VascularCell - http://vascularcell.com/

REVIEW | OPEN ACCESS

Targeting lymphatic vessel functions through tyrosine kinases

Journal of Angiogenesis Research 2:13 | DOI: 10.1186/2040-2384-2-13 | © Li et al.; licensee Publiverse Online S.R.L. 2010
Received: 21 Jun 2010 | Accepted: 11 Jun 2010 | Published: 11 Jun 2010

Williams Steven P, Karnezis Tara, Achen Marc G, Stacker Steven A[@]

[+] Contributed equally[@] Corresponding author

Abstract

The lymphatic vascular system is actively involved in tissue fluid homeostasis, immune surveillance and fatty acid transport. Pathological conditions can arise from injury to the lymphatics, or they can be recruited in the context of cancer to facilitate metastasis. Protein tyrosine kinases are central players in signal transduction networks and regulation of cell behavior. In the lymphatic endothelium, tyrosine kinases are involved in processes such as the maintenance of existing lymphatic vessels, growth and maturation of new vessels and modulation of their identity and function. As such, they are attractive targets for both existing inhibitors and the development of new inhibitors which affect lymphangiogenesis in pathological states such as cancer. RNAi screening provides an opportunity to identify the functional role of tyrosine kinases in the lymphatics. This review will discuss the role of tyrosine kinases in lymphatic biology and the potential use of inhibitors for anti-lymphangiogenic therapy.

Introduction

A number of human diseases have been linked to abnormal or defective lymphatic vessels [1]. While the theory of anti-angiogenesis therapy has been extensively studied [2], the concept of targeting lymphangiogenesis to gain a therapeutic advantage in human disease is only a recent development [1]. Advances in our understanding of the molecular signaling pathways that control lymphatic vessel formation therefore provide an opportunity to explore the value of inhibiting these processes.

A good example of this is cancer biology, where the spread of tumor cells appears highly dependent on the vessels of the lymphatic system and the protein factors which drive their growth and differentiation [3]. As a consequence, therapeutic options which target these cellular pathways may provide a means to prevent growth or metastasis from the primary tumor. Therapeutics may be either anti-lymphatic (targeting functions of the existing vessels) and/or anti-lymphangiogenic (targeting the generation of new lymphatic vessels). An understanding of the key signaling components and cellular processes that are critical for lymphatic vessel function and growth is essential to enable the rational design of effective inhibitors.

One family of molecules, the protein tyrosine kinases, are known to be key drivers of angiogenesis [4], and studies have shown they also play a pivotal role in lymphatic biology/lymphangiogenesis [5]. In this review we explore the potential for this family of molecules to be used as targets for anti-lymphatic/anti-lymphangiogenesis and the ways in which we can gain insight into how these family members might contribute to key signaling pathways within the lymphatic endothelium.

The lymphatic system in health and disease

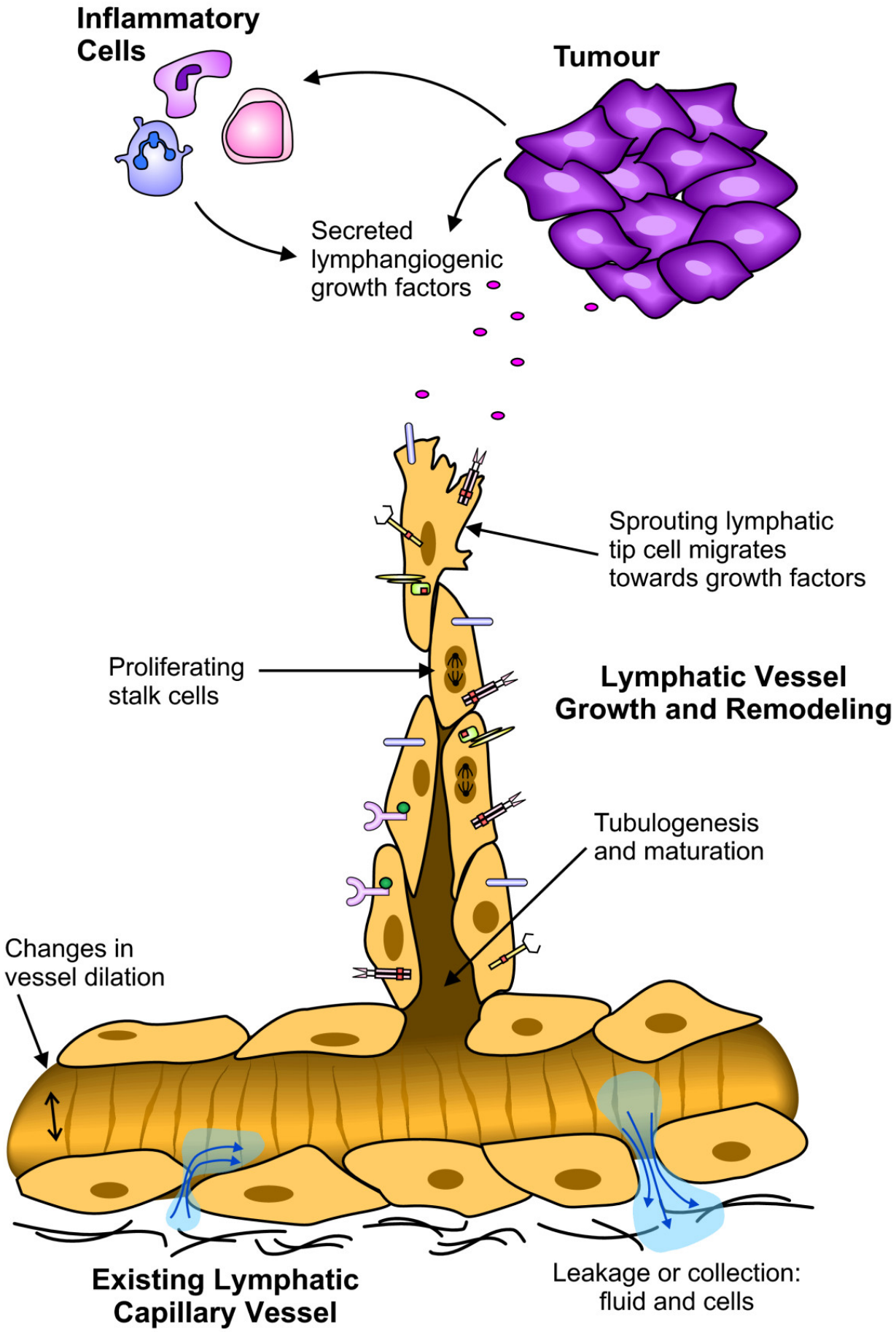
While blood vessels carry oxygenated blood and nutrients to cells within the body, the lymphatic vessels act to maintain fluid homeostasis by draining excess fluid from the tissues, as well as contributing to immune surveillance and fatty acid transport. Fluid and cells released by the blood vessels are returned to the circulation via protein-rich lymph fluid that is drained by blind-ended capillaries in the superficial dermis. This is fed into the deeper, larger caliber lymphatic collecting vessels via lymph nodes and the thoracic duct and back to the circulation. All of these vessels have a specialized lining of endothelial cells. Both blood and lymphatic endothelial cells originate from common developmental precursors. Yet, it is now clear that the lymphatic endothelial cells differ in their molecular and physiological behavior to the "classical" blood endothelial cell [6, 7].

Similarly, the endothelial cells of small lymphatic capillary vessels are distinct in function and gene expression from the lymphatic endothelial cells (LEC) that line the major collecting lymphatic vessels [8]. Interestingly, Baluk et al. recently described the presence of unique cell-cell junctions in lymphatic vessels [9]. They found lymphatic capillaries had discontinuous 'button-like' junctions that would allow flaps of the vessel to open and allow fluid entry. In contrast, collecting lymphatics had continuous 'zipper' junctions, yet in both vessel types the junctions appeared to have the same molecular components. How this organisation is achieved is unknown, but it presumably stems from the functional differences of the lymphatic vessel subtypes.

Florence Sabin's pioneering work of the early 20th century mapped the development of the lymphatic vasculature by injecting blue dye into pig embryos, allowing the vessels to be visualized [10, 11]. This foundation led to recent discoveries showing that early in embryonic development, lymphatic progenitor cells migrate away from the cardinal vein [12]. The process of developmental lymphangiogenesis proceeds with vessels sprouting from the lymph sacs formed from the progenitor cells. Many molecular signals are required to stimulate the correct lymphatic network development and maturation, some of which are discussed below.

In the context of human disease, both blood and lymphatic vessels play important roles. For example, in cancer, tumor progression relies on the angiogenic switch, or the induction of new blood vessel growth [13, 14] for the supply of oxygen required for the tumor to grow. Blood vessels also provide a route for tumor dissemination to distant sites, via invasion of the bloodstream and homing to organs such as the brain, lungs, liver and bone [15]. Tumor angiogenesis (the growth of new blood vessels in a tumor) is therefore a valid target for cancer therapeutics. Recent work has shown that the lymphatic network also plays a central role in the metastasis of cancer, allowing spread to draining lymph nodes [16– 18]. Clinically, many carcinomas are commonly seen to metastasize initially via the lymphatic vasculature to the lymph nodes [15], with the lymphatic vessels providing a key initial entry point for metastatic cancer cells. Numerous studies have shown a significant correlation between levels of the lymphangiogenic vascular endothelial growth factor C (VEGF-C), lymphatic vessel invasion, lymph node metastasis and/or overall survival (reviewed in [3, 15, 19]). Targeting the induction of tumor lymphangiogenesis (the generation of new lymphatic vessels within a tumor), and the signaling that drives functional changes in both new and existing lymphatic vessels (Figure 1), may help to prevent a route for tumor metastasis.

Figure 1



Different functions of LECs in active lymphatic vessels. This schematic outlines some of the cellular processes that occur in lymphatic vessels under pathological conditions such as cancer. In this diagram a tumor (and/or infiltrating immune cells) secretes factors that induce changes in the lymphatic vasculature. Growth factors binding to the different receptors expressed on the surface of the LECs may induce sprouting of new lymphatic vessels from existing lymphatic capillaries. The

leading 'tip cell' detects a gradient of growth factors by means of cell surface receptors, and migrates towards the tumor. Behind the tip cell are the stalk cells, responding to proliferation stimuli. The formation of a lumen and maturation of the vessel is required to create a functional vessel. Other aspects of the vessel such as vessel dilation and vessel permeability to fluid and cells may also be altered. These characteristics may be exaggerated in the context of a tumor, to create the abnormal vessels often associated with cancer and enhance the ease with which lymphogenous metastasis occurs. Many of these responses are induced by signaling pathways involving tyrosine kinases.

In addition to cancer, there are a range of pathological conditions associated with defective or abnormal lymphatic vessels. Lymphedema results from inadequate drainage of fluid from a limb, and can be primary or acquired. Primary lymphedema is rare, but patients are often found to harbour point mutations in key lymphatic genes such as vascular endothelial growth factor receptor 3 (VEGFR-3). Acquired lymphedema can be caused by damage or trauma to the lymphatic vessels (eg sentinel lymph node biopsy), or infection with the parasitic worms that cause filariasis (elephantiasis). Recent work by Tammela et al. [20] demonstrated that by stimulating the VEGFR-3 tyrosine kinase by treatment with the lymphangiogenic vascular endothelial growth factors C or D (VEGF-C or VEGF-D) it is possible to regenerate functional collecting lymphatic vessels in mice following lymph node dissection.
Lymphangioma or lymphangiectasia can result from a build up of fluid, causing an excessive dilation/distension of the lymphatic vessels that is not resolved. Patients (often children) may present with a group of skin lesions that discharge milky fluid, or cystic masses of the head, neck or genitals. Current treatments rely on compression bandages or surgery, although more recently sclerosing agents have been used with some success to induce fibrous obliteration of the vessel [21].
Therefore, understanding the biology of the lymphatics and lymphatic endothelium may provide new options for the treatment of diseases involving the lymphatics, such as cancer, lymphangioma, lymphedema and wound healing.

Tyrosine kinases in vascular biology

Current strategies for targeting tyrosine kinases

Therapeutic targeting of PTKs has been approached from a number of angles, with varying success. Humanized monoclonal antibodies (mAb) raised against the extracellular domains of an RTK have been used. The first FDA approved PTK inhibitor was trastuzumab, a mAb directed to the HER2/neu RTK [63, 64] for use against metastatic breast cancer. Since then, several others have made their way into the clinic; bevacizumab [65, 66], and cetuximab [67, 68] being the most significant examples.
Monoclonal antibody inhibitors of RTKs act via prevention of receptor dimerization and ligand binding, and in some cases may cause receptor internalization and immune cell recruitment [64]. Antibodies generally allow much more specific blocking and thus have the advantage of specificity that small molecule inhibitors tend to lack. Inhibitory antibodies are however, only effective against cell surface receptors, and not against non-receptor tyrosine kinases.
Recent developments in medicinal chemistry and crystallography have led to the possibility of tailor-made small molecule inhibitors that are designed to fit perfectly into the active site of the kinase. These small molecules are able to enter the cell and it is therefore possible to target them to either the intracellular kinase domain of RTKs or the cytoplasmic tyrosine kinases. However one of the caveats of small molecule PTK inhibitors is that kinase domains are highly similar across the families, making selective inhibition difficult. This does mean that multiple pathways may be blocked simultaneously, which may have therapeutic benefit in some cases [27, 69]. The disadvantage of a less selective small molecule PTK inhibitor is greater toxicity and risk of adverse effects. Some PTK inhibitors are well tolerated, however reported effects are anemia, rash, diarrhea, nausea, fatigue, weight loss and hypertension [70, 71].
The prototype small molecule PTK inhibitor is imatinib; targeted to the chimeric protein that occurs in 95% of chronic myeloid leukemia patients as a result of the t(9;22)(q34;q11) translocation [72]. This fusion of the *BCR* gene to *ABL* , creates a constitutively active kinase [73]. Imatinib is able to selectively inhibit BCR-ABL driven cell proliferation at submicromolar concentrations, while having minimal effects on cells that do not have the translocation [74, 75]. Imatinib's mechanism of action is now thought to be one of allosteric inhibition [76], binding to a site adjacent to the ATP pocket. More 'Type II' allosteric inhibitors are now being designed, that act by locking the kinase into an inactive state and preventing signal transduction (reviewed in [77]).

Available strategies for anti-lymphangiogenesis therapy via PTK family

First proposed by Folkman in 1971 [2], anti-angiogenic therapy has now become accepted for cancer treatment [78]. Current strategies for targeting the blood vasculature are focused on inhibition of VEGF and/or blockade of the VEGFRs which activate the downstream pathways [71, 79]. Bevacizumab, also known as Avastin (Genentech), is a monoclonal anti-VEGF antibody that has been approved in combination with chemotherapies for colorectal cancer, non-squamous non-small cell lung cancer, metastatic renal cell carcinoma and metastatic HER2-negative breast cancer [65, 66]. Despite this, there is a risk of side effects such as gastrointestinal perforation, bleeding and impaired wound healing. Bevacizumab's exact mechanism of action is somewhat unclear, and while it may have some anti-angiogenic properties, the key may actually lie in the stabilization of tumor vessels. By normalizing the tumor vessels, the blood flow is increased and interstitial pressure is reduced allowing conventional chemotherapy better access to the tumor.

Other approaches have used soluble forms of VEGFR to create the 'VEGF-trap' (Regeneron), a recombinant chimeric decoy receptor which is in clinical trials [80, 81]. Similarly, ImClone has developed inhibitory antibodies for VEGFR-1 [82] and VEGFR-2 [83– 86], both of which are in clinical trials. A human neutralizing anti-VEGFR-3 antibody has also been generated [87]; in mouse experiments an equivalent antibody to mouse VEGFR-3 was shown to completely block tumor lymphangiogenesis with no effect on preexisting vessels [88] (Table 1). Soluble VEGFR-3 and antibodies targeted to VEGF-C and VEGF-D are in commercial development. Recently, several groups have had success creating peptidomimetics in a form that are resistant to degradation [89, 90]. One of these is targeted to VEGFR-1 and NRP1, and appears effective at blocking angiogenesis in mouse models of retinopathy and cancer [90].

In contrast there are a large number of small molecule inhibitors available that inhibit VEGFR signaling [71]. However many of them also inhibit the activity of other related RTKs such as platelet derived growth factor receptors (PDGFRs), c-KIT and colony stimulating factor 1 receptor (CSF1R) due to similarity in the kinase site, and it is not uncommon to show activity against a wider range of kinases. The VEGF receptor inhibitors that have been FDA approved as chemotherapeutics are sorafenib (Bayer) [91, 92], sunitinib (Pfizer) [93– 95] and pazopanib (GlaxoSmithKline) [96]. One of the commonly seen issues associated with all anti-VEGF treatments is resistance, as alternative proangiogenic pathways are switched on. Small molecule inhibitors that target multiple pathways (e.g. VEGFRs, FGFRs and PDGFRs) simultaneously may avoid this problem, but also increase the risk of associated side-effects. Sorafenib was originally designed to inhibit B-Raf, and was found to be effective in renal and hepatocellular cancers. However, this is now attributed not to the inhibition of B-Raf, but to its activity against VEGFR-2 and PDGFRβ [69]. This leads to blockade of angiogenesis through VEGFR-2, and PDGFRβ inhibition prevents the recruitment of pericytes for vessel stabilization. Recently Murphy et al. [97] reported a second generation 'Type II' inhibitor, designed to be highly selective for PDGFRβ and B-Raf. Oral administration of this compound was able to suppress growth of orthotopic kidney and pancreatic tumors in mice, with significant anti-angiogenic effects.

Eph-Ephrin signaling is a promising anti-angiogenic/anti-lymphangiogenic target. A number of small molecule inhibitors are available [59], including EXEL-7647. EXEL-7647 is currently in clinical trials, and inhibits epidermal growth factor receptor (EGFR), ErbB2, VEGFRs and EphB4 [98, 99]. Other inhibitors in the form of peptidomimetics, inhibitory monoclonal antibodies, and soluble receptors are being tested [59]. It also remains to investigate in more detail the contribution of other Eph receptors to vascular biology; EphA2 signaling has been shown to contribute to tumor angiogenesis, while the ligand ephrinA1 can be upregulated by VEGF [100]. This complex field of Eph signaling, if well understood, could give rise to a range of useful therapeutics.

The nine Src family kinases are cytoplasmic PTKs closely associated with the cell membrane and both RTKs and non-PTK receptors (Figure 2). Src family kinases mediate signal transduction pathways relating to many critical functions of a cell; proliferation, apoptosis, cell adhesion and migration [25]. A number of small molecule inhibitors are available, and several are in clinical trials [25]. Inhibitors of Src family kinases may be useful both to reduce the expression of growth factors in tumor cells [101], as well as having direct effects on the endothelium. Src is known to interact with VEGF receptors, and a selective Src inhibitor significantly reduced human umbilical vein endothelial cell (HUVEC) proliferation and migration *in vitro* [102]. Recently Ischenko et al. showed that the Src inhibitor AZM475271 was effective at blocking VEGF-C driven lymphangiogenesis *in vivo* [103] (Table 1). Previously this inhibitor had been demonstrated to have potent anti-tumor and anti-angiogenic effects in mouse pancreatic cancer models [104]. This suggests a common mechanism that could be targeted to simultaneously block lymphangiogenesis, angiogenesis and tumorigenesis.

Currently there are no PTK inhibitors specifically targeting the lymphatics. Even VEGFR-3, which was thought to be specific to LECs, has now been shown to be expressed at the leading edge of sprouting

blood vasculature [105]. Therefore this remains an attractive target for dual inhibition of blood and lymphatic growth [105]. Encouragingly, it was recently shown that inhibition of the coreceptor NRP2 specifically blocked lymphatic vessel sprouting and migration but did not affect cell proliferation [40, 106]. As many of the trials of PTK inhibitors have been focused on anti-angiogenic efficacy, it remains to be determined whether any possess significant anti-lymphangiogenic potential. Evaluation of specific inhibitors will be required to identify those that have activity in *in vitro* and *in vivo* lymphangiogenesis assays.

Identifying new targets for anti-lymphatic treatment

In order to identify new targets for anti-lymphangiogenic treatments efficiently, screening strategies must be successfully employed. The recent and exciting advent of RNAi technology and high throughput screening systems have allowed researchers to investigate the functional importance of a large number of genes in *in vitro* assays [107– 109]. RNAi screens have been successfully used to identify new molecules involved in many processes including cell cycle [110, 111], apoptosis [112], endocytosis [113], cell migration [114– 116], morphology [117], neural outgrowth [118] and drug resistance [119]. It has also been useful in dissecting molecular pathways to identify new regulators and downstream mediators [120– 124]. Yet this powerful technique has hardly been utilized in studying endothelial cell biology. RNAi screens could potentially identify new anti-lymphangiogenic targets by screening for LEC migration and proliferation genes, or by screening for regulators of key molecular pathways. Many commercial companies now offer siRNA libraries covering the human kinome, making RNAi screening feasible for research laboratories. RNAi screens are primarily considered a target identification tool, as there are still some obstacles to be overcome to the clinical application of siRNA therapy. In addition, hits from a screen may not be easily druggable, or a drug may give a different phenotype to the siRNA [27]. Nonetheless, a recent study does show that there are feasible and effective methods for specific targeting and delivery of siRNAs in humans [125], suggesting the RNAi screen may soon be used as a direct therapeutic agent identification tool.

High throughput screening of chemical libraries offers the opportunity to screen thousands of compounds to identify small molecule inhibitors of a cell process of interest [126– 129]. If a key kinase target is known, the assay readout can be set to indicate whether the compound is on-target [130]. Chemical library screens are commonly performed *in vitro* , however the use of model organisms such as Xenopus and Zebrafish has enabled high throughput chemical screens to be carried out *in vivo* . Kälin et al. recently screened 1280 compounds looking for modulators of angiogenesis and lymphangiogenesis in Xenopus [131]. Interestingly, several compounds known to inhibit tyrosine kinases were identified as having selective anti-lymphangiogenic activity.

Alternatively, once a target has been identified, rational drug design can be used to develop a compound that binds with high specificity [77]. This approach has been used to create drugs such as imatinib, but also more recently a selective inhibitor of both B-Raf and PDGFRβ [97]. Finding the balance between highly selective compounds and still inhibiting the multiple necessary pathways to see maximal effect without causing severe side-effects will require a combination of approaches. RNAi screening allows the entire genome to be screened, including the thousands of virtually unannotated genes. Similarly, chemical libraries now comprise hundreds of thousands of compounds, many of which are unknown. These platform technologies may soon provide targets and lead compounds, and eventually give rise to reagents targeting protein tyrosine kinases for anti-lymphangiogenic therapy that have clinical application.

Conclusions

The recent renaissance in lymphatic endothelial biology has led to a better understanding of the important role these vessels play in health and disease. It is now apparent that specific targeting of protein tyrosine kinases is an effective way to elicit anti-angiogenic responses in the context of cancer therapy. Similar approaches could be used to target lymphatics to prevent metastasis, while in other pathological conditions such as lymphedema, targeted therapy may be used to restore their growth and subsequent function. Some of these treatments have been developed to existing targets such as the VEGFRs and their ligands. Further testing will be required to fully determine their efficacy, but there are also potentially many novel targets not yet discovered or not currently associated with lymphatic biology.

Declarations

Acknowledgements

This work was funded partly by a Program Grant from the National Health and Medical Research Council of Australia (NH&MRC). SAS and MGA are supported by Senior Research Fellowships from the NH&MRC. SAS would like to acknowledge the support of the Pfizer Australia Fellowship. SPW is supported by a National Breast Cancer Foundation Doctoral Research Scholarship. The authors thank J. Taylor for assistance in generating figures.

Authors' original submitted files for images

Below are the links to the authors' original submitted files for images.

Authors' original file for figure 1

Authors' original file for figure 2

Competing interests

SAS and MGA are consultants for Vegenics Ltd, a company which develops inhibitors of VEGF receptors.

Authors' contributions

SPW, TK, MGA and SAS were involved in preparation of the manuscript. All the authors read and approved the final manuscript.

References

1. Tammela T, Alitalo K. Lymphangiogenesis: Molecular mechanisms and future promise. Cell. 2010;140:460-476.
View Article Google Scholar

2. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971;285:1182-1186.
View Article Google Scholar

3. Stacker SA, Achen MG, Jussila L, Baldwin ME, Alitalo K. Lymphangiogenesis and cancer metastasis. Nat Rev Cancer. 2002;2:573-583.
View Article Google Scholar

4. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. Nat Rev Mol Cell Biol. 2006;7:359-371.
View Article Google Scholar

5. Adams RH, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. Nat Rev Mol Cell Biol. 2007;8:464-478.
View Article Google Scholar

6. Kriehuber E Breiteneder-Geleff S Groeger M Soleiman A Schoppmann SF Stingl G Kerjaschki D Maurer D Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages J Exp Med 2001 194 797 8082195953 10.1084/jem.194.6.797

7. Podgrabinska S Braun P Velasco P Kloos B Pepper MS Skobe M Molecular characterization of lymphatic endothelial cells Proc Natl Acad Sci USA 2002 99 16069 16074138566 10.1073/pnas.242401399

8. Shayan R, Achen MG, Stacker SA. Lymphatic vessels in cancer metastasis: bridging the gaps. Carcinogenesis. 2006;27:1729-1738.
View Article Google Scholar

9. Baluk P Fuxe J Hashizume H Romano T Lashnits E Butz S Vestweber D Corada M Molendini C Dejana E McDonald DM Functionally specialized junctions between endothelial cells of lymphatic vessels J Exp Med 2007 204 2349 23622118470 10.1084/jem.20062596

10. Sabin F. On the origin of the lymphatic system from the veins and the development of the lymph hearts and thoracic duct in the pig. American Journal of Anatomy. 1902;1:367-389.
View Article Google Scholar

11. Sabin F. On the development of the superficial lymphatics in the skin of the pig. American Journal of Anatomy. 1904;3:183-195.
View Article Google Scholar

12. Oliver G. Lymphatic vasculature development. Nat Rev Immunol. 2004;4:35-45.
View Article Google Scholar

13. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. Semin Cancer Biol. 2009;19:329-337.
View Article Google Scholar

14. Naumov GN, Akslen LA, Folkman J. Role of angiogenesis in human tumor dormancy: animal models of the angiogenic switch. Cell Cycle. 2006;5:1779-1787.
View Article Google Scholar

15. Leong SP, Cady B, Jablons DM, Garcia-Aguilar J, Reintgen D, Jakub J, Pendas S, Duhaime L, Cassell R, Gardner M, et al. Clinical patterns of metastasis. Cancer Metastasis Rev. 2006;25:221-232.
View Article Google Scholar

16. Mandriota SJ Jussila L Jeltsch M Compagni A Baetens D Prevo R Banerji S Huarte J Montesano R Jackson DG Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis EMBO J 2001 20 672 682145430 10.1093/emboj/20.4.672

17. Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, Velasco P, Riccardi L, Alitalo K, Claffey K, Detmar M. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med. 2001;7:192-198.
View Article Google Scholar

18. Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, Jackson DG, Nishikawa S, Kubo H, Achen MG. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat Med. 2001;7:186-191.
View Article Google Scholar

19. Ran S, Volk L, Hall K, Flister MJ. Lymphangiogenesis and lymphatic metastasis in breast cancer. Pathophysiology. 2009;:-.
View Article Google Scholar

20. Tammela T, Saaristo A, Holopainen T, Lyytikka J, Kotronen A, Pitkonen M, Abo-Ramadan U, Yla-Herttuala S, Petrova TV, Alitalo K. Therapeutic differentiation and maturation of lymphatic vessels after lymph node dissection and transplantation. Nat Med. 2007;13:1458-1466.
View Article Google Scholar

21. Okazaki T, Iwatani S, Yanai T, Kobayashi H, Kato Y, Marusasa T, Lane GJ, Yamataka A. Treatment of lymphangioma in children: our experience of 128 cases. J Pediatr Surg. 2007;42:386-389.
View Article Google Scholar

22. Robinson DR, Wu YM, Lin SF. The protein tyrosine kinase family of the human genome. Oncogene. 2000;19:5548-5557.
View Article Google Scholar

23. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science. 2002;298:1912-1934.
View Article Google Scholar

24. Yeatman TJ. A renaissance for SRC. Nat Rev Cancer. 2004;4:470-480.
View Article Google Scholar

25. Kim LC, Song L, Haura EB. Src kinases as therapeutic targets for cancer. Nat Rev Clin Oncol. 2009;6:587-595.
View Article Google Scholar

26. Sardi SP, Murtie J, Koirala S, Patten BA, Corfas G. Presenilin-dependent ErbB4 nuclear signaling regulates the timing of astrogenesis in the developing brain. Cell. 2006;127:185-197.
View Article Google Scholar

27. Knight ZA Lin H Shokat KM Targeting the cancer kinome through polypharmacology Nat Rev Cancer 2010 10 130 1372880454 10.1038/nrc2787

28. Achen MG, Stacker SA. Targeting tumor stroma. Curr Cancer Drug Targets. 2008;8:446-.
View Article Google Scholar

29. Bahram F, Claesson-Welsh L. VEGF-mediated signal transduction in lymphatic endothelial cells. Pathophysiology. 2009;:-.
View Article Google Scholar

30. Terman BI, Dougher-Vermazen M, Carrion ME, Dimitrov D, Armellino DC, Gospodarowicz D, Bohlen P. Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem Biophys Res Commun. 1992;187:1579-1586.
View Article Google Scholar

31. de Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT. The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science. 1992;255:989-991.
View Article Google Scholar

32. Achen MG Jeltsch M Kukk E Makinen T Vitali A Wilks AF Alitalo K Stacker SA Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4) Proc Natl Acad Sci USA 1998 95 548 55318457 10.1073/pnas.95.2.548

33. Joukov V Pajusola K Kaipainen A Chilov D Lahtinen I Kukk E Saksela O Kalkkinen N Alitalo K A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases Embo J 1996 15 290 298449944

34. Makinen T Veikkola T Mustjoki S Karpanen T Catimel B Nice EC Wise L Mercer A Kowalski H Kerjaschki D Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the VEGF-C/D receptor VEGFR-3 EMBO J 2001 20 4762 4773125596 10.1093/emboj/20.17.4762

35. Mandriota SJ Jussila L Jeltsch M Compagni A Baetens D Prevo R Banerji S Huarte J Montesano R Jackson DG Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis EMBO J 2001 20 672 682145430 10.1093/emboj/20.4.672

36. Haiko P Makinen T Keskitalo S Taipale J Karkkainen MJ Baldwin ME Stacker SA Achen MG Alitalo K Deletion of vascular endothelial growth factor C (VEGF-C) and VEGF-D is not equivalent to VEGF receptor 3 deletion in mouse embryos Mol Cell Biol 2008 28 4843 48502493372 10.1128/MCB.02214-07

37. Dixelius J, Makinen T, Wirzenius M, Karkkainen MJ, Wernstedt C, Alitalo K, Claesson-Welsh L. Ligand-induced vascular endothelial growth factor receptor-3 (VEGFR-3) heterodimerization with VEGFR-2 in primary lymphatic endothelial cells regulates tyrosine phosphorylation sites. J Biol Chem. 2003;278:40973-40979.
View Article Google Scholar

38. Nilsson I Bahram F Li X Gualandi L Koch S Jarvius M Soderberg O Anisimov A Kholova I Pytowski B VEGF receptor 2/-3 heterodimers detected in situ by proximity ligation on angiogenic sprouts EMBO J 2010 29 1377 13882868571 10.1038/emboj.2010.30

39. Whitaker GB, Limberg BJ, Rosenbaum JS. Vascular endothelial growth factor receptor-2 and neuropilin-1 form a receptor complex that is responsible for the differential signaling potency of VEGF(165) and VEGF(121). J Biol Chem. 2001;276:25520-25531.
View Article Google Scholar

40. Xu Y Yuan L Mak J Pardanaud L Caunt M Kasman I Larrivee B Del Toro R Suchting S Medvinsky A Neuropilin-2 mediates VEGF-C-induced lymphatic sprouting together with VEGFR3 J Cell Biol 2010 188 115 1302812843 10.1083/jcb.200903137

41. Favier B, Alam A, Barron P, Bonnin J, Laboudie P, Fons P, Mandron M, Herault JP, Neufeld G, Savi P, et al. Neuropilin-2 interacts with VEGFR-2 and VEGFR-3 and promotes human endothelial cell survival and migration. Blood. 2006;108:1243-1250.
View Article Google Scholar

42. Karpanen T, Heckman CA, Keskitalo S, Jeltsch M, Ollila H, Neufeld G, Tamagnone L, Alitalo K. Functional interaction of VEGF-C and VEGF-D with neuropilin receptors. FASEB J. 2006;20:1462-1472.
View Article Google Scholar

43. Wang L Dutta SK Kojima T Xu X Khosravi-Far R Ekker SC Mukhopadhyay D Neuropilin-1 modulates p53/caspases axis to promote endothelial cell survival PLoS One 2007 2 e11612048754 10.1371/journal.pone.0001161

44. Achen MG, Stacker SA. Tumor lymphangiogenesis and metastatic spread-new players begin to emerge. Int J Cancer. 2006;119:1755-1760.
View Article Google Scholar

45. Cao R, Bjorndahl MA, Gallego MI, Chen S, Religa P, Hansen AJ, Cao Y. Hepatocyte growth factor is a lymphangiogenic factor with an indirect mechanism of action. Blood. 2006;107:3531-3536.
View Article Google Scholar

46. Kajiya K Hirakawa S Ma B Drinnenberg I Detmar M Hepatocyte growth factor promotes lymphatic vessel formation and function EMBO J 2005 24 2885 28951187946 10.1038/sj.emboj.7600763

47. Shin JW Min M Larrieu-Lahargue F Canron X Kunstfeld R Nguyen L Henderson JE Bikfalvi A Detmar M Hong YK Prox1 promotes lineage-specific expression of fibroblast growth factor (FGF) receptor-3 in lymphatic endothelium: a role for FGF signaling in lymphangiogenesis Mol Biol Cell 2006 17 576 5841356570 10.1091/mbc.E05-04-0368

48. Bjorndahl M Cao R Nissen LJ Clasper S Johnson LA Xue Y Zhou Z Jackson D Hansen AJ Cao Y Insulin-like growth factors 1 and 2 induce lymphangiogenesis in vivo Proc Natl Acad Sci USA 2005 102 15593 155981266150 10.1073/pnas.0507865102

49. Thurston G. Role of Angiopoietins and Tie receptor tyrosine kinases in angiogenesis and lymphangiogenesis. Cell Tissue Res. 2003;314:61-68.
View Article Google Scholar

50. Nguyen VP Chen SH Trinh J Kim H Coomber BL Dumont DJ Differential response of lymphatic, venous and arterial endothelial cells to angiopoietin-1 and angiopoietin-2 BMC Cell Biol 2007 8 101828055 10.1186/1471-2121-8-10

51. Dumont DJ, Gradwohl G, Fong GH, Puri MC, Gertsenstein M, Auerbach A, Breitman ML. Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo. Genes Dev. 1994;8:1897-1909.
View Article Google Scholar

52. Sato TN, Tozawa Y, Deutsch U, Wolburg-Buchholz K, Fujiwara Y, Gendron-Maguire M, Gridley T, Wolburg H, Risau W, Qin Y. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. Nature. 1995;376:70-74.
View Article Google Scholar

53. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell. 1996;87:1171-1180.
View Article Google Scholar

54. Puri MC Rossant J Alitalo K Bernstein A Partanen J The receptor tyrosine kinase TIE is required for integrity and survival of vascular endothelial cells EMBO J 1995 14 5884 5891394706

55. D'Amico G, Korhonen EA, Waltari M, Saharinen P, Laakkonen P, Alitalo K. Loss of endothelial Tie1 receptor impairs lymphatic vessel development-brief report. Arterioscler Thromb Vasc Biol. 2010;30:207-209.
View Article Google Scholar

56. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature. 1995;376:62-66.
View Article Google Scholar

57. Gale NW, Thurston G, Hackett SF, Renard R, Wang Q, McClain J, Martin C, Witte C, Witte MH, Jackson D, et al. Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1. Dev Cell. 2002;3:411-423.
View Article Google Scholar

58. Kuijper S, Turner CJ, Adams RH. Regulation of angiogenesis by Eph-ephrin interactions. Trends Cardiovasc Med. 2007;17:145-151.
View Article Google Scholar

59. Pasquale EB Eph receptors and ephrins in cancer: bidirectional signalling and beyond Nat Rev Cancer 2010 10 165 1802921274 10.1038/nrc2806

60. Makinen T Adams RH Bailey J Lu Q Ziemiecki A Alitalo K Klein R Wilkinson GA PDZ interaction site in ephrinB2 is required for the remodeling of lymphatic vasculature Genes Dev 2005 19 397 410546518 10.1101/gad.330105

61. Kim YH Hu H Guevara-Gallardo S Lam MT Fong SY Wang RA Artery and vein size is balanced by Notch and ephrin B2/EphB4 during angiogenesis Development 2008 135 3755 37642596923 10.1242/dev.022475

62. Mishima K Watabe T Saito A Yoshimatsu Y Imaizumi N Masui S Hirashima M Morisada T Oike Y Araie M Prox1 induces lymphatic endothelial differentiation via integrin alpha9 and other signaling cascades Mol Biol Cell 2007 18 1421 14291838981 10.1091/mbc.E06-09-0780

63. Goldenberg MM. Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer. Clin Ther. 1999;21:309-318.
View Article Google Scholar

64. Hudis CA. Trastuzumab--mechanism of action and use in clinical practice. N Engl J Med. 2007;357:39-51.
View Article Google Scholar

65. Presta LG, Chen H, O'Connor SJ, Chisholm V, Meng YG, Krummen L, Winkler M, Ferrara N. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res. 1997;57:4593-4599.
View Article Google Scholar

66. Yang JC Haworth L Sherry RM Hwu P Schwartzentruber DJ Topalian SL Steinberg SM Chen HX Rosenberg SA A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer N Engl J Med 2003 349 427 4342275324 10.1056/NEJMoa021491

67. Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. Clin Cancer Res. 1995;1:1311-1318.
View Article Google Scholar

68. Herbst RS, Hong WK. IMC-C225, an anti-epidermal growth factor receptor monoclonal antibody for treatment of head and neck cancer. Semin Oncol. 2002;29:18-30.
View Article Google Scholar

69. Bergers G Song S Meyer-Morse N Bergsland E Hanahan D Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors J Clin Invest 2003 111 1287 1295154450 10.1172/JCI200317929

70. Smith JK, Mamoon NM, Duhe RJ. Emerging roles of targeted small molecule protein-tyrosine kinase inhibitors in cancer therapy. Oncol Res. 2004;14:175-225.
View Article Google Scholar

71. Ivy SP, Wick JY, Kaufman BM. An overview of small-molecule inhibitors of VEGFR signaling. Nat Rev Clin Oncol. 2009;6:569-579.
View Article Google Scholar

72. Hermans A, Heisterkamp N, von Linden M, van Baal S, Meijer D, van der Plas D, Wiedemann LM, Groffen J, Bootsma D, Grosveld G. Unique fusion of bcr and c-abl genes in Philadelphia chromosome positive acute lymphoblastic leukemia. Cell. 1987;51:33-40.
View Article Google Scholar

73. Konopka JB, Watanabe SM, Witte ON. An alteration of the human c-abl protein in K562 leukemia cells unmasks associated tyrosine kinase activity. Cell. 1984;37:1035-1042.
View Article Google Scholar

74. Buchdunger E, Zimmermann J, Mett H, Meyer T, Muller M, Druker BJ, Lydon NB. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res. 1996;56:100-104.
View Article Google Scholar

75. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996;2:561-566.
View Article Google Scholar

76. Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. Science. 2000;289:1938-1942.
View Article Google Scholar

77. Liu Y, Gray NS. Rational design of inhibitors that bind to inactive kinase conformations. Nat Chem Biol. 2006;2:358-364.
View Article Google Scholar

78. Ellis LM, Hicklin DJ. VEGF-targeted therapy: mechanisms of anti-tumour activity. Nat Rev Cancer. 2008;8:579-591.
View Article Google Scholar

79. Schneider BP, Sledge GW. Drug insight: VEGF as a therapeutic target for breast cancer. Nat Clin Pract Oncol. 2007;4:181-189.
View Article Google Scholar

80. Konner J, Dupont J. Use of soluble recombinant decoy receptor vascular endothelial growth factor trap (VEGF Trap) to inhibit vascular endothelial growth factor activity. Clin Colorectal Cancer. 2004;4:S81-85.

View Article Google Scholar

81. Lockhart AC Rothenberg ML Dupont J Cooper W Chevalier P Sternas L Buzenet G Koehler E Sosman JA Schwartz LH Phase I study of intravenous vascular endothelial growth factor trap, aflibercept, in patients with advanced solid tumors J Clin Oncol 2010 28 207 2142815710 10.1200/JCO.2009.22.9237

82. Wu Y, Zhong Z, Huber J, Bassi R, Finnerty B, Corcoran E, Li H, Navarro E, Balderes P, Jimenez X, et al. Anti-vascular endothelial growth factor receptor-1 antagonist antibody as a therapeutic agent for cancer. Clin Cancer Res. 2006;12:6573-6584.
View Article Google Scholar

83. Hsu JY, Wakelee HA. Monoclonal antibodies targeting vascular endothelial growth factor: current status and future challenges in cancer therapy. BioDrugs. 2009;23:289-304.
View Article Google Scholar

84. Krupitskaya Y, Wakelee HA. Ramucirumab, a fully human mAb to the transmembrane signaling tyrosine kinase VEGFR-2 for the potential treatment of cancer. Curr Opin Investig Drugs. 2009;10:597-605.
View Article Google Scholar

85. Mackey J, Gelmon K, Martin M, McCarthy N, Pinter T, Rupin M, Youssoufian H. TRIO-012: a multicenter, multinational, randomized, double-blind phase III study of IMC-1121B plus docetaxel versus placebo plus docetaxel in previously untreated patients with HER2-negative, unresectable, locally recurrent or metastatic breast cancer. Clin Breast Cancer. 2009;9:258-261.
View Article Google Scholar

86. Spratlin JL Cohen RB Eadens M Gore L Camidge DR Diab S Leong S O'Bryant C Chow LQ Serkova NJ 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 7872834394 10.1200/JCO.2009.23.7537

87. Persaud K, Tille JC, Liu M, Zhu Z, Jimenez X, Pereira DS, Miao HQ, Brennan LA, Witte L, Pepper MS, Pytowski B. Involvement of the VEGF receptor 3 in tubular morphogenesis demonstrated with a human anti-human VEGFR-3 monoclonal antibody that antagonizes receptor activation by VEGF-C. J Cell Sci. 2004;117:2745-2756.
View Article Google Scholar

88. Pytowski B, Goldman J, Persaud K, Wu Y, Witte L, Hicklin DJ, Skobe M, Boardman KC, Swartz MA. Complete and specific inhibition of adult lymphatic regeneration by a novel VEGFR-3 neutralizing antibody. J Natl Cancer Inst. 2005;97:14-21.
View Article Google Scholar

89. Cardo-Vila M Giordano RJ Sidman RL Bronk LF Fan Z Mendelsohn J Arap W Pasqualini R From combinatorial peptide selection to drug prototype (II): Targeting the epidermal growth factor receptor pathway Proc Natl Acad Sci USA 2010 107 5118 51232841862 10.1073/pnas.0915146107

90. Giordano RJ Cardo-Vila M Salameh A Anobom CD Zeitlin BD Hawke DH Valente AP Almeida FC Nor JE Sidman RL From combinatorial peptide selection to drug prototype (I): Targeting the vascular endothelial growth factor receptor pathway Proc Natl Acad Sci USA 2010 107 5112 51172841949 10.1073/pnas.0915141107

91. Strumberg D, Clark JW, Awada A, Moore MJ, Richly H, Hendlisz A, Hirte HW, Eder JP, Lenz HJ, Schwartz B. Safety, pharmacokinetics, and preliminary antitumor activity of sorafenib: a review of four phase I trials in patients with advanced refractory solid tumors. Oncologist. 2007;12:426-437.
View Article Google Scholar

92. Strumberg D, Richly H, Hilger RA, Schleucher N, Korfee S, Tewes M, Faghih M, Brendel E, Voliotis D, Haase CG, et al. Phase I clinical and pharmacokinetic study of the Novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors. J Clin Oncol. 2005;23:965-972.
View Article Google Scholar

93. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, Schreck RE, Abrams TJ, Ngai TJ, Lee LB, et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. ClinCancer Res. 2003;9:327-337.
View Article Google Scholar

94. Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, Ginsberg MS, Kim ST, Baum CM, DePrimo SE, et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol. 2006;24:16-24.
View Article Google Scholar

95. Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, Bello C, Deprimo S, Brega N, Massimini G, et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol. 2006;24:25-35.
View Article Google Scholar

96. Kumar R, Knick VB, Rudolph SK, Johnson JH, Crosby RM, Crouthamel MC, Hopper TM, Miller CG, Harrington LE, Onori JA, et al. Pharmacokinetic-pharmacodynamic correlation from mouse to human with pazopanib, a multikinase angiogenesis inhibitor with potent antitumor and antiangiogenic activity. Mol Cancer Ther. 2007;6:2012-2021.
View Article Google Scholar

97. Murphy EA Shields DJ Stoletov K Dneprovskaia E McElroy M Greenberg JI Lindquist J Acevedo LM Anand S Majeti BK Disruption of angiogenesis and tumor growth with an orally active drug that stabilizes the inactive state of PDGFRb/B-RAF Proc Natl Acad Sci USA 2010 107 4299 43042840076 10.1073/pnas.0909299107

98. Gendreau SB, Ventura R, Keast P, Laird AD, Yakes FM, Zhang W, Bentzien F, Cancilla B, Lutman J, Chu F, et al. Inhibition of the T790 M gatekeeper mutant of the epidermal growth factor receptor by EXEL-7647. Clin Cancer Res. 2007;13:3713-3723.
View Article Google Scholar

99. Pennell NA, Lynch TJ. Combined inhibition of the VEGFR and EGFR signaling pathways in the treatment of NSCLC. Oncologist. 2009;14:399-411.
View Article Google Scholar

100. Cheng N, Brantley DM, Liu H, Lin Q, Enriquez M, Gale N, Yancopoulos G, Cerretti DP, Daniel TO, Chen J. Blockade of EphA receptor tyrosine kinase activation inhibits vascular endothelial cell growth factor-induced angiogenesis. Mol Cancer Res. 2002;1:2-11.
View Article Google Scholar

101. Summy JM, Trevino JG, Lesslie DP, Baker CH, Shakespeare WC, Wang Y, Sundaramoorthi R, Metcalf CA, Keats JA, Sawyer TK, Gallick GE. AP23846, a novel and highly potent Src family kinase inhibitor, reduces vascular endothelial growth factor and interleukin-8 expression in human solid tumor cell lines and abrogates downstream angiogenic processes. Mol Cancer Ther. 2005;4:1900-1911.
View Article Google Scholar

102. Ali N, Yoshizumi M, Fujita Y, Izawa Y, Kanematsu Y, Ishizawa K, Tsuchiya K, Yano S, Sone S, Tamaki T. A novel Src kinase inhibitor, M475271, inhibits VEGF-induced human umbilical vein endothelial cell proliferation and migration. J Pharmacol Sci. 2005;98:130-141.
View Article Google Scholar

103. Ischenko I, Seeliger H, Camaj P, Kleespies A, Guba M, Eichhorn ME, Jauch KW, Bruns CJ. Src tyrosine kinase inhibition suppresses lymphangiogenesis in vitro and in vivo. Curr Cancer Drug Targets. 2010;10:546-553.
View Article Google Scholar

104. Ischenko I, Guba M, Yezhelyev M, Papyan A, Schmid G, Green T, Fennell M, Jauch KW, Bruns CJ. Effect of Src kinase inhibition on metastasis and tumor angiogenesis in human pancreatic cancer. Angiogenesis. 2007;10:167-182.
View Article Google Scholar

105. Tammela T, Zarkada G, Wallgard E, Murtomaki A, Suchting S, Wirzenius M, Waltari M, Hellstrom M, Schomber T, Peltonen R, et al. Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation. Nature. 2008;454:656-660.
View Article Google Scholar

106. Caunt M, Mak J, Liang WC, Stawicki S, Pan Q, Tong RK, Kowalski J, Ho C, Reslan HB, Ross J, et al. Blocking neuropilin-2 function inhibits tumor cell metastasis. Cancer Cell. 2008;13:331-342.
View Article Google Scholar

107. Boutros M, Ahringer J. The art and design of genetic screens: RNA interference. Nat Rev Genet. 2008;9:554-566.
View Article Google Scholar

108. Carpenter AE, Sabatini DM. Systematic genome-wide screens of gene function. Nat Rev Genet. 2004;5:11-22.
View Article Google Scholar

109. Echeverri CJ, Perrimon N. High-throughput RNAi screening in cultured cells: a user's guide. Nat Rev Genet. 2006;7:373-384.
View Article Google Scholar

110. Mukherji M Bell R Supekova L Wang Y Orth AP Batalov S Miraglia L Huesken D Lange J Martin C Genome-wide functional analysis of human cell-cycle regulators Proc Natl Acad Sci USA 2006 103 14819 148241595435 10.1073/pnas.0604320103

111. Kittler R, Pelletier L, Heninger AK, Slabicki M, Theis M, Miroslaw L, Poser I, Lawo S, Grabner H, Kozak K, et al. Genome-scale RNAi profiling of cell division in human tissue culture cells. Nat Cell Biol. 2007;9:1401-1412.
View Article Google Scholar

112. Aza-Blanc P, Cooper CL, Wagner K, Batalov S, Deveraux QL, Cooke MP. Identification of modulators of TRAIL-induced apoptosis via RNAi-based phenotypic screening. Mol Cell.

2003;12:627-637.
View Article Google Scholar

113. Pelkmans L, Fava E, Grabner H, Hannus M, Habermann B, Krausz E, Zerial M. Genome-wide analysis of human kinases in clathrin-and caveolae/raft-mediated endocytosis. Nature. 2005;436:78-86.
View Article Google Scholar

114. Collins CS Hong J Sapinoso L Zhou Y Liu Z Micklash K Schultz PG Hampton GM A small interfering RNA screen for modulators of tumor cell motility identifies MAP4K4 as a promigratory kinase Proc Natl Acad Sci USA 2006 103 3775 37801383649 10.1073/pnas.0600040103

115. Vitorino P Meyer T Modular control of endothelial sheet migration Genes Dev 2008 22 3268 32812600767 10.1101/gad.1725808

116. Simpson KJ, Selfors LM, Bui J, Reynolds A, Leake D, Khvorova A, Brugge JS. Identification of genes that regulate epithelial cell migration using an siRNA screening approach. Nat Cell Biol. 2008;10:1027-1038.
View Article Google Scholar

117. Kiger AA Baum B Jones S Jones MR Coulson A Echeverri C Perrimon N A functional genomic analysis of cell morphology using RNA interference J Biol 2003 2 27333409 10.1186/1475-4924-2-27

118. Sepp KJ Hong P Lizarraga SB Liu JS Mejia LA Walsh CA Perrimon N Identification of neural outgrowth genes using genome-wide RNAi PLoS Genet 2008 4 e10001112435276 10.1371/journal.pgen.1000111

119. Mullenders J, Bernards R. Loss-of-function genetic screens as a tool to improve the diagnosis and treatment of cancer. Oncogene. 2009;28:4409-4420.
View Article Google Scholar

120. Friedman A, Perrimon N. High-throughput approaches to dissecting MAPK signaling pathways. Methods. 2006;40:262-271.
View Article Google Scholar

121. Lum L, Yao S, Mozer B, Rovescalli A, Von Kessler D, Nirenberg M, Beachy PA. Identification of Hedgehog pathway components by RNAi in Drosophila cultured cells. Science. 2003;299:2039-2045.
View Article Google Scholar

122. Berns K, Hijmans EM, Mullenders J, Brummelkamp TR, Velds A, Heimerikx M, Kerkhoven RM, Madiredjo M, Nijkamp W, Weigelt B, et al. A large-scale RNAi screen in human cells identifies new components of the p53 pathway. Nature. 2004;428:431-437.
View Article Google Scholar

123. Friedman A, Perrimon N. A functional RNAi screen for regulators of receptor tyrosine kinase and ERK signalling. Nature. 2006;444:230-234.
View Article Google Scholar

124. Muller P, Kuttenkeuler D, Gesellchen V, Zeidler MP, Boutros M. Identification of JAK/STAT signalling components by genome-wide RNA interference. Nature. 2005;436:871-875.
View Article Google Scholar

125. Davis ME Zuckerman JE Choi CH Seligson D Tolcher A Alabi CA Yen Y Heidel JD Ribas A Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles Nature 2010 464 1067 10702855406 10.1038/nature08956

126. Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA, Lander ES. Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell. 2009;138:645-659.
View Article Google Scholar

127. Yarrow JC, Totsukawa G, Charras GT, Mitchison TJ. Screening for cell migration inhibitors via automated microscopy reveals a Rho-kinase inhibitor. Chem Biol. 2005;12:385-395.
View Article Google Scholar

128. Yarrow JC, Feng Y, Perlman ZE, Kirchhausen T, Mitchison TJ. Phenotypic screening of small molecule libraries by high throughput cell imaging. Comb Chem High Throughput Screen. 2003;6:279-286.
View Article Google Scholar

129. Melnick JS Janes J Kim S Chang JY Sipes DG Gunderson D Jarnes L Matzen JT Garcia ME Hood TL An efficient rapid system for profiling the cellular activities of molecular libraries Proc Natl Acad Sci USA 2006 103 3153 31581413928 10.1073/pnas.0511292103

130. Fong TAT, Shawver LK, Sun L, Tang C, App H, Powell TJ, Kim YH, Schreck R, Wang X, Risau W, et al. SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. Cancer Research. 1999;59:99-106.
View Article Google Scholar

131. Kalin RE Banziger-Tobler NE Detmar M Brandli AW An in vivo chemical library screen in Xenopus tadpoles reveals novel pathways involved in angiogenesis and lymphangiogenesis Blood 2009 114 1110 11222721788 10.1182/blood-2009-03-211771

132. Albuquerque RJ Hayashi T Cho WG Kleinman ME Dridi S Takeda A Baffi JZ Yamada K Kaneko H Green MG Alternatively spliced vascular endothelial growth factor receptor-2 is an essential endogenous inhibitor of lymphatic vessel growth Nat Med 2009 15 1023 10302882165 10.1038/nm.2018

133. Schomber T, Zumsteg A, Strittmatter K, Crnic I, Antoniadis H, Littlewood-Evans A, Wood J, Christofori G. Differential effects of the vascular endothelial growth factor receptor inhibitor PTK787/ZK222584 on tumor angiogenesis and tumor lymphangiogenesis. Mol Cancer Ther. 2009;8:55-63.
View Article Google Scholar

134. Makinen T, Jussila L, Veikkola T, Karpanen T, Kettunen MI, Pulkkanen KJ, Kauppinen R, Jackson DG, Kubo H, Nishikawa S, et al. Inhibition of lymphangiogenesis with resulting lymphedema in transgenic mice expressing soluble VEGF receptor-3. Nat Med. 2001;7:199-205.
View Article Google Scholar

135. Hos D, Bock F, Dietrich T, Onderka J, Kruse FE, Thierauch KH, Cursiefen C. Inflammatory corneal (lymph)angiogenesis is blocked by VEGFR-tyrosine kinase inhibitor ZK 261991, resulting in improved graft survival after corneal transplantation. Invest Ophthalmol Vis Sci. 2008;49:1836-1842.
View Article Google Scholar

136. Heckman CA, Holopainen T, Wirzenius M, Keskitalo S, Jeltsch M, Yla-Herttuala S, Wedge SR, Jurgensmeier JM, Alitalo K. The tyrosine kinase inhibitor cediranib blocks ligand-induced vascular endothelial growth factor receptor-3 activity and lymphangiogenesis. Cancer Res. 2008;68:4754-4762.
View Article Google Scholar

137. Falcon BL Hashizume H Koumoutsakos P Chou J Bready JV Coxon A Oliner JD McDonald DM Contrasting actions of selective inhibitors of angiopoietin-1 and angiopoietin-2 on the normalization of tumor blood vessels Am J Pathol 2009 175 2159 21702774078 10.2353/ajpath.2009.090391

138. Cao R, Bjorndahl MA, Religa P, Clasper S, Garvin S, Galter D, Meister B, Ikomi F, Tritsaris K, Dissing S, et al. PDGF-BB induces intratumoral lymphangiogenesis and promotes lymphatic metastasis. Cancer Cell. 2004;6:333-345.
View Article Google Scholar

139. Heath VL, Bicknell R. Anticancer strategies involving the vasculature. Nat Rev Clin Oncol. 2009;6:395-404.
View Article Google Scholar