

The lymphangiogenic growth factors VEGF-C and VEGF-D

Part 2: The role of VEGF-C and VEGF-D in diseases involving the lymphatic system

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Summary

VEGF-C and VEGF-D are the two central signaling molecules that govern the development and growth of the lymphatic system. The presence or absence of lymphangiogenesis plays a central and sometimes causative role in a variety of diseases. Therefore, the molecules that govern lymphangiogenesis – especially VEGF-C and VEGF-R3 – offer the possibility of therapeutic intervention.

Although lymphangiogenesis blockade doesn't exist as an independent therapeutical concept, several anti-lymphangiogenic drugs are tested at the moment in clinical trials. The rationale is that by targeting VEGF-C and VEGF-D, the present antiangiogenic treatment would be improved since tumors may deploy the angiogenic forms of VEGF-C and VEGF-D when VEGF-A-mediated angiogenesis is blocked.

Despite many attempts there has been no breakthrough in the pro-angiogenic therapies. Furthermore, pro-lymphangiogenic, VEGF-C- or VEGF-D-based therapies have practically never made it to the clinical trial phase. At least one clinical study with VEGF-C is now in preparation, namely in combination with lymph node transplantation to treat postmastectomy edema.

Here, we review the roles that VEGF-C, VEGF-D and their receptors play in diseases that involve the lymphatic system and we present opportunities to utilize these molecules to stimulate lymphatic vessel growth to fight lymphedema or to block their growth in order to inhibit tumor angiogenesis and tumor lymphangiogenesis.

Keywords: VEGF-C, VEGF-D, growth factors, lymphangiogenesis, lymphedema, lymphatic metastasis

Die lymphangiogenen Wachstumsfaktoren VEGF-C und VEGF-D Teil 2: Die Rolle von VEGF-C und VEGF-D bei lymphatischen Erkrankungen

Zusammenfassung

VEGF-C und VEGF-D sind die beiden zentralen Signalmoleküle, die für die Entwicklung und das Wachstum des Lymphgefäßsystems verantwortlich sind. Fehlende oder überschüssige Lymphangiogenese spielt in einer Reihe von Krankheiten eine zentrale und manchmal auch ursächliche Rolle. Deshalb stellen die die Lymphangiogenese steuernden Signalmoleküle, insbesondere VEGF-C und VEGF-Rezeptor-3, eine Möglichkeit zur therapeutischen Intervention dar.

Obwohl Lymphangiogenese-Blockierung nicht als eigenständiges Therapiekonzept existiert, werden eine ganze Reihe von antilymphangiogenen Wirkstoffen zur Zeit in klinischen Versuchen getestet. Man erhofft sich von ihnen eine Verbesserung der existierenden antiangiogenen Tumorthherapie, weil bei der Blockierung von VEGF-A Tumore auf die angiogenen Formen von VEGF-C und VEGF-D ausweichen können, um ihre Versorgung mit Blutgefäßen sicherzustellen.

Trotz vieler Versuche konnte auf der anderen, proangiogenen Seite noch keine Therapie einen entscheidenden Durchbruch vermelden, und prolymphangiogene, auf VEGF-C oder VEGF-D basierende Therapien wurden bisher so gut wie nie in klinischen Studien untersucht. Zumindest eine klinische Studie mit VEGF-C befindet sich jetzt al-

Endothelial cells are mostly in the resting phase

During embryonic development, organ growth goes hand in hand with vascularization by blood vessels (angiogenesis) and lymphatic vessels (lymphangiogenesis; see Figure 1). Only a few organs are excluded; e.g. the brain contains no lymphatic vessels and the cornea of the eye is completely avascular. Together with organ growth angiogenesis and lymphangiogenesis cease in adulthood, and apart from a few exceptions, the endothelial cells of the adult organism are not actively dividing. However, a re-entry into the active cell cycle can be observed during wound healing [1], in cardiac and skeletal muscle during athletic training [2], periodically within the hair cycle [3] and female reproductive organs, and during placental development [4]. Common to these angiogenic processes is, that the endothelial cells exit from the cell cycle into the resting state after the physiological angiogenesis target has been reached. This is in sharp con-

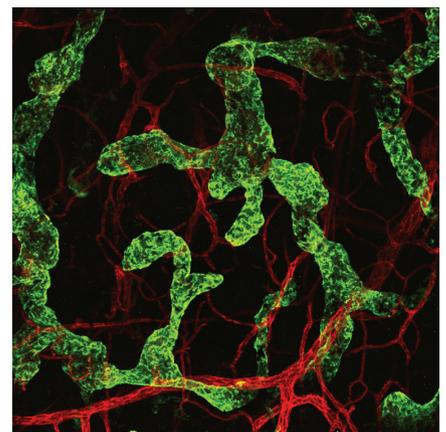


Figure 1.

Blood and lymphatic vessels are found in most organs. Shown here are blood and lymphatic capillaries in the skin of a mouse ear (Mus musculus). The vasculature is specifically stained with fluorescent antibodies. Lyve-1 (green) shows the lymphatic capillaries in the foreground, PECAM-1 (red) the blood capillaries. Due to the intensive LYVE-1 staining, the PECAM-1 staining of the lymphatic capillaries is not visible. Note the larger diameter of the lymphatic capillaries. We thank Harri Nurmi for providing this image.

lerdings in Vorbereitung, und zwar in Kombination mit Lymphknoten-Transplantation für die Therapie des postoperativen Lymphödems.

In diesem Artikel geben wir einen Überblick über die Rolle, die VEGF-C, VEGF-D und ihre Rezeptoren bei Krankheiten des Lymphgefäßsystems spielen und wir präsentieren verschiedene Möglichkeiten, diese Moleküle therapeutisch für das Wachstum von Lymphgefäßen für die Lymphödemtherapie zu nutzen oder sie zu blockieren, um z. B. die für das Tumorstadium notwendige Angiogenese und die für die Tumorausbreitung förderliche Lymphangiogenese zu hemmen.

Schlüsselwörter: VEGF-C, VEGF-D, Lymphangiogenese, Lymphödem, lymphatische Metastasierung

trast to the uncontrolled, pathological angiogenesis, which plays an important and partly causal role in many diseases, especially during tumor growth.

The role of VEGF-C and VEGF-D for tumor angiogenesis and tumor lymphangiogenesis

The dependence of tumor growth on tumor vascularization has been recognized long ago [5], and a whole range of therapeutic concepts has been based on this dependence. In many experimental cancer models, the tumors initially grow without the need for a blood supply. At a certain tumor size, the supply of oxygen by diffusion alone becomes insufficient, and eventually the tumor cells begin to stimulate blood vessel growth. This switch from avascular to vascular growth can take place quite early during tumor development [6, 7].

VEGF-A and its associated receptor VEGFR-2 transmit the main signals for tumor angiogenesis [8]. Accordingly, the first clinically available anti-angiogenesis drug was a monoclonal antibody directed against VEGF-A (bevacizumab, brand name "Avastin", see Figure 4). Contrary to initial expectations, anti-angiogenic therapy appeared far from universally effective. Some tumors were found to be generally resistant; but also when the anti-angiogenic therapy is initially successful, the tumors eventually develop resistance [see Figure 2; 9, 10, 11].

The development of resistance against VEGF-A-blocking anti-angiogenic therapy is multifactorial: the tu-

mor can increase the production of VEGF-A. Alternatively, it could deploy a different ligand (e.g. VEGF-C) and/or an alternative receptor (e.g. VEGF receptor-3).

VEGF receptor-3, which is expressed by tumor blood vessels, can be activated by VEGF-C or VEGF-D. In addition, in their mature form, VEGF-C and VEGF-D can activate VEGF receptor-2 [12, 13]. Other growth factors such as FGFs [14], other angiogenic mechanisms [co-option; 15, 16] and the mobilization of myeloid cells from the

bone marrow [17] may contribute to the development of resistance. A future anti-angiogenic therapy will therefore need to block several angiogenic factors and mechanisms at the same time to prevent the development of resistance.

Hematogenous metastasis versus lymphatic

Tumors spread either through the vascular system (hematogenous metastasis) or via the lymphatic vessels (lymphatic metastasis). The blood vessel density of tumor is correlated with hematogenous metastasis [18, 19] and, not unexpectedly, VEGF-A and VEGFR-2 were shown to be prognostic markers for tumor metastasis [20, 21].

A similar relationship exists between VEGF-C/VEGF-D and lymphatic metastasis. VEGF-C and VEGF-D stimulate the growth of lymphatic vessels within (intratumoral) and around (peritumoral) the tumor and accelerate

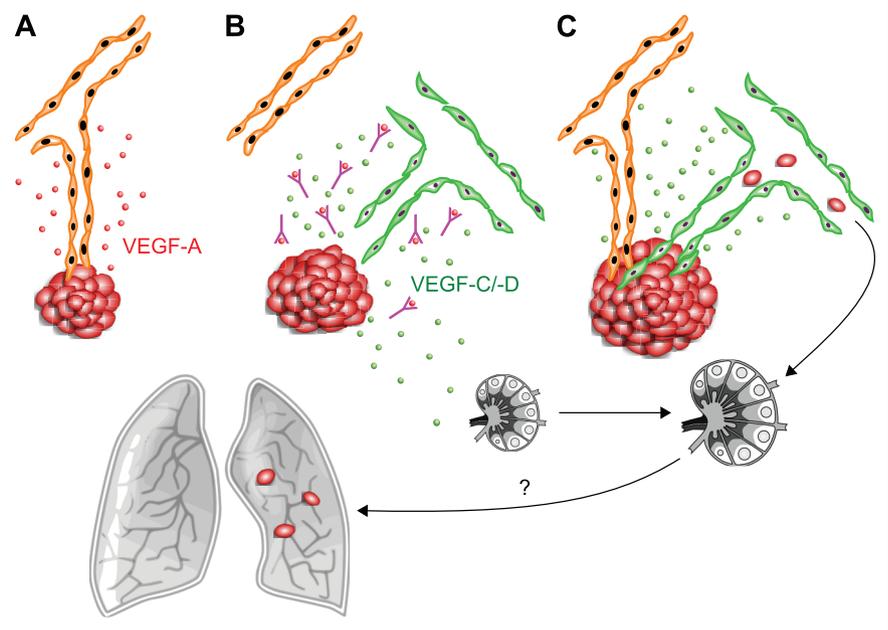


Figure 2.

Scenario for the roles of VEGF-C and VEGF-D for tumor angiogenesis and metastasis. (A) The tumor secretes the primary angiogenesis factor VEGF-A, and thus initiates its own vascularization. (B) The antiangiogenic effect of the anti-VEGF-A therapy slows or stops tumor growth. In the following, the compensatory microevolution leads to increased production and activation of VEGF-C and/or VEGF-D. (C) VEGF-C and/or VEGF-D mediate tumor angiogenesis and tumor lymphangiogenesis. The disease progresses in form of lymph node metastasis and eventually distant metastases.

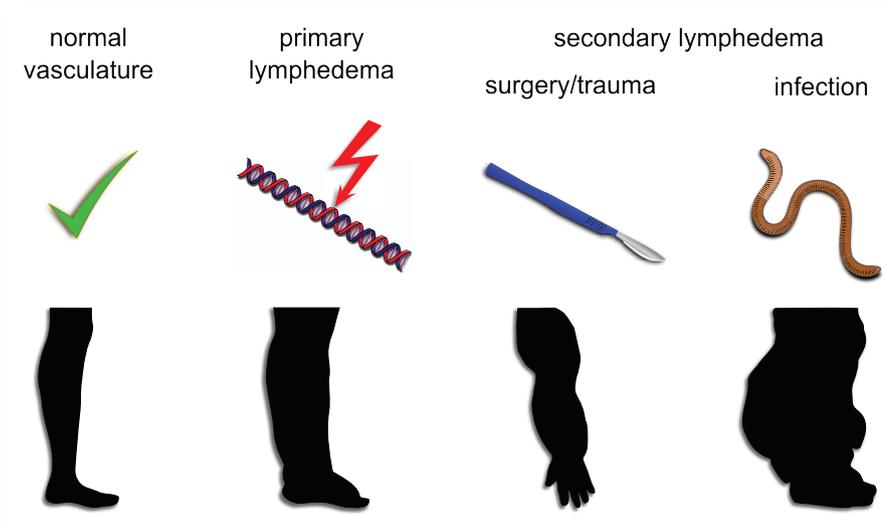


Figure 3.

Lymphedema causes and classification. For several types of primary lymphedema, the causative gene defects have been identified (see Table 1). Globally, most secondary lymphedemas are caused by parasites. In industrialized countries, however, the main causes are surgery and traumata.

lymphatic metastasis [see Figure 2; 22, 23, 24]. Lymphatic vessels within the tumor are probably rare and unlikely to be functional, but have been demonstrated in several tumors, and could be - at least in some studies - correlated with VEGF-C/VEGF-D expression and the disease progression [25]. Peritumoral lymphatics develop around the tumor, originating from the surrounding lymphatic vasculature [23, 26, 27]. In experimental tumor models in mice, mostly these peritumoral vessels appear to be responsible for lymphatic metastasis [26, 28, 29]. In these scenarios, the lymphatic system appears not only as a passive recipient of egressing tumor cells, but also as an active accomplice. Produced by the tumor, VEGF-C causes the lymph system to collaborate in the processes of lymph node metastasis by causing an enlargement of the regional lymph nodes, an increased lymph flow and increased lymph node lymphangiogenesis within the sentinel nodes [see Figure 2; 30, 31, 32].

Pro-angiogenic therapy

Excessive or insufficient angiogenesis or lymphangiogenesis are characteristic of certain diseases. The lack of a

robust angiogenic response to hypoxia in coronary heart diseases seems paradoxical since hypoxia is the main angiogenic stimulus. The growth of new, collateral blood vessels to compensate for the narrowings is therapeutically desired. Unfortunately, stimulation of angiogenesis and arteriogenesis appears insufficient in most ischemic diseases [33, 34].

There has been no shortage of attempts to therapeutically stimulate angiogenesis and arteriogenesis [35]. The most straightforward approach used VEGF growth factors directly to activate the VEGF receptors and thus the endothelial cells. Although some early attempts to treat cardiovascular diseases with VEGF-A [36] and VEGF-C [37, 38] looked promising, these strategies have not matured into useful therapeutic tools despite of more than a decade of preclinical and clinical studies [39, 40]. It is possible that VEGFs alone are not able to stimulate a clinically relevant angiogenesis and that additional signals from other growth factors or cytokines are required. Another possible explanation for the ultimate failure of direct VEGF growth factor therapy may be the technological limitations of the early attempts [41]. If the a concerted action of several pro-angiogenic signals is needed in order to

provoke a positive response in the patient, a therapy would be preferable which aims at an angiogenic master switch, such as the hypoxia-induced factor HIF [42].

Lymphedema and lymphatic hypo- and aplasia

Tissue swellings due to insufficient lymphatic drainage are divided into primary lymphedema, which has hereditary causes, and secondary lymphedema, which is an acquired disease [see Figure 3; 43]. Table 1 lists the hereditary lymphedemas in which the edema is a dominant or the only symptom. Besides these, there are a number of inherited diseases in which the lymphedema is part of a syndrome. Two of these diseases that are interesting due to the molecular etiology can also be found in Table 1. One of these syndromes is responsible for the most common form of hereditary lymphedema: lymphedema-distichiasis syndrome (OMIM 153400). It can be traced to mutations of the transcription factor FOXC2, which lead to a malformation of the lymphatic valves, to abnormal recruitment of smooth muscle to the lymph capillaries and the undesirable formation of a basal lamina for the lymphatic capillary bed [44, 45].

About 70% of the type I hereditary lymphedema sufferer have an inhibiting gene mutation that inactivates the intracellular kinase of the VEGF receptor-3 [OMIM 153100; 46, 47] and thus lymphangiogenesis. The disease is autosomal dominant, because mutant receptors can dimerize with the remaining intact receptors and thereby render them non-responsive leading to an overall reduction of lymphangiogenic signaling [48]. For medical research, it is interesting to note that there is a breeding line of mice (the so-called Chymice), which also has a mutation in the VEGF receptor-3 and type I hereditary lymphedema-like symptoms [49].

Although all endothelial cells carry the mutant gene, not all lymphatic ves-

| Disease | | Gene (Protein) | Phenotype | Temporal manifestation | Molecular mechanism | OMIM | Inheritance | Reference |
|--|-------------------------------|---------------------------|---|----------------------------------|--|------------------------|-------------|-----------|
| Nonne-Milroy-lymphedema | Hereditary lymphedema type 1A | <i>FLT4</i> (VEGFR-3) | Lymphedema of predominantly the lower extremities | congenital | The mutated VEGFR-3 has lost the ability to transmit a proliferation signal. | 153100 | ad | [46] |
| | Hereditary lymphedema type 1B | 6q16.2-q22.1 | Lymphedema of predominantly the lower extremities | childhood to puberty | ? | 611944 | ad | [101] |
| | Hereditary lymphedema type 1C | <i>GJC2</i> (Connexin 47) | Lymphedema of the lower extremities | childhood to puberty | Connexin communication between LECs or LECs and their environment is perturbed. | 613480 | ad | [102] |
| | * | <i>VEGFC</i> (VEGF-C) | Lymphedema of the lower extremities | congenital or in early childhood | The mutated VEGF-C is not anymore secreted. | * | ad | [55] |
| Meige lymphedema, Hereditary lymphedema type 2 | | ? | Lymphedema of predominantly the lower extremities | puberty | ? | 153200 | ? | [103] |
| Lymphedema-Distichiasis-Syndrome | | <i>FOXC2</i> (FOXC2) | Lymphedema of predominantly the lower extremities, second row of eye lashes, varicose veins | puberty or later | Lymphatic valve malformation and pathological pericyte coverage of lymphatic capillaries | 153400 | ad | [44, 104] |
| Hennekam-Syndrome | | <i>CCBE1</i> (CCBE1) | Lymphedema of the extremities, lymphangiectasia of the internal organs, mental retardation | congenital | The mutated CCBE1 cannot synergize with VEGF-C. | 235510 | ar | [51] |

Table 1.

Hereditary lymphedemas and selected syndromes with a consistent lymphedema component. OMIM, Online Mendelian Inheritance in Man; ad, autosomal dominant; ar, autosomal recessive; *, not yet assigned; LEC, lymphatic endothelial cell

sels and body parts are equally affected. Strongly hypoplastic or aplastic lymph capillaries are mainly found in the periphery [50]. A possible explanation could be the increased need for drainage in the extremities, where more lymph is produced due to a higher hydrostatic pressure in the blood vasculature. Against this explanation argues the fact that in mice (where hydrostatic pressure differences are negligible) the edema prefers as well the extremities [49]. Possibly, different lymphatic structures might exhibit a differential dependency on VEGF-C signaling.

A very rare form of hereditary lymphedema, the Hennekam Syndrome (HS; OMIM 235510), can be caused by mutations in the CCBE1 gene [51]. VEGF-C-induced lymphangiogenesis is impaired in HS [52, 53]. A disease phenotype similar to HS can be induced in mice by blocking the signaling of VEGF receptor-3 [54]. Possibly similarly rare and so far only described once is a secretion-inhibiting mutation in the coding sequence of the VEGF-C gene, which results in a clinical picture very similar to type I hereditary lymphedema [55]. A functionally analogous

mutation was also identified in zebrafish and characterized in detail [56].

However, the overall most common causes of lymphedema are not of genetic, but of infectious and traumatic nature. In tropical countries, filariasis causes the majority of lymphedema cases. Filarial nematodes are parasitic infections with roundworms such as *Wuchereria bancrofti* or *Brugia malayi*, whose untreated final stages are known as elephantiasis. The transmission of the pathogen happens by mosquito bite, and the lymphatic vessels serve as conduit and habitat for the parasite [57]. The resulting enlargement of the lymph vessels seems to be directly controlled by parasitic antigens and indirectly via VEGF-C [58, 59].

Pro-lymphangiogenesis therapy

In industrialized countries, iatrogenic postoperative lymphedema is probably the most common type of lymphedema, particularly lymphedema following the evacuation of lymph nodes associated with cancer opera-

tions. The extent to which the surgical procedure itself is responsible for the edema is unknown, but it is noteworthy that postoperative edema may emerge after the acute phase edema has resolved even years after surgery [60, 61]. Therapeutically, lymph node transplantation seems to become the treatment of choice [62]. In experimental models, the integration of transplanted lymph nodes into the regional lymph system could be improved by VEGF-C treatment [63, 64]. Based on these findings, a clinical study is being planned to test the combined therapy of lymph node transplantation with Lymfactin™ (the biopharmaceutical brand name for VEGF-C; http://www.contractpharma.com/contents/view_breaking-news/2012-10-15/ark-to-make-lymfactin-for-laurantis). A similarly high dose pro-lymphangiogenesis gene therapy with VEGF-C was successful in the Chy mouse model of Nonne-Milroy lymphedema and in other experimental models, where it could stimulate the growth of lymphatic vessels [49, 65]. Whether and to what extent these results can be transferred to the hereditary lymphedema of humans is unclear due to the

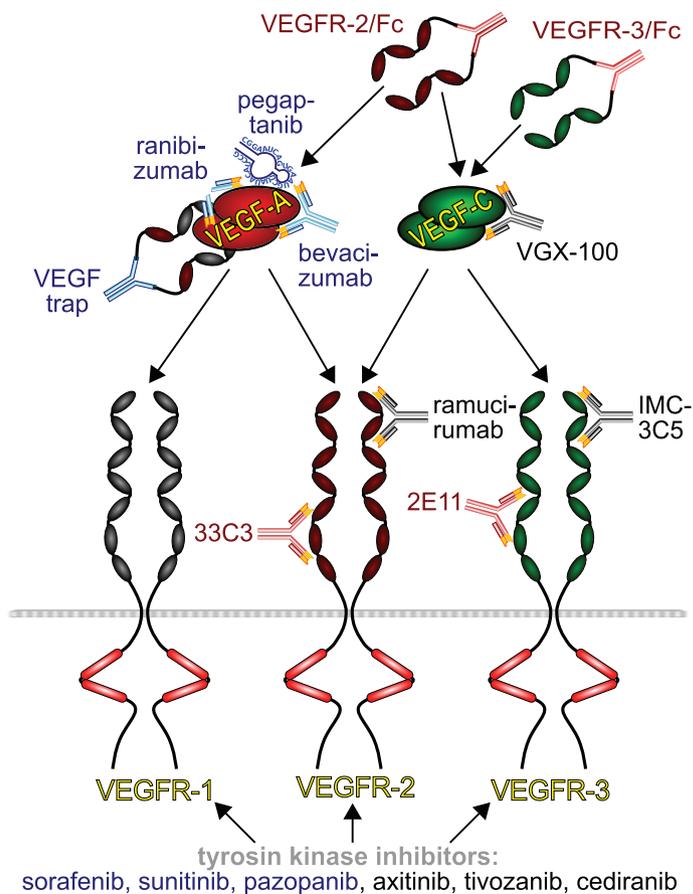


Figure 4.

Drugs, that act on the signal transduction of VEGFs. Biopharmaceuticals and tyrosine kinase inhibitors with marketing approval in blue, in clinical trials in black, in preclinical testing in red. All pharmaceuticals in this figure block signal transduction and act anti-angiogenic and/or anti-lymphangiogenic. The only pro-lymphangiogenic biological drug is Lymfactivin™ (which is identical with VEGF-C).

molecular differences between humans and mice (see Figure 5 in part 1 of this article). However, in this regard, clinical trials would be simplified considerably if the Phase I study of Lymfactivin™ successfully completed. In this regard, it is of interest that in several recent large studies, the orthodox procedure to evacuate tumor cell infiltrated sentinel nodes was not associated with a survival benefit [66-68].

Lymphangiogenesis in inflammatory diseases

Inflammation does not only stimulate angiogenesis [69], but also lymphangiogenesis. Blood and lymph vessels supply the inflammation with immune

cells, which in turn produce a number of growth factors, not in the least VEGF-C itself [70]. Therefore, it is not surprising that in addition to angiogenesis, lymphangiogenesis is often characteristic for chronic inflammatory situations. In autoimmune diseases such as type 1 diabetes [71] and other conditions associated with inflammatory features, such as in chronic respiratory diseases [72, 73], psoriasis [74], and possibly obesity [75], pathological lymphangiogenesis can be observed. Although lymphangiogenesis is not causally involved in the pathogenesis of these diseases, an anti-lymphangiogenic therapy could prove useful because the lymphatic vessels are the preferred trails of disease-promoting immune cells [24].

Tumors of the endothelium and vascular malformations

Hemangiomas

Hemangioma is one of the more common tumors that develop from uncontrolled endothelial cell proliferation. Hemangiomas require treatment only in cases in which the tumor is a larger cosmetic problem or presents a functional risk to the surrounding organs [76]. Hemangiomas regress almost always spontaneously within a few years. It is unknown, why hemangiomas arise, but it is believed that hypoxia-induced changes in the expression of VEGFs and VEGF receptors are involved in causing the uncontrolled angiogenesis [77, 78].

Lymphangiomas

Lymphangiomas are vascular malformations of the lymphatic vessels. They seem to arise exclusively sporadically and thus differ from capillary and venous malformations, of which both sporadic and inherited variants are known [79]. However, the genetic component of venous malformations was not found in the VEGF-receptor genes, but in the genes of the Tie receptors, which are also specific for endothelial cells [80, 81].

Malignant endothelial cell tumors

In contrast to epithelial tumors (carcinomas), the tumors that develop from endothelial cells are usually benign. However, hemangiosarcomas and lymphangiosarcomas are rare, malignant endothelial cell tumors. It is not clear how exactly these tumors develop, but it is interesting that lymphangiosarcoma occurs as rare complication of postmastectomy edema [82].

A more frequently occurring tumor, whose origin is suspected in the reprogramming of lymphatic endothelial cells by herpes virus-8, is Kaposi's sarcoma [83].

Anti-lymphangiogenesis therapy

Anti-lymphangiogenesis therapy does not exist as an independent tool in the treatment of tumors because - unlike angiogenesis - lymphangiogenesis is not a prerequisite for tumor growth. The situation is somewhat different for metastasis, because anti-lymphangiogenesis therapy could play a preventive function [84]. However, clinical studies do not exist. Nevertheless, anti-lymphangiogenesis therapies are used indirectly in clinical trials. Firstly, because tumor angiogenesis co-opts the lymphangiogenic signaling pathways (especially when the primary angiogenic signaling through VEGF-A and VEGFR-2 is therapeutically blocked), and secondly, because the anti-angiogenic tyrosine kinase inhibitors have also an effect on the lymphangiogenic VEGFR-3. Important drugs that target VEGFs and VEGF receptors are shown in Figure 4.

Tyrosine kinase inhibitors

Receptor tyrosine kinase inhibitors are generally analogues of adenosine triphosphate (ATP). A number of them (among others sorafenib, sunitinib and pazopanib) inhibit the enzymatic activity of the intracellular tyrosine kinase domain of the VEGF receptors [85]. In addition to the VEGF receptors, these drugs do inhibit to a variable degree other kinases that are not involved in angiogenesis or lymphangiogenesis, which is a major cause of their side effects.

Clinical trials with the so-called second-generation VEGF receptor tyrosine kinase inhibitors (Tivozanib, Axitinib and Cediranib) showed that they act still anti-angiogenic despite having reduced side effects [85]. Due to the high homology within the VEGF receptor family, also these new RTK inhibitors do not inhibit the angiogenic VEGFR-2 without affecting the lymphangiogenic VEGFR-3. Therefore, it is conceivable that therapies with these inhibitors could also act anti-lymphangiogenic and prevent the formation of secondary tumors.

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VEGF and VEGF receptor blocker blockers

Combining traditional cytotoxic therapy with a blocking VEGF-A by bevacizumab is now standard medical care for certain types of cancers [86, 87]. Despite this, the clinical benefit remains limited, which underlines the complex nature of tumor angiogenesis and the need to simultaneously block the various angiogenesis mechanisms, e.g. VEGF-C-induced angiogenesis.

Therapies directed against VEGF-C have been tested in preclinical studies. Although antibodies against VEGF-C (e. g. VGX-100) have been available for some time and although preliminary reports have been presented at conferences (http://www.circadian.com.au/sites/default/files/VGX-100%20Poster%20Presentation%20at%20EORTC_0.pdf), no positive results of preclinical tumor studies with anti-VEGF-C antibodies have been published in the peer-reviewed literature. In contrast, the metastasis in experimental mouse tumor models could be prevented with the soluble VEGF receptor-3, which also targets VEGF-C [84]. Soluble VEGF receptors bind VEGF growth factors and thus prevent them from binding and activating the VEGF receptors on the cell membrane of endothelial cells.

Soluble variants are available for all VEGF receptors. For example, the fusion protein consisting of the ligand binding domain of VEGF receptor-1 and the constant region of immunoglobulin G (Flt(1-3)IgG) effectively blocked VEGF-A [88]. The fusion protein consisting of parts of the VEGF receptor-1, VEGF receptor-2 and IgG (VEGF-A-Trap) is also an anti-angiogenic molecule [89], that was recently approved for the treatment of wet macular degeneration and oxaliplatin-resistant metastatic colorectal cancer (<http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs>).

Since mature VEGF-C can also bind

the VEGF receptor-2, a soluble VEGF receptor-2 might also inhibit lymphangiogenesis. An endogenous soluble splice variant of VEGF receptor-2 appears to act in the same way as a lymphangiogenesis repressor [90] as the soluble splice variant of VEGF receptor-1 acts as endogenous angiogenesis inhibitors [91].

Whether therapies directed against VEGF-C trials will be successful in clinical as independent anti-angiogenesis drugs is questionable, because for the tumor VEGF-C is likely only a reserve factor to fall back on if the primary angiogenesis factor VEGF-A is therapeutically blocked. In light of this hypothesis, only a combined block of VEGF-A and VEGF-C seems reasonable. The only current clinical study relevant for this question therefore investigates the combination therapy of a monoclonal antibody against VEGF-C (VGX-100) with bevacizumab (<http://clinicaltrials.gov>). That the elimination of multiple angiogenic pathways is promising, has already been shown in preclinical studies by simultaneously blocking VEGF receptor-2 and VEGF receptor-3 [12]. The same VGX-100 antibody that is directed against VEGF-C, showed also efficacy in the treatment of dry eye disease (keratoconjunctivitis sicca) [92].

Theoretically, blocking a receptor that can bind multiple ligands should be more effective in preventing its activation than blocking any individual ligand. Therefore, it is surprising that no antibodies against VEGF receptors are found among the approved anti-angiogenesis drugs. Activation of VEGF receptors requires first the binding of the ligand and thereafter the dimerization of the receptor. Therefore, the receptor activation can be blocked either at the level of ligand binding or at the level of receptor dimerization.

The antibodies against VEGF receptors that are currently in clinical studies prevent the first step: the binding of the ligand [93, 94]. Antibodies that block the second step (receptor dimerization) have the advantage that they do not have to compete with the

ligand for binding to the receptor, and in preclinical studies such antibodies have demonstrated their superiority [95-97].

Therapies with such an antibody against VEGFR-3 (IMC-3C5) are at the moment tested in clinical studies (<http://clinicaltrials.gov/ct2/show/NCT01288989>). In the preclinical studies, the strength of an anti-VEGFR-3 therapies seem to lie rather in the prevention of early metastasis and relapse [98], and the output of the anti-VEGFR-3 studies is therefore uncertain. Another target disease for the same antibody could be lymphangiomyomatosis (LAM). Caused by mutations in the tumor suppressors TSC1 or TSC2, the smooth muscle-like LAM cells infiltrate and obstruct the interstitium and lymphatic vessels of the lungs with the inevitable ultimate consequence of lung transplantation [99]. LAM-cells typically secrete high levels of VEGF-D, which might be related to their affinity for the lymphatic system [100].

Concluding remarks

The proteins of the VEGF family are the key molecules that stimulate vessel growth. VEGF-A is the most important growth factor in the blood vessels, and VEGF-C is the most important for the lymphatic vessels. Because lymphatic vessels can be found almost everywhere in the body, they are involved in many disease processes. The knowledge of the VEGF growth factors and VEGF receptors has led to the development of new therapeutic approaches that belong already to the standard of care, e.g. the anti-angiogenic treatment of some cancers. In contrast, pro-angiogenesis, pro-lymphangiogenesis and anti-lymphangiogenesis therapies are still absent from clinical routine. Because such therapies could provide substantial benefits for patients in many common diseases, this therapeutic gap requires some filling.

But also the existing antiangiogenic therapies do seemingly not reach their full potential and therefore the search for better drugs and drug combinations for tumor treatment is still actively pur-

sued.

Because pro- and anti-angiogenic therapies aim at contradictory objectives, there may also be limits their applicability. At least theoretically, it could be, that a pro-angiogenic therapy of coronary heart disease or a pro-lymphangiogenic therapy of lymphedema increases the risk of cancer. Vice-versa, a long-term cancer therapy with antiangiogenic or anti-lymphangiogenic drugs could affect the blood or lymphatic system.

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The 1st part of this article was published in the 1/2013 issue of this journal.

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