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Introduction

One of the main causes of cancer evasion is the ability of tumor cells to spread to local and distant tissues and organs. Therefore, for several decades, a major focus of cancer research was to understand the mechanisms which unlock the ability of tumor cells to form metastases. Many studies showed that the lymphatic development can be re-activated during tumor lymphangiogenesis and, as it was widely reviewed before, the tumor lymphangiogenesis strongly depends on vascular endothelial growth factor C/D (VEGF-C/D) and vascular endothelial growth factor receptor 3 (VEGFR-3) pathway. In lymphangiogenesis, the pivotal role is played by the SRY-related HMG-box (SOX18), which through activation of prospero homeobox 1 (Prox-1) transcription, induces expression of lymphatic-specific genes, e.g. podoplanin.

Recent research has been focused on uncovering the active role of lymphatic endothelium in tumor cells transport and modulation of antitumor immune response. The communication between immune system and tumor is initiated with blind-ended capillaries of afferent lymphatics that merge into larger collecting vessels and connect tissue with lymph nodes. This lymphatic link between tumor and the distant microenvironment is associated with flow of tumor antigens (Ags), cytokines and enzymes but is also crucial for the progression and dissemination of tumor cells. In face of the discovery of several key lymphatic-specific molecular markers, the number of studies on lymphatic biology has been recently augmented. Therefore, we review the role of lymphatic endothelial cells (LECs) in tumor progression and immunity as well as the requirements for tumor cells to enter initial afferent lymphatic vessels. We discuss the newest achievements in anti-lymphatic therapies underlying some innovative treatments such as photodynamic therapy (PDT).

Structure and function of lymphatic vessels

Normal tissue fluid homeostasis in the human body is dependent on mutual reinforcing functions of blood and lymph...
vessels system. In contrast to blood vessels responsible for delivery of oxygen, nutrients, hormones and cells to the body tissues, lymphatic vessels are specialized in uptake of tissue fluid with macromolecules, microbes and other substances from interstitial space.

The lymphatic vessels are present in tissues that frequently come in contact with foreign Ags, such as the skin and mucous membranes. Moreover, lymphatic vessels are found in all vascularized tissues, including recently discovered central nervous system lymphatic vasculature, with notable exception of bone marrow. Tissue fluid first enters lymphatic capillaries, comprised of SMCs organized in highly permeable single-layered, blind-ended and thin-walled sacs. LECs are attached to the extracellular matrix through elastic anchoring filaments. These fibrillin-stranded structures protect vessels from collapse and stretch under high-interstitial pressure. This leads to the opening of endothelial flaps, allowing fluid and macromolecules to enter the vessel’s lumen. The lymph is further transferred to larger pre-collector vessels that contain occasional valves, a basement membrane and sparse coverage of smooth muscle cells (SMCs). The pre-collectors converge into the lymphatic collecting vessels, with LECs forming continuous “zipper-like” junctions, surrounded by a basement membrane and supported by incessant layer of SMCs. The lymph flow is achieved by SMCs contractility, vasoemotion and the activity of surrounding skeletal muscles. The bileaflet valves in collecting vessels prevent the backflow of lymph. The collectors pass the lymph to the lymph nodes and further to lymphatic trunks and right lymphatic and thoracic ducts, where lymph is eventually drained back into the venous circulation at the venous angles. Therefore, the central function of lymphatic vascular system is to sustain tissue-fluid homeostasis. Additionally, lacteal lymphatic vessels absorb and transport fat soluble vitamins and dietary fat, such as chylomicrons, from the small intestine, bypassing the liver that normally clear hydrophilic substances collected by blood from the intestine. In addition, the lymphatic system is essential for proper functioning of the immune system. Its role is especially invaluable in the immune response as lymphatic vessels enable leukocytes trafficking and transport of Ags and antigen-presenting cells (APCs) from the intestine to the lymph nodes, where they communicate with naive lymphocytes. Until recently, the role of lymphatic endothelium in immune response modulation was underestimated. It was thought that LECs’ function was restricted only to the passive transport of immune cells and Ags. Conversely, it was shown that interstitial pressure and fluid flow can activate LECs, leading to increase of fluid and solute permeability, uptake and expression of adhesion molecules required for immune cell migration. Furthermore, current data suggest that LECs suppress dendritic cells (DCs) maturation and further priming of CD8 T cells, express components of antigen-presenting machinery, major histocompatibility complex (MHC) class I and II molecules, and may be significant contributors to peripheral tolerance. Lately, Swartz group provided data suggesting that LECs can constantly uptake and cross-present exogenous Ags to CD8 T cells, under normal conditions, implying the contribution of LECs in sustaining of CD8 T-cell tolerance to exogenous Ags present in the lymph.

The lymphatic system plays an important role not only in physiological but also in pathological conditions. The fluid production reaches up to two-thirds of the total volume of interstitial fluid every day. Hence, any dysregulation of extracellular fluid balance, caused by insufficient lymphatic vessel function, leads to interstitial accumulation of fluid and to lymphedema. Additionally, a high-protein edema fluid triggers an inflammatory reaction, a subsequent fibrosis, an adipose tissue augmentation, an impaired immune response and wound healing. Moreover, during the inflammation, the gene expression profile of LECs is changed and the enhancement of C-C chemokine receptor type 7 (CCR7)-positive DCs migration to lymphatic vessels is mainly induced by increased secretion of a CCL21 chemokine.

The tumor lymphatic vessels development

Recent research has defined a tumor lymphangiogenesis as a multifactorial process occurring due to the interactions between tumor, endothelial and immune cells. These cells are the source of protein factors that lead to LECs proliferation, migration and vessels development. Due to a high-interstitial fluid pressure of most solid tumors, the lymphatic vessels in the tumor mass are collapsed and have a limited functionality. Thus, they are unable to transport cancer cells to distant organs and their role has remained unclear, whereas lymphatics at the tumor margin (peritumoral) facilitate the spread of cancer cells (Fig. 1A).

Tumor cells and tumor-infiltrating myeloid cells stimulate direct formation of peritumoral lymphatic vessels via secretion of IL-10, VEGF, TGF-β and PGE2 (Fig. 1B). However, the central role in the embryonic and postnatal lymphatic formation is played by VEGFR-3, that is phosphorylated through the interaction with secreted proteins: VEGF-C and VEGF-D. Nevertheless, the lymphangiogenesis can be additionally initiated by the activation of multiple types of receptor tyrosine kinases such as VEGFR-2, insulin-like growth factor receptor, fibroblast growth factor receptor 3 and angiotropin receptors (Tie1 and Tie2). However, in view of the fact that tumor cells as well as tumor-associated cells (fibroblasts, immune cells) can overexpress VEGF-C, the VEGF-C-VEGFR-3 pathway has been so far best described. The overexpression of VEGF genes seems to be related directly to conditions of tumor environment. The physiological environment of even microscopic tumors can be characterized by high-interstitial pressure and hypoxia that promote tumor growth and metastatic dissemination. The correlation between hypoxia-inducible transcription factor (HIF-1) and lymphangiogenesis was extensively studied. HIF-1 was shown to stimulate transcription of important lymphangiogenic factors: platelet derived growth factor B, Prox-1 and SOX18.

However, it seems that hypoxia can augment the VEGF-C protein levels also via a HIF-1 independent effect on VEGF-C IRES-dependent initiation of translation. Moreover, this hypoxia-induced switching from cap-dependent to IRES-dependent VEGF-C translation was even higher in tumor cells that had metastasized to the lymph nodes compared to tumor cells.
that were present in the primary tumor. These results are in line with the pre-clinical studies on melanoma xenografts showing that lymph nodes metastases correlate with the size of hypoxic tissue fraction in the center of tumor as well as microvascular density in the tumor periphery. Interestingly, VEGFR-3 and VEGF-C expressed by metastatic tumor cells, acts as autocrine stimulation mechanisms that may induce tumor cells proliferation and invasiveness. Therefore, much focus has been placed lately on VEGF-C implication in the lymphangiogenesis and lymphatic vessels enlargement. Secretion of a variety of cytokines and growth factors mobilize tumor cells as well as dendritic cells to get inside the initial lymphatic vessels. Another, newly described secreted protein, involved in the process of tumor lymphangiogenesis is transforming growth factor-β-induced protein (TGFBIp). Tumors expressing TGFBIp develop more metastases via induction of LECs migration and tube formation as well as increase of lymphatic vessels permeability. Interestingly, the TGFBIp-induced effect on tumor dissemination can be abolished by lithium treatment, that suppress the metastatic potential of colon cancer without affecting the growth rate of tumor cells.

**The tumor-generated unique conditions of draining lymph nodes, promoting metastases**

It has been evident that cancer cells coordinate the pre-metastatic niche formation through the secretion of a variety of cytokines, enzymes and growth factors. Pre-treatment of animals with tumor-conditioned media (TCM) not only increases lymphangiogenesis in draining lymph nodes and peripheral areas of tumors but also accelerates metastasis of MDA-MB-231 and
SUM-149 in mammary fat pad xenografts models. Moreover, it is suggested that in lymph nodes lymphangiogenesis occurs even before the arrival of tumor cells and leads to the formation of the pre-metastatic niche. Interestingly, tumor cells may influence the gene expression profile of LECs. In rat model of gastric cancer, the significant differences between expression profile of LECs, from control and metastatic tumor, were detected in over 800 of genes. In LECs from pre-metastatic organs, treated with TCM, genes.28 In LECs from pre-metastatic organs, treated with TCM, from control and metastatic tumor, were detected in 800 of Table 1.

| Molecule involved in cell migration | Role in leucocytes trafficking by lymphatic vessels | Expression on tumor cells | Influence on tumor progression |
|-----------------------------------|-----------------------------------------------|--------------------------|-----------------------------|
| CCR7                              | Upregulated CCR7 during activation and maturation of DCs to respond to lymphatic-secreted CCL21 and elicits directional migration.52 | Melanoma, colorectal, mammary, gastric, non-small cell lung, head and neck cancers, thyroid and squama cell carcinomas.42,63 | Expression is correlated with lymph node metastasis. |
| CCR4                              | Selectively expressed on Th2 cells and regulatory T cells | Breast, lung, gastric cancer.54 | Expression is associated with lung and lymph nodes metastasis. In breast cancer, correlated with HER2 positive tumors and poor prognosis. The results are controversial. In some cases CCR4 leads to actin polymerization and pseudopodia formation of breast tumor cells and have a significant influence on metastasis to regional lymph nodes and lungs.42 |
| ICAM-1                            | ICAM-1 knockout mice have defects in lymph node recruitment of DCs.43 | Melanoma, breast cancer, gastric cancer, esophageal cancer, colorectal carcinoma.65 | Expression is correlated with lymph node metastasis. In breast cancer, correlated with HER2 positive tumors and poor prognosis. The results are controversial. In some cases CCR4 plays a major role in invasion of cancerous cells while in others decreased expression inhibits formation of metastases.56 |
| COX-2                             | COX-2 plays an important role in leucocytes migration and adhesion, likely by modulating p110g P13K–mediated cell signaling.67 | Breast cancer, esophageal cancer, pancreatic cancer, various colorectal tumors, adenocarcinoma, prostate and bladder cancers.68 | Expression is associated with lung and lymph nodes metastasis. In breast cancer, correlated with HER2 positive tumors and poor prognosis. The results are controversial. In some cases CCR4 plays a major role in invasion of cancerous cells while in others decreased expression inhibits formation of metastases.56 |
| CD99                              | Involved in transmigration of monocytes, neutrophils, lymphocytes and DCs | Ewing sarcoma, synovial sarcoma and low-grade fibromyxoid sarcoma.19 | Knocking down CD99 in Ewing sarcoma reduces tumor ability to form metastases |
Table 2. Overview of lymphangiogenesis inhibitors investigated in pre-clinical studies.

| Drug name               | Molecular target | Treatment                  | Mechanism of action                                                                 | Outcome                                      |
|-------------------------|------------------|----------------------------|-------------------------------------------------------------------------------------|----------------------------------------------|
| Soluble VEGFR-3         | VEGF-C           | Pre-clinical studies in endometrial cancer model | Inhibits lymphatic endothelial cell growth in vitro                                   | Suppresses in vivo lymph node and lung metastasis |
| Canstatin               | Ang1/Ang2        | Pre-clinical studies in colon carcinoma model | Reduces tumor blood and lymphatic vessel densities                                    | Reduces final volume and weight of tumors     |
| Endostatin              | VEGF-3           | Pre-clinical                | Causes inhibition of bFGF-induced corneal angiogenesis and lymphangiogenesis          | Prevents lymphatic metastasis                |
| 16K hPRL                | VEGF-3           | Pre-clinical studies in melanoma model | Induces apoptosis and inhibits proliferation, migration and tube formation of human dermal lymphatic microvascular endothelial cells |                                               |
| SAR13167S               | VEGFR-3          | Pre-clinical studies in breast cancer model | Reduces TAM infiltration                                                              |                                               |
| cVE-199                 | VEGF-D           | Pre-clinical studies in neuroblastoma model | Inhibits lymphangiogenesis                                                            | Prevents lymphatic metastasis of neuroblastoma|
| Nrp2                    | Semaphorin       | Pre-clinical studies in breast cancer model | Inhibits VEGF-C-induced phosphorylation of VEGFR-3, ERK1/2, and AKT                  | Tumor cells expressing sema3C contained a lower concentration of lymph vessels and form lymph nodes metastasis much less effectively |
| Biomimetic peptide SP2012 | c-MET             | Pre-clinical studies in breast cancer model | Inhibits blood and lymphatic endothelial cell viability, migration, adhesion and tube formation |                                               |
| Rapamycin               | mTOR             | Pre-clinical studies in head and neck cancer model | Inhibits lymphangiogenesis                                                             | Prevents dissemination to the cervical lymph nodes |

Although the chemokine-dependent migration mechanism of tumor cells appears to be similar to those used by DCs, this similarity to DCs cannot be established as a rule for other molecules. For example, ICAM-1 promotes the exit of leukocytes from tissue to lymphatics. However, in case of tumor, the role of adhesion molecules in metastatic dissemination has not been yet fully understood. In melanoma, the high expression of ICAM-1 correlates with the risk of metastasis, while breast cancer patients with CAMs-positive tumors have a better prognosis. Nevertheless, it is probable that some molecules like CD99 may be involved in migration of leukocytes as well as tumor cells. CD99 has been described as a transmigration mediator of monocytes, neutrophils, lymphocytes, and DCs. The results with anti-CD99 antibodies suggest a homophilic interaction between CD99 on the leukocytes and CD99 on the endothelial cells. Interestingly, the truncated isoform of CD99 enhances migration and metastasis ability of cancer cells, while the full length of CD99 acts as an onco-suppressor in a wider group of tumors. However, the direct mechanism of tumor cells and LECs interaction mediated by CD99 has not been yet investigated.

The anti-lymphangiogenic therapies

Monoclonal antibodies and recombinant proteins

Angiogenesis and lymphangiogenesis play a pivotal role in tumor cells growth and dissemination, therefore, factors involved in these processes are potential targets in antitumor therapies. Since VEGF-C/VEGF-D-VEGFR-3 signaling axis is the most prominent in metastatic disease, provided with either anti-VEGF or anti-VEGFR antibodies or soluble form of VEGF receptor—sVEGFR-3. Interestingly, VEGFR-3 and VEGFR-2 together promote proliferation and migration of LECs, therefore simultaneously blocking both of the receptors seems to be more effective in inhibition of peritumoral lymphangiogenesis than single anti-VEGF treatment (Table 2). Bevacizumab, an anti-VEGF antibody, is applied in the treatment of colon, non-small cell lung, kidney and ovarian cancer. Moreover, there are some ongoing clinical trials using bevacizumab in combination with different drugs, such as dexamethasone or VGX-100. VGX-100 is an anti-VEGF-C antibody, which is currently in phase I of clinical trials. However, in the mentioned combined therapies, VEGF-D is still active and may induce lymphatic vessels formation, therefore these therapies only partially block peritumoral lymphangiogenesis. Another agent with promising effect in pre-clinical studies is AMG-386 (Trembananib) an Angiopoietin-1/2-neutralizing peptibody, consisting of Ang-binding sequence fused with Fc region of antibody. It prevents Ang1/2 binding to Tie-2 receptor, therefore inhibits the growth of tumor in mouse xenograft models and currently is in phase III of clinical trials in ovarian cancer treatment. Moreover, AMG-386 is under current investigation in glioblastoma therapy combined with bevacizumab, however this study has not been completed yet. There are some interesting results indicating that sVEGFR-3 poses soluble extracellular ligand-binding domain, able to trap VEGF-C and leads to its inactivation, what results in inhibition of lymphangiogenesis. Also, ramucirumab, the fully human monoclonal antibody that binds to VEGFR-2 inhibits not only angiogenesis but may affect lymphangiogenesis.

Chemotherapy

Another group of antitumor lymphangiogenesis drugs are small molecule receptor tyrosine kinase inhibitors targeting VEGFR-3 including regorafenib used in the treatment of metastatic colorectal cancer and gastrointestinal stromal tumors and axitinib applied in renal cell carcinoma therapy. A promising choice for anti-lymphangiogenic therapy is lenalidomide (LEN). This immunomodulatory agent currently is used in the treatment of multiple myeloma, transfusion-dependent
myelodysplastic syndrome and mantle cell lymphoma. In LECs, LEN is shown to reduce levels of PROX-1 factor, podoplanin and VEGFR-3. Several studies indicate that LEN affects not only LECs but also tumor-associated macrophages (TAMs), which are primarily responsible for the secretion of VEGF-C. Additionally, LEN triggers various effects on the immune system, which may contribute to its therapeutic outcome. It stimulates CD4+ and CD8+ T lymphocytes and also increases the expression of IL-2 and IFN-γ.52

Novel anti-lymphatic agent, collagen IV biomimetic peptide (SP2012), inhibits metastases to lungs in breast cancer tumor xenograft model and leads to LECs apoptosis.53 Moreover, other well-known kinase inhibitors including sorafenib, sunitinib and pazopanib are already approved for the treatment of various cancer by Food and Drug Administration (Table 3). These drugs, well known for their anti-angiogenic action, also prevent phosphorylation of VEGFR-3, leading to lymphangiogenesis inhibition.54,55

All these experimental and clinical studies highlighted the critical role of lymphatic vasculature in tumor metastatic spreading and point them as antitumor therapies target. Although lymphatic vessels are an important element of human immune system function, little is known so far about the impact of lymphatic destruction on immune response to cancer cells.

**Photodynamic therapy as a new anti-lymphatic approach**

Recently described approach used to damage lymphatic vessels is PDT. PDT is a light-based therapeutic modality approved for the treatment of various solid tumors as well as non-oncological conditions such as age macular degeneration (AMD). In the clinical settings, PDT procedure requires administration of a photosensitizing drug, that selectively accumulates in the tumor tissue, and irradiation of the lesion with a visible light of an appropriate wavelength.56 Light-excited photosensitizer transfers its energy to the molecular oxygen, leading to formation of reactive oxygen species. Antitumor effects of PDT result from direct tumor damage, collapse of tumor vasculature and induction of antitumor immune response.57 Antivascular effect of PDT has been extensively studied during the past decades, whereas anti-lymphatic action of PDT is a recently described phenomenon. While pre-existing lymphatic vessels cannot be eradicated with anti-lymphangiogenic agents, PDT can be applied for the destruction of even pre-existing tumor lymphatic vessels. Tammela et al. have been the first to publish that verteporfin-PDT can damage tumor-associated lymphatic vessels in mice and pigs. Moreover, PDT of tumor lymphatic vasculature led to the eradication of intra-lymphatic tumor cells and prevented metastasis of mouse melanoma cells and subsequent recurrence. Interestingly, Tammela et al. also have shown that combination treatment of PDT and AdVEGFR-3-Ig reduces the surface area of peritumoral lymphatic capillaries when compared with single treatment.58 Furthermore, as PDT is an established procedure in various ophthalmological diseases such as AMD, Bucher et al. have used PDT to induce regression of corneal lymphatic vessels without affecting blood vessels in mouse model.

In our previous study, we have identified the optimal conditions to selectively close lymphatic collecting vessels without...
injuring the blood vasculature in a mouse ear dermis.\textsuperscript{59} We have shown that PDT selectively ablates lymphatic vessels and subsequently leads to closure of lymphatic drainage in a particular region. According to our recent results, both apoptosis and autophagy are involved in cell death induced by verteporfin-PDT in LECs.\textsuperscript{60} In addition to Tammela studies presenting that PDT-damaged lymphatic vessels regrowth after stimulation with VEGF-C, we have shown that lymphatic vessels eventually regenerate, without any additional treatment, by recanalization of blocked collectors leading to restoration of lymphatic drainage.\textsuperscript{58,59} Therefore, in order to avoid tumor metastasis and further relapse, treatment combining PDT with anti-lymphangiogenic agents seems to be rational. Such combination therapy would be a promising approach as, according to current literature, there are several known pharmacological inhibitors of lymphangiogenesis as well as it is possible to obtain recombinant proteins able to suppress formation of lymphatic vasculature.

However, we would like to emphasize an undisputed problem concerning anti-lymphangiogenic therapies’ influence on development of specific antitumor immune response. Since Burnet and Thomas extended “immune surveillance hypothesis,” suggesting that cancer cells are recognized as “foreign” by the immune system, various immune therapies have been extensively studied. Therefore, new effective therapies, stimulating immune system and overcoming tumor suppression, become available including, exciting recent developments, immune checkpoint blockade.\textsuperscript{61} PDT is also thought to be an immune therapy as it can induce strong local inflammatory reaction that under some unique circumstances and in combination with immune-modulating drugs can lead to the development of systemic antitumor immune response and the formation of memory responses.\textsuperscript{57} A prolonged damage of tumor lymphatic vasculature may totally abrogate immune cell trafficking from the tumor to the draining lymph nodes and can subsequently lead to impaired development of antitumor immune response (unpublished results). Thus, we speculate that, even though anti-lymphangiogenic therapies can overcome tumor tolerance and prevent metastasis, it may affect antitumor immune response leading to poor PDT outcome.

**Conclusions**

In the light of the latest findings, the lymphatic vessels are not only passive channels but also can participate in the development of antitumor immune response. It is becoming clear that the process of cells’ entry into the lymphatic vessels requires active and highly complex interactions with LECs. However, in contrast to blood vessels the mechanisms of tumor cell intravasation has not been well described. Hence, it is not known what type of junctions are preferred between tumor cell interactions and whether the initial or/and collecting vessels are the gates for metastatic cells entry. Therefore, to better understand the molecular control of immune and tumor cells interaction with LECs, further research is warranted.

The latest discoveries have demonstrated the potential of novel therapeutic strategies to prevent formation of tumor metastases. As we discussed here, the VEGF-C-stimulated tumor lymphangiogenesis may have important bearing on formation of pre-metastatic niche in draining lymph nodes, and promotes progression and dissemination of tumor cells. Nevertheless, the lymphatic vessels’ role in the process of antigen presentation and antitumor adaptive immune response development should not be diminished, especially in the light of recently described effective immunotherapies. Thus, the currently generated inhibitors of VEGF-C-VEGFR-3 pathway are probably just the beginning of therapies modulating lymphatic function and hopefully the nearest future will bring great advances in this field.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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The experiments (Fig. 1A) were carried out in mice according to a protocol approved by the Committee for Animal Experiments for the Canton Vaud, Switzerland (authorization 2687).

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**References**

1. Stacker SA, Williams SP, Karnezis T, Shayan R, Fox SB, Achen MG. Lymphangiogenesis and lymphatic vessel remodelling in cancer. Nat Rev Cancer 2014; 14:159-72; PMID:24561443; http://dx.doi.org/10.1038/nrc3677
2. Francois M, Caprini A, Hosking B, Orsenigo F, Wilhelm D, Browne C, Paaoven K, Karnezis T, Shayan R, Downes M et al. Sox18 induces development of the lymphatic vasculature in mice. Nature 2008; 456:643-7; PMID:18931657; http://dx.doi.org/10.1038/nature07391
3. Lund AW, Duraes FV, Hirosue S, Raghavan VR, Nembrini C, Thomas SN, Issa A, Hugues S, Swartz MA. VEGF-C promotes immune tolerance in B16 melanomas and cross-presentation of tumor antigen by lymph node lymphatics. Cell Rep 2012; 1:191-9; PMID:22832193; http://dx.doi.org/10.1016/j.celrep.2012.01.005
4. Maby-El Hajjami H, Petrova TV. Developmental and pathological lymphangiogenesis: from models to human disease. Histochem Cell Biol 2008; 130:1063-78; PMID:18946678; http://dx.doi.org/10.1007/s00418-008-0525-5
5. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Decker NC, Castle D, Mandell JW, Lee KS et al. Structural and functional features of central nervous system lymphatic vessels. Nature 2015; S23:337-41; PMID:26030524; http://dx.doi.org/10.1038/nature14432
6. Baluk P, Fuxe J, Hashizume H, Romano T, Lashnits E, Butz S, Vestweber D, Corada M, Molendini C, Dejana E et al. Functionally specialized junctions between endothelial cells of lymphatic vessels. J Exp Med 2007; 204:2349-62; PMID:17846148; http://dx.doi.org/10.1084/jem.20062596
7. Dieterich LC, Detmar M. Tumor lymphangiogenesis and new drug development. Adv Drug Deliv Rev 2016 Apr 1; 99(PT B):148-160; PMID:26705849; http://dx.doi.org/10.1016/j.addr.2015.12.011
8. Hirosue S, Vokali E, Raghavan VR, Rincon-Restrepo M, Lund AW, Corthesy-Henrioud P, Capotosti F, Halin Winter C, Hugues S, Swartz MA. Steady-state antigen scavenging, cross-presentation, and CD8+ T cell priming: a new role for lymphatic endothelial cells. J Immunol 2014; 192:5002-11; PMID:24795456; http://dx.doi.org/10.4049/jimmunol.1302492
9. Lund AW, Swartz MA. Role of lymphatic vessels in tumor immunity: passive conduits or active participants? J Mammary Gland Biol
10. Fletcher AL, Malhotra D, Turley SJ. Lymph node stroma broaden the peripheral tolerance paradigm. Trends Immunol 2011; 32:12-8; PMID:21147035; http://dx.doi.org/10.1016/j.it.2010.11.002

12. Warren Ag, Bronson H, Borud LJ, Slavin SA. Lymphedema: a comprehensive review. Ann Plast Surg 2007; 59:464-72; PMID:17901744; http://dx.doi.org/10.1097/01.sap.0000257149.42922.7e

16. Achen MG, McColl BK, Stacker SA. Focus on lymphangiogenesis in breast cancer cells. Proc Natl Acad Sci USA 2012; 109:E2707-16; PMID:22980544; http://dx.doi.org/10.1073/pnas.1214019109

18. Schito L, Rey S, Tafani M, Zhang H, Wong CC, Russo A, Russo MA, Rose-John S, Lee KM, Baker AH, Wheat R, Blackbourn DJ et al. An IL-35:IL-27 axis promotes cancer cell survival by driving antitumor immune tolerance. Cancer Cell 2015; 30:391-404; http://dx.doi.org/10.1016/j.ccell.2015.06.017

20. Schafer ZT, Brugge JS. IL-6 involvement in epithelial cancers. J Clin Invest 2007; 117:3660-3; PMID:18060028; http://dx.doi.org/10.1182/jci34237

22. Denkert C, Winzer KJ, Muller BM, Weichert W, Pest S, Kobel M, Kristiansen G, Reles A, Siegert A, Guski H et al. Elevated expression of cyclooxygenase-2 is a negative prognostic factor for disease free survival. J Pathol 2003; 201:544-54; PMID:14648657; http://dx.doi.org/10.1002/j.1600-2812.2003.tb00074.x

23. Ji H, Cao R, Yang Y, Zhang Y, Iwamoto H, Lim S, Nakamura M, Andersson P, Wang J, Sun Y et al. TNFR1 mediates TNF-alpha-induced tumour lymphangiogenesis and metastasis through the inhibition of TGFBIp expression in cancer cells. Sci Rep 2016; 6:20739; PMID:26857144; http://dx.doi.org/10.1038/srep20739

24. Maeng YS, Aguilar B, Choi SI, Kim EK. Inhibition of TGFBIp expression reduces lymphangiogenesis and tumor metastasis. Oncogene 2016; 35:196-205; PMID:25772247; http://dx.doi.org/10.1038/onc.2015.73

25. Maeng YS, Lee R, Lee B, Choi SI, Kim EK. Lithium inhibits tumor lymphangiogenesis and metastasis through the inhibition of TGFBIp expression in cancer cells. Scientific Rep 2016; 6:20739; PMID:26857144; http://dx.doi.org/10.1038/srep20739

28. Xu J, Zhang C, He Y, Wu H, Wang Z, Song W, Li W, He W, Cai S, Zhang W. Lymphatic endothelial cell-secreted CXCL1 stimulates lymphangiogenesis and metastasis of gastric cancer. Int J Cancer J Int du Cancer 2012; 130:787-97; PMID:21387301; http://dx.doi.org/10.1002/ijc.26035

29. Lee E, Fertig EJ, Jin K, Sukumar S, Pandey NB, Popel AS. Breast cancer cell condition lymphatic endothelial cells within pre-metastatic niches to promote metastasis. Nat Commun 2014; 5:4715; PMID:25178650; http://dx.doi.org/10.1038/ncomms5715

30. Schafer ZT, Brugge JS. IL-6 involvement in epithelial cancers. J Clin Invest 2007; 117:3660-3; PMID:18060028; http://dx.doi.org/10.1182/jci34237

32. Chayama K. Vascular endothelial growth factor-C expression predicts lymph node metastasis of breast cancer cells. J Biol Chem 2008; 283:11155-63; PMID:18319253; http://dx.doi.org/10.1074/jbc.M802419200

33. Oh K, Lee OY, Shon SY, Nam O, Ryu PM, Seo MW, Lee DS. A mutual activation loop between breast cancer cells and myeloid-derived suppressor cells facilitates spontaneous metastasis through IL-6 trans-signaling in a murine model. Breast Cancer Res 2013; 15:R79; PMID:24021059; http://dx.doi.org/10.1186/bcr3473

34. Van Trappen PO, Steele D, Lowe DG, Baithun S, Beasley N, Thiele W, Weich H, Krishnan J, Shepherd JH, Pepper MS et al. Expression of vascular endothelial growth factor (VEGF)-C and VEGF-D, and their receptor VEGFR-3, during different stages of cervical carcinogenesis. J Pathol 2003; 201:544-54; PMID:14648657; http://dx.doi.org/10.1002/j.1600-2812.2003.tb00074.x

35. Tutunea-Fatan E, Majumder M, Xin X, Lala PK. The role of CCL21/CCR7 chemokine axis in breast cancer-induced lymphangiogenesis and metastasis through the inhibition of TGFBIp expression. Cancer Cell 2015; 30:196-205; PMID:26713622; http://dx.doi.org/10.1038/srep12133

36. Shahid J, Emmett MS, Dunn DB, Joyd KD, Sage LM, Rigby H, Mortimer PS, Orlando A, Levick JR, Bates DO. Chemokine-mediated migration of melanoma cells towards lymphatics—a mechanism contributing to metastasis. Oncogene 2007; 26:2997-3005; PMID:17130836; http://dx.doi.org/10.1038/sj.onc.1210114

37. Pan MR, Hou MF, Chang HC, Hung WC. Cyclooxygenase-2 up-regulates CCR7 via EP2/EP4 receptor signaling pathways to enhance lymphatic invasion of breast cancer cells. J Biol Chem 2008; 283:11155-63; PMID:18319253; http://dx.doi.org/10.1074/jbc.M802419200

38. Bovina C, Wang JF, Sun Y et al. TNFR1 mediates TNF-alpha-induced tumour lymphangiogenesis and metastasis through the inhibition of TGFBIp expression in cancer cells. Scientific Rep 2016; 6:20739; PMID:26857144; http://dx.doi.org/10.1038/srep20739

39. Shields JD, Fleury ME, Yong C, Tomei AA, Randolph GJ, Swartz MA. Autologous chemotaxis as a mechanism of tumour cell homing to lymphatics via interstitial fluid flow and autocrine CCR7 signaling. Cancer Cell 2007; 11:526-38; PMID:17560334; http://dx.doi.org/10.1016/j.ccr.2007.08.020

40. McKimmie CS, Singh MD, Hewit K, Lopez-Franco O, Le Brocq M, Rose-John S, Lee KM, Baker AH, Wheat R, Blackbourn DJ et al. Autologous chemotaxis as a mechanism of tumour cell homing to lymphatics via interstitial fluid flow and autocrine CCR7 signaling. Cancer Cell 2007; 11:526-38; PMID:17560334; http://dx.doi.org/10.1016/j.ccr.2007.08.020

41. Zhou C, de Ruyck J, Schijns V. Pro-inflammatory cytokines stimulate lymphangiogenesis through stimulation of HIF-1α-dependent translation-mediated mechanism. Cell Rep 2016; 6:20739; http://dx.doi.org/10.1016/j.celrep.2016.02.017

42. Christofoletti R, Klussmann J, Neumann P, Novack V, Pabst O, Krueger T, Wagner S, Lipp H, Friedewald K, Madsen K, et al. Pro-inflamatory cytokines stimulate lymphangiogenesis through stimulation of HIF-1-dependent translation-mediated mechanism. Cell Rep 2016; 6:20739; http://dx.doi.org/10.1016/j.celrep.2016.02.017

43. Widder S, Klussmann J, Neumann P, Novack V, Pabst O, Krueger T, Wagner S, Lipp H, Friedewald K, Madsen K, et al. Pro-inflamatory cytokines stimulate lymphangiogenesis through stimulation of HIF-1-dependent translation-mediated mechanism. Cell Rep 2016; 6:20739; http://dx.doi.org/10.1016/j.celrep.2016.02.017
Muller A, Homye B, Soto H, Ge N, Catron D, Buchanan ME, McCla-
nahan T, Murphy E, Yuan W, Wagner SN et al. Involvement of che-
mokine receptors in breast cancer metastasis. Nature 2001; 410:30-6; 
PMD:11242036; http://dx.doi.org/10.1038/35065016

Johnson LA, Casper S, Holt AP, Lalor PF, Bahan D, Jackson DG. An 
inflammation-induced mechanism for leukocyte transmigration 
among lymphatic vessel endothelium. J Exp Med 2006; 203:2763-77; 
PMD:17171672; http://dx.doi.org/10.1084/jem.20051759

Yan J, Jiang Y, Ye M, Liu W, Feng L. The clinical value of lymphatic vessel 
density, intercellular adhesion molecule 1 and vascular cell adhesion 
molecule 1 expression in patients with oral tongue squamous cell carci-
noma. J Cancer Res Therapeutics 2014; 10 Suppl:CL25-30; 
PMD:25450269; http://dx.doi.org/10.4103/0973-1482.145827

Winger RC, Harp CT, Chiang MY, Sullivan DP, Watson RL, Weber 
EW, Poodjo RJ, Miller SD, Muller WA. Cutting edge: CD99 is a novel 
therapeutic target for control of T cell-mediated central nervous sys-
tem autoimmune disease. J Immunol 2016; 196:1443-3; 
PMD:26773145; http://dx.doi.org/10.4049/jimmunol.1501634

Lou O, Alcaide P, Lucinskas FW, Muller WA. CD99 is a key mediator 
of the transendothelial migration of neutrophils. J Immunol 2007; 
178:1136-43; http://dx.doi.org/10.4049/jimmunol.178.2.1136

 Scotlandi K, Zuntini M, Manara MC, Scandra M, Rocchi A, Renini S, Nic-
oletti G, Bernard G, Nanni P, Lollini PL, et al. CD99 isoforms dictate oppo-
site functions in tumour malignancy and metastases as activating or 
repressing c-Src kinase activity. Oncogene 2007; 26:6604-18; 
PMD:17471235; http://dx.doi.org/10.1038/sj.oni.1210481

Hajrasoulia HR, Funaki T, Sadrai Z, Hattori T, Chauhan SK, Dana R. 
Vascular endothelial growth factor-C promotes alloimmunity by 
amplifying antigen-presenting cell maturation and lymphangiogene-
sis. Invest Ophthalmol Visual Sci 2012; 53:1244-50; 
PMD:22288120; http://dx.doi.org/10.1167/iovs.11-8866

Mita AC, Takimoto CH, Mita M, Tolcher A, Sankhala K, Sarantopou-
los J, Valdivieso M, Wood L, Rasmussen E, Sun YN et al. Phase 1 
study of AMG 386, a selective angiopoietin 1/2-neutralizing pepti-
dide, in combination with chemotherapy in adults with advanced 
solid tumors. Clin Cancer Res 2010; 16:3044-56; PMID:20501621; 
http://dx.doi.org/10.1158/1078-0432.CCR-09-3368

Dieras V, Wildiers H, Jassem J, Dirix LY, Guastalla JP, Bono P, 
Hurvitz SA, Goncalves A, Romieu G, Limentani SA et al. Trebana-
tib (AMG 386) plus weekly paclitaxel with or without bevacizumab 
as first-line therapy for HER2-negative locally recurrent or met-
astatic breast cancer: a phase 2 randomized study. Breast 2015; 
24:182-90; PMID:25747197; http://dx.doi.org/10.1016/j.
breast.2014.11.003

Spreatlin JL, Cohen RB, Eadens M, Gore L, Camridge DR, Diab S, 
Leong S, O’Bryant C, Chow LC, Serkova NJ et al. Phase I pharmacologic and 
biologic study of ramucirumab (IMC-1121B), a fully human immunoglobulin 
G1 monoclonal antibody targeting the vascular endothelial growth factor 
receptor-2. J Clin Oncol 2010; 28:780-7; PMID:20048182; http://dx.doi.
org/10.1200/JCO.2009.23.7537

Song K, Herzog BH, Sheng M, Fu J, McDaniel JM, Chen H, Ruan J, 
Xia L. Lenalidomide inhibits lymphangiogenesis in preclinical models of 
mantle cell lymphoma. Cancer Res 2013; 73:7254-64; 
PMD:24158094; http://dx.doi.org/10.1158/0008-5472.CAN-13-0750

Lee E, Lee SJ, Koskimaki JE, Han Z, Pandey NB, Popel AS. Inhibition 
of breast cancer growth and metastasis by a biomimetic peptide. Scientific 
Rep 2014; 4:7139; PMID:25409905; http://dx.doi.org/10.1038/srep07139

Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. 
Preclinical overview of sorafenib, a multikinase inhibitor that targets 
the molecular study of ramucirumab (IMC-1121B), a fully human immunoglobulin 
G1 monoclonal antibody targeting the vascular endothelial growth factor 
receptor-2. J Clin Oncol 2010; 28:780-7; PMID:20048182; http://dx.doi.
org/10.1200/JCO.2009.23.7537

Song K, Herzog BH, Sheng M, Fu J, McDaniel JM, Chen H, Ruan J, 
Xia L. Lenalidomide inhibits lymphangiogenesis in preclinical models of 
mantle cell lymphoma. Cancer Res 2013; 73:7254-64; 
PMD:24158094; http://dx.doi.org/10.1158/0008-5472.CAN-13-0750

Lee E, Lee SJ, Koskimaki JE, Han Z, Pandey NB, Popel AS. Inhibition 
of breast cancer growth and metastasis by a biomimetic peptide. Scientific 
Rep 2014; 4:7139; PMID:25409905; http://dx.doi.org/10.1038/srep07139

Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. 
Preclinical overview of sorafenib, a multikinase inhibitor that targets 
both Raf and VEGF and PDGF receptor tyrosine kinase signaling. 
Mol Cancer Ther 2008; 7:3129-40; PMID:18582116; http://dx.doi.
org/10.1158/1535-7163.MCT-08-0138

Mihaly Z, Sztpinski Z, Szurowicz P, Gryforff B. A comprehensive 
overview of targeted therapy in metastatic renal cell carcinoma. Cur-
cent cancer drug targets 2012; 12:857-72; PMID:22515521; http://dx.doi.
org/10.1016/j.cct.2015.06.008

Wachowska M, Muchowicz A, Golab J. Targeting epigenetic processes in 
photodynamic therapy-induced anticancer immunity. Front Oncol 
2015; 5:176; PMID:26284197; http://dx.doi.org/10.3389/ 
fonc.2015.00176

Agostinis P, Berg K, Cengel KA, Foster TH, Girotti AW, Gollick SO, 
Hahn SM, Hamblin MR, Juuzeniene A, Kessel D et al. Photodynamic therapy 
of cancer: an update. CA Cancer J Clin 2011; 61:250-81; 
PMD:21617154; http://dx.doi.org/10.1016/S0008-543X(10)007799

Lee E, Lee SJ, Koskimaki JE, Han Z, Pandey NB, Popel AS. Inhibition 
of breast cancer growth and metastasis by a biomimetic peptide. Scientific 
Rep 2014; 4:7139; PMID:25409905; http://dx.doi.org/10.1038/srep07139

Hwang-Bo J, Yoo KH, Park JH, Jeong HS, Chung IS. Recombinant 
canstatin inhibits angiopoietin-1-induced angiogenesis and lymph-
angiogenesis. Int J Cancer Int J du Cancer 2012; 131:298-309; 
PMD:21823121; http://dx.doi.org/10.1002/ijc.26353

Han KY, Azar DT, Sabri A, Lee H, Jain S, Lee BSChang JH. Character-
ization of the interaction between endostatin short peptide and VEGF
receptor 3. Protein and Peptide Letters 2012; 19:969-74; PMID:22512651; http://dx.doi.org/10.2174/092986612802084465

73. Kinet V, Castermans K, Herkenne S, Maillard C, Blacher S, Lion M, Noël A, Martial JA, Struman I. The angiostatic protein 16K human prolactin significantly prevents tumor-induced lymphangiogenesis by affecting lymphatic endothelial cells. Endocrinology 2011; 152:4062-71; PMID:21862622; http://dx.doi.org/10.1210/en.2011-1081

74. Espagnolle N, Barron P, Mandron M, Blanc I, Bonnin J, Agnel M, Kerbelec E, Herault JP, Savi P, Bono F et al. Specific inhibition of the VEGFR-3 tyrosine kinase by SAR131675 reduces peripheral and tumor associated immunosuppressive myeloid cells. Cancers 2014; 6:472-90; PMID:24589997; http://dx.doi.org/10.3390/cancers6010472

75. Kashima K, Watanabe M, Satoh Y, Hata J, Ishii N, Aoki Y. Inhibition of lymphatic metastasis in neuroblastoma by a novel neutralizing antibody to vascular endothelial growth factor-D. Cancer Sci 2012; 103:2144-52; PMID:22937829; http://dx.doi.org/10.1111/cas.12100

76. Mumblat Y, Kessler O, Ilan N, Neufeld G. Full-length Semaphorin-3C is an inhibitor of tumor lymphangiogenesis and metastasis. Cancer Res 2015; 75:2177-86; PMID:25808871; http://dx.doi.org/10.1158/0008-5472.CAN-14-2464

77. Patel V, Marsh CA, Dorsam RT, Mikels CM, Masedunskas A, Amornphimoltham P, Nathan CA, Singh B, Weigert R, Molinolo AA et al. Decreased lymphangiogenesis and lymph node metastasis by mTOR inhibition in head and neck cancer. Cancer Res 2011; 71:7103-12; PMID:21975930; http://dx.doi.org/10.1158/0008-5472.CAN-10-3192

78. Li XP, Jing W, Sun J, Liu ZY, Zhang JT, Sun W, Zhu W, Fan YZ. A potential small-molecule synthetic antilymphangiogenic agent nor-cantharidin inhibits tumor growth and lymphangiogenesis of human colonic adenocarcinomas through blocking VEGF-A,-C,-D/VEGFR-2,-3 "multi-points priming" mechanisms in vitro and in vivo. BMC Cancer 2015; 15:527; PMID:26187792; http://dx.doi.org/10.1186/s12885-015-1521-5

79. Kodera Y, Katanasaka Y, Kitamura Y, Tsuda H, Nishio K, Tamura T, Koizumi F. Sunitinib inhibits lymphatic endothelial cell functions and lymph node metastasis in a breast cancer model through inhibition of vascular endothelial growth factor receptor 3. Breast Cancer Res 2011; 13:R66; PMID:21693010; http://dx.doi.org/10.1186/bcr2903

80. Schmieder R, Hoffmann J, Becker M, Bhargava A, Muller T, Kahmann N, Ellinghaus P, Adams R, Rosenthal A, Thierach KH et al. Regorafenib (BAY 73-4506): antitumor and antimetastatic activities in preclinical models of colorectal cancer. Int J Cancer J Int du Cancer 2014; 135:1487-96; PMID:24347491; http://dx.doi.org/10.1002/ijc.28669