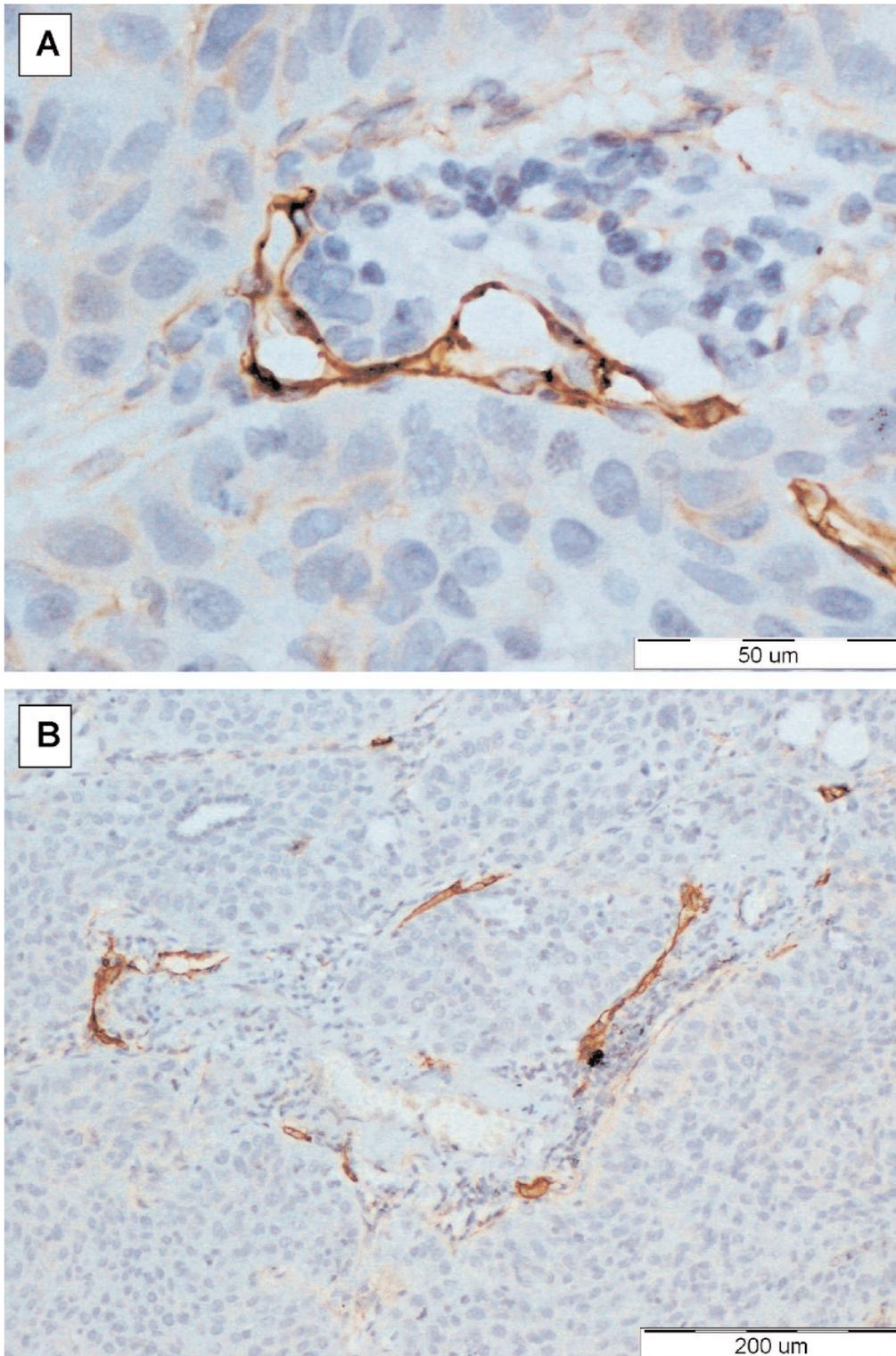


Fig. 1. Expression of LYVE-1 (brown) in lymphatic vessels in non-small cell lung cancer. A – magnification $\times 400$; B – magnification $\times 100$.

to the group of mucoproteins, the expression of which in endothelial cells of lymphatic vessels is controlled by Prox-1 [34]. Recently conducted studies indicate that podoplanin may participate in adhesion and migration of lymphatic endothelium cells [35].

Characterization of the proteins resulted not only in a design of novel techniques of identifying lymphatic vessels but permitted also to suggest a new approach to a lymphangiogenesis-modifying therapy of diseases associated with an abnormal structure and/or function

of lymphatic vessels [36]. Currently, a great challenge is linked to definition of the detailed role played by LYVE-1 and other proteins engaged in immunological function of lymphoid system and recognition of the ways in which tumour cells take advantage of lymphatic vessels for development of metastases and, thus, for progression of the tumour.

Factors which stimulate lymphangiogenesis

Vascular endothelial growth factors (VEGF) are glycoproteins, the family of which encompasses VEGF-A, VEGF-B, VEGF-C, VEGF -D, VEGF- E and Placenta Growth Factor (PIGF) [37].

The vascular endothelial growth factor (VEGF), earlier termed the vascular permeability factor (VPF), capable of increasing vascular permeability, was discovered in 1989 and plays an important role in control of the of angiogenesis process [38, 39]. All proteins of the VEGF family stimulate cell-mediated immunity by binding to their receptors (VEGFR), located on the surface of endothelial cells. VEGFR receptors consist each of the extracellular, transmembrane and intracellular domain, the latter manifesting activity of tyrosine kinase [40].

VEGF-A encompasses 10 isoforms: VEGF-A₁₂₁, VEGF-A₁₃₈, VEGF-A₁₄₅, VEGF-A₁₄₈, VEGF-A₁₆₂, VEGF-A_{162b}, VEGF-A₁₆₅, VEGF-A₁₈₃, VEGF-A₁₈₉ and VEGF-A₂₀₆. It is synthesized by various cell types, including mast cells, smooth muscle cells of vascular walls, macrophages, fibroblasts, neoplastic cells, cells of endothelium, monocytes, keratinocytes, eosinophiles and lymphocytes T [41]. VEGF-A plays a key role in the process of angiogenesis [42].

VEGF-B in 88% resembles the structure of VEGF-A and also participates in processes of angiogenesis and neurogenesis [43-46].

VEGF-C is indispensable in embryonal development in the process of formation of lymphatic vessels [47]. It manifests a mitogenic and a protective role related to both lymphatic and blood vessels [48]. A pronounced expression of VEGF-C is noted in the heart, placenta, muscles, ovaries, intestines and in certain tumours [49]. The factor is responsible also for increases in permeability and diameter of lymphatic vessels [50]. It seems to be of key importance in the process of lymphangiogenesis [51].

VEGF-D is expressed in the lungs, skin, heart, skeletal muscles, alimentary tract and in certain tumours [52]. It stimulates growth and migration of lymphatic endothelium cells [53]. Similarly to VEGF-C, VEGF-D participates also in the process of lymphangiogenesis [54].

VEGF-E, which till now has been located only in orf viruses, may be involved in the process of angiogenesis [55]. The orf virus, termed also the virus of ovine small-

pox, belongs to the family of *Poxviridae*. The family encompasses large and structurally complex DNA viruses with a strong immunogenic potential.

The placenta growth factor (PIGF) fulfils an important role in both physiological and pathological angiogenesis. Similarly to VEGF it stimulates proliferation of endothelial cells. Originally, the factor was isolated for the first time from human placenta but later it was identified also in various tumours [56].

VEGFR-3 (Vascular Endothelial Growth Factor Receptor 3) proved to be the first specific receptor protein detected in lymphatic endothelium [57]. It fulfils the role of a receptor for lymphatic vessel endothelial growth factors VEGF-C and VEGF-D, belonging to the IIIrd class of tyrosine receptors [53]. Activation of VEGFR-3 by its ligands, VEGF-C and VEGF-D as well as the subsequent activation of its intracellular domain of tyrosine kinase stimulates proliferation of lymphatic endothelial cells [8]. Activity of lymphangiogenesis taking advantage of the VEGFR-3/VEGF-C/VEGF-D axis was demonstrated in numerous investigations both *in vivo* and *in vitro* [58]. On the other hand, VEGFR-1 and VEGFR-2 receptors are characteristic for endothelium of blood vessels. The VEGFR-2 receptor serves as the most important transmitter of signals in the process of angiogenesis but its presence was demonstrated also in lymphatic cisternae and lymphatic capillaries in the course of lymphangiogenesis [18]. The interaction between endothelial growth factors and VEGFR receptors have been presented by several authors [11, 59, 60]. In our review we would like to suggest a simple scheme of the above described relationships (Fig. 2).

Lymphangiogenesis in malignant tumours

The high mortality associated with neoplastic diseases used to ascribed, first of all, to spread in the body of neoplastic cells in the form of metastases. Clinical and anatomopathological reports confirm that metastases by lymphatic vessels to sentinel (regional) lymph nodes represent the early manifestation of the disease spread in cases of many solid tumours in humans [61].

Presence of neoplastic cells in the draining lymph nodes represents a significant index in evaluation of tumour advancement and in selection of the appropriate surgery, chemotherapy and radiotherapy. As mentioned earlier, the discovery of lymphatic vessel markers promoted development of studies using experimental models which allow for isolation of lymphatic endothelial cells. In recent years, increasing numbers of reports have appeared related to the role of lymphangiogenesis in mammary cancer, cancers of pancreas, prostate, stomach, in melanoma and pulmonary tumours as well as to growth factors specific for the processes [62-67].

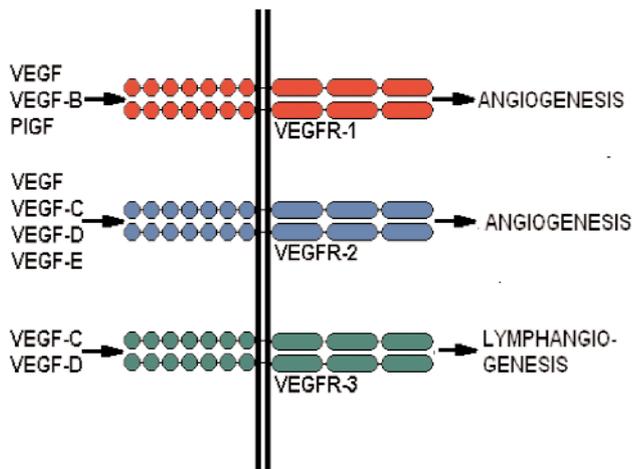


Fig. 2. Interactions between vasculoendothelial growth factors and their VEGFR receptors (60) in modification of our own. VEGF (VEGF-A) binds to VEGFR-1 and VEGFR-2. PlGF and VEGF-B are specific ligands for VEGFR-1. VEGF-E activates VEGFR-2. VEGF-C and VEGF-D interact with VEGFR-2 and VEGFR-3.

Dissemination of neoplastic cells from the primary tumour may take place by invasion of the local tissues, by the networks of lymphatic and blood vessels and by dissemination within body cavities. The most common method involves development of metastases due to spread of neoplastic cells with the circulating blood and within lymphatic vessels [67]. Schematic presentation of spread mechanisms and development of metastases from the tumour cells is shown in Fig. 3.

In 2005, He *et al.* [68] investigated the manner in which neoplastic cells enter lymphatic vessels and may form neoplastic metastases. The current concepts related to the mechanisms of metastasis formation are illustrated in Fig. 4A (in our own modification of the concept given in [67]). The neoplastic cells and macrophages which surround the tumour release VEGF-C and VEGF-D [8]. Activation of VEGFR-3 receptor by its ligands, VEGF-C and VEGF-D, leads to activation of its intracellular domain of tyrosine kinase, which stimulates proliferation and migration of lymphatic endothelial cells [69]. The mechanisms which promote the invasion of lymphatic vessels by neoplastic cells may include intra- and perineoplastic lymphangiogenesis as well as interactions between neoplastic cells and lymphatic endothelial cells (LEC). They result in an increase in penetrability of lymphatic vessels, altered adhesive properties of vascular endothelial cells, in this way facilitating development of metastases in lymph nodes [70].

The potential for inhibition of metastatic colonization of lymph nodes by tumour cells by inhibition of the VEGF-C/VEGF-D/ VEGFR-3 axis is shown in Fig. 4B. However, investigations of He *et al.* [68] demonstrated that inhibition of the VEGF-C/VEGF-D/

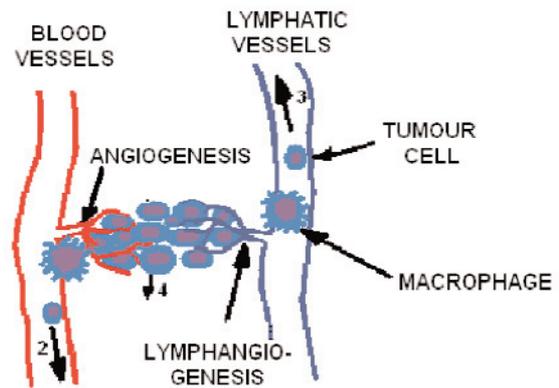


Fig. 3. Development of metastases (1) in continuity, in surrounding tissues, (2) by blood vessels, (3) by lymphatic vessels, (4) dissemination in body cavities (60, in modification of our own).

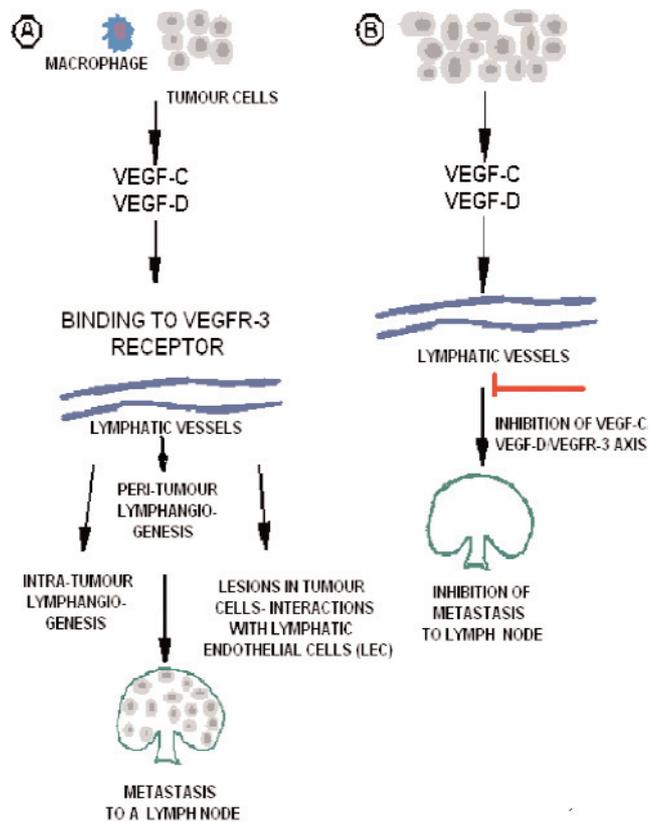


Fig. 4. A. Current concepts related to mechanisms in which neoplastic metastases develop using VEGFR-3/VEGF-C/VEGF-D axis. B. Potential for inhibition of metastases to lymph nodes by blocking VEGFR-3/VEGF-C/VEGF-D axis (67; in modification of our own).

VEGFR-3 axis was not sufficient to block development of metastases and the tumour cells still were present in the lymph nodes. Therefore, it seemed purposeful to inhibit lymphangiogenesis in parallel to blocking of the direct invasion of neoplastic cells from

the primary tumour [68]. Activation of the VEGF-C/VEGF-D/VEGFR-3 axis increases motility and invasiveness of neoplastic cells and in this way promotes development of neoplastic metastases in several types of tumours, *e.g.*, in lung adenocarcinoma, cancers of mammary gland, neck, prostate and large intestine [71]. Most of the reports documented positive correlation noted between overexpression of VEGF-C and VEGF-D on one hand and invasion of lymphatic vessels, involvement of lymph nodes, development of distant metastases and an unfavourable clinical course on the other in various histological types of malignant tumours [72].

Also other lymphangiogenesis-stimulating factors were described as factors which negatively affect the course and prognosis in neoplastic diseases [65].

Cyclooxygenase 2 (COX-2) and its overexpression are associated with an unfavourable course of gastric as well as pulmonary adenocarcinoma and, in parallel, they stimulate lymphangiogenesis [73,74]. Also VEGF-C and COX-2 have been found to exhibit positive correlation with density of lymphatic microvessels and the extent of invasion of lymphatic vessels by neoplastic cells in gastric cancer [75].

Fibroblast growth factor 2 (FGF-2) induces both angiogenesis and lymphangiogenesis through the control of VEGF-C and VEGF-D expression, which has been confirmed in animal experiments [76].

Angiopoetin-1 and insulin-like growth factor (IGF) represent mediators in the action of VEGF-C/VEGF-D/VEGFR-3 axis [77,78].

Even if VEGF-A plays a significant role in angiogenesis, some investigations point also to effects of VEGF-A on induction of lymphangiogenesis directly through activation of VEGFR-2 and indirectly, inducing an augmented secretion of VEGF-C and VEGF-D by macrophages in vicinity of neoplastic cells [79]. In view of the investigations it seems that treatment of tumours might yield improved effects upon parallel administration of VEGF-A/VEGFR-2 mechanism blockers, *e.g.* Avastin, and inhibitors of VEGFR-3/VEGF-C/VEGF-D/ axis [57]. Recent studies on animal models, related to fibrosarcoma have demonstrated that platelet-derived growth factor (PDGF) induced neoplastic lymphangiogenesis and is responsible for development of intraneoplastic lymphatic vessels, which augments their potential to form intraneoplastic lymphatic vessels, thus stimulating their potential to develop metastases in lymph nodes [80]. In view of the above, extensive hope is linked to application of antagonists of platelet-derived growth factor, such as, *e.g.*, Gleevec in treatment of neoplastic diseases [75].

Other new studies have shown that hepatocyte growth factor (HGF) participates in lymphangiogenesis by stimulating growth of peritumoral lymphatics (PTLs), due to activation of VEGFR-3 receptor [81].

Other currently conducted investigations also have demonstrated that nitrogen oxide (NO) is produced and released by cells of lymphatic endothelium and, probably due to its effect on penetrability of lymphatic endothelium, leads to stimulation of one of the isoforms of nitrogen oxide synthase (iNOS). In turn, activity of nitrogen oxide synthase in a tumour has been found to manifest positive correlation with expression of VEGF-C and VEGF-D [82].

Lymphangiogenesis in lung cancer

Lung cancer represent one of the most frequent causes of death due to neoplasia. Despite the progress in diagnosis and treatment prognosis in patients with lung cancer remains poor. Among many prognostic indices in non-small cell lung cancer presence of metastases to lymph nodes remains to be of principal prognostic significance [83]. Also, few data are available on mediating mechanisms in development of metastases to lymph nodes in lung cancer. The role of lymphangiogenesis in development of the tumour type continues to be studied. Studies on animal models related to breast and renal carcinomas unequivocally indicate that an increase in the level of VEGF-C or VEGF-D induces lymphangiogenesis [51,84]. Studies from the recent few years have indicated a positive correlation between expressions of VEGF-C/D and VEGFR-3 in non-small cell lung cancer [85].

In lung adenocarcinoma increase in the level of mRNA for VEGF-C is indicative of metastases to lymph nodes [86]. Moreover, a significantly longer survival has been shown in patients with non-small cell lung cancer manifesting low expression of VEGF-C as compared to patients with high expression of VEGF-C [87].

In 2008 Kawai *et al.* [1] in patients with non-small cell lung cancer detected significant role of VEGF-A 121 isoform in promotion of lymphangiogenesis in sentinel lymph nodes situated most closely to the tumour. In order to evaluate lymphangiogenesis in the lymph nodes antibodies specific for LYVE-1 were used. In such patients, the authors detected a significantly elevated levels of LYVE-1 and VEGF-A 121 in sentinel lymph nodes as compared to the other lymph nodes. In the patients VEGF-A 121 was found to promote proliferation of lymphatic endothelium cells [1]. Moreover, the same authors detected a significant positive correlation between expression of VEGF-C and VEGF-D on one hand and expression of LYVE-1 expression in sentinel lymph nodes with neoplastic metastases. The relationship was absent from sentinel lymph nodes free of metastases [1].

Also in the year of 2008 Kadota *et al.* [88] evaluated lymphatic vessel density (LVD) and extent of invasion to lymphatic vessels (LVI) using antibodies specific for

D2-40 (an equivalent of podoplanin) in patients with various types of non-small cell lung cancer. In the same patients density of intra-tumour microvessels (MVD) was also evaluated. A significantly higher lymphatic vessel density (LVD) was detected in squamous cell carcinoma as compared to adenocarcinoma. Shorter survival of the patients was linked to high LVD and high MVD. Lymphangiogenesis was found to be correlated with a high LVD. The data indicated that LVD and MVD represent independent prognostic indices in patients with non-small cell lung cancer [88].

The till now obtained results allow to conclude that high expression of VEGF-C correlates with presence of metastases to lymph nodes and with poor prognosis in patients with non-small cell lung cancer. The poor prognosis in such patients is also linked to high density of lymphatic vessels and a high density of intra-tumour microvessels.

Extensive hopes are associated also with drugs which inhibit lymphangiogenesis. Hopefully, in future improved results of pulmonary carcinoma treatment will be obtained due to application of a combined chemotherapy, consisting of, *i.a.*, inhibitors of epidermal growth factor receptors and inhibitors of VEGFR-3 receptor [89].

Inhibition of lymphangiogenesis

VEGFR-3 for VEGF represented the first to be described specific receptor protein for lymphatic endothelium [57]. It plays role of a receptor for the lymphatic endothelium growth factors, VEGF-C and VEGF-D. Recent investigations on animal models unequivocally proved that an increased level of VEGF-C and/or VEGF-D promotes lymphangiogenesis in neoplastic diseases and a lymphatic vessel-mediated tumour spread to regional lymph nodes. The phenomenon may be inhibited by blocking VEGFR-3 receptor [92].

Application of a soluble form of the VEGFR-3 receptor in lung cancer reduces the amount of intra-tumour lymphatic vessels as well as decreases frequency of metastases to regional lymph nodes and to the lungs [69,92,93]. Inhibitors of VEGFR-2 receptor proved to function also as inhibitors of VEGFR-3 receptor, such as Surafenib (Nexavar, BAY 43-9006) [89] and Cediranib (Recentin, AZD2171) [90] studied on lung cancer, SU 11248, studied on renal carcinoma [94]. Cediranib, examined by Heckman in 2008, significantly inhibited both angiogenesis and lymphangiogenesis in experimental models with adenoviruses [91]. MAZ 51 inhibited growth of breast cancer in studies on animal models [95]. Promising results were also obtained in studies on a recombinant protein received from adenoviruses, which affected expression of the soluble form of VEGFR-3 receptor. The protein helped to inhibit metastases to lymph nodes and to

decrease the amount of peri-tumour lymphatic vessels in mice [51,96]. In studies on animals with tumours secreting recombinant VEGF-D, in which monoclonal antibodies to VEGF-D were used, which blocked ligand binding to both VEGFR-2 and VEGFR-3, the inhibited processes involved angiogenesis, lymphangiogenesis and spread of lymphatic vessel-mediated tumour metastases [84,97]. Also in recent years clinical studies succeeded in inhibiting growth of metastatic colonic and anal cancer due to application of Avastin preparation (Bevacizumab), the monoclonal antibody specific for VEGF (an inhibitor of angiogenesis) [98]. Duration of an average survival till progression of the disease and mean survival of the patients were found to be extended in the patients.

Summary

The problems which remain to be clarified include molecular mechanisms of lymphangiogenesis, specificity of growth factors, markers of lymphatic vessels and numerous phenomena responsible for tumour growth and its spread by lymphatic vessels. Recognition of angiogenesis and lymphangiogenesis and design of drugs which effectively inhibit the processes may significantly improve results of therapy in neoplastic diseases.

In recent years, lymphangiogenesis in pulmonary cancer constitutes a topic of intense studies. In the non-small cell lung cancer expressions of VEGF-C/D and of VEGFR-3 have been found to be positively correlated with each other.

A significantly abbreviated survival is noted in lung cancer patients with high expression of VEGF-C. Investigations conducted in 2008 have demonstrated positive correlation between expression of VEGF-C and VEGF-D on one hand and expression of LYVE-1 in sentinel lymph nodes with metastases of neoplastic cells on the other in patients with non-small cell lung cancer [1]. Also, high density of lymphatic vessels and a high density of intra-tumour microvessels have been demonstrated to represent independent unfavourable prognostic indices in patients with non-small cell lung cancer [88].

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