Published in final edited form as:

Cancer Res. 2012 April 15; 72(8): 1909–1914. doi:10.1158/0008-5472.CAN-11-3406.

Anti-VEGF/VEGFR therapy for cancer: Reassessing the target

Basel Sitohy^{1,2}, Janice A. Nagy^{1,2}, and Harold F. Dvorak^{1,2}

¹The Center for Vascular Biology Research, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215

²Departments of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215

Abstract

Judah Folkman recognized that new blood vessel formation is important for tumor growth and proposed anti-angiogenesis as a novel approach to cancer therapy. Discovery of vascular permeability factor/vascular endothelial growth factor (VEGF-A) as the primary tumor angiogenesis factor prompted the development of a number of drugs that targeted it or its receptors. These agents have often been successful in halting tumor angiogenesis and in regressing rapidly growing mouse tumors. However, results in human cancer have been less impressive. A number of reasons have been offered for the lack of greater success, and we here call attention to the heterogeneity of the tumor vasculature as an important issue. Human and mouse tumors are supplied by at least six well-defined blood vessel types that arise by both angiogenesis and arterio-venogenesis. All six types can be generated in mouse tissues by an adenoviral vector expressing VEGF-A¹⁶⁴. Once formed, four of the six types lose their VEGF-A dependency and so their responsiveness to anti-VEGF/VEGFR therapy. If therapies directed against the vasculature are to have a greater impact on human cancer, targets other than VEGF and its receptors will need to be identified on these resistant tumor vessels.

Keywords

Angiogenesis; Ad-VEGF-A¹⁶⁴; VEGF; Aflibercept (VEGF Trap); VEGF-therapy resistance

It is just 40 years since Judah Folkman published his classic New England Journal of Medicine paper entitled “Tumor angiogenesis: therapeutic implications” (1). In that paper he set forth three bold postulates: i. Angiogenesis is essential for tumor growth beyond minimal size. ii. Tumors secrete a “tumor angiogenesis factor” (TAF) that is responsible for inducing angiogenesis. and iii. Anti-angiogenesis is a potential therapeutic strategy for treating cancer. Now, four decades later, after considerable debate and not a little controversy, all three of these postulates are widely accepted. Generally speaking, tumors do need to induce a new vascular supply if they are to grow, though some make do, at least for a time, by co-opting the existing host vasculature (2). Folkman's RNA-like TAF candidate proved to be a blind alley, but the discovery of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF or more simply VEGF-A) and the preparation of antibodies against it provided an opportunity for testing Folkman's hypotheses (3, 4). VEGF-A is widely expressed by nearly all malignant tumors and is generally agreed to be the most important TAF. It acts on vascular endothelial cells (EC) within minutes to induce vascular

Corresponding author: Harold F. Dvorak M.D., Department of Pathology, Beth Israel Deaconess Medical Center, 330 Brookline Ave, RN227C, Tel: 617-667-8529, Fax: 617-667-3591, hdvorak@bidmc.harvard.edu.

Conflict of interest: none

permeability, and, longer term, reprograms gene expression, leading to EC proliferation and migration in vitro, and, in vivo, generation of new blood vessels. Moreover, a variety of drugs that target VEGF-A or its receptors effectively prevent the growth of many mouse tumors and tumor xenografts (2, 5-7). Among these drugs are antibodies against VEGF-A or its receptors, engineered proteins that mimic VEGF receptors, and small molecule receptor tyrosine kinase (RTK) inhibitors that preferentially target VEGF receptor-2 (VEGFR-2/flk-1/KDR) with high affinity.

Unfortunately, however, the striking benefits of anti-VEGF/VEGFR therapy observed in treating mouse tumors have not been translated to the clinic. All of these drugs have had only modest effects on human cancers and have not lived up to the great expectations that Folkman and many others anticipated. For example, Bevacizumab (Avastin™, Genentech), a humanized antibody against VEGF-A, prolongs the life of patients with advanced colon cancer by, on average, 4-5 months, and then only when combined with triple chemotherapy (8). In fact, a recent editorial has questioned whether Bevacizumab was “boon or bust” because of its limited effectiveness, serious (though uncommon) side effects, and high cost (9). Other FDA-approved drugs that bind VEGF-A or that target VEGF-A receptors, have fared no better (10). Thus, we are left with the question, why doesn't anti-VEGF-A/VEGFR therapy work better against human cancers? But, before tackling that question, it will be helpful to address some others first: What are tumor blood vessels? How do they form? What does anti-VEGF-A/VEGFR therapy do to tumor blood vessels?

What are tumor blood vessels and how do they form?

Despite the great sums of money that companies, the NIH, and charitable organizations have invested in anti-VEGF/VEGFR approaches to cancer therapy, relatively little attention has been paid to the types of new blood vessels that these drugs are expected to target. Tumor angiogenesis has often been regarded as if it were a single entity in which all tumor blood vessels were identical. However, it has long been known that tumor blood vessels are heterogeneous and differ considerably among themselves with respect to organization, structure and function (11). Studying several important human carcinomas, we identified six distinctly different types of blood vessels (12, 13). Further, to avoid the complexity of the tumor environment, we developed a means of generating surrogate forms of tumor blood vessels in the absence of tumor cells, making use of a non-replicating adenovirus (engineered by Richard Mulligan) to express the most common VEGF-A isoform, mouse VEGF-A¹⁶⁴ (human VEGF-A¹⁶⁵) (14).

When injected into the tissues of immunodeficient mice, Ad-VEGF-A¹⁶⁴ generates each of the six vessel types we had found in human cancers and does so by two distinct but interrelated processes, angiogenesis and arterio-venogenesis. On the angiogenesis side, preexisting venules transform over the course of a few days into “mother” vessels (MV); MV are greatly enlarged, pericyte-poor vessels that result from proteolytic degradation of venule basement membranes and from pericyte detachment (12-15) (Fig. 1). Freed of these restraints, normally cuboidal venules undergo dramatic enlargement that is made possible by extensive EC thinning; in this process, EC discharge the cytoplasmic vesicles and vacuoles comprising their vesiculo-vacuolar organelles (VVOs) to the cell surface, providing the greatly expanded plasma membrane that is needed to line a 4-5-fold expanded lumen (14, 16). MV are highly permeable to plasma proteins, largely by transcellular routes that involve residual vesicles and newly formed fenestrae. Over the course of days to weeks, MV evolve into daughter vessels of three types: 1. glomeruloid microvascular proliferations (GMP), poorly organized structures that, as their name implies, resemble renal glomeruli macroscopically. GMP result from intraluminal proliferation of EC and pericytes and the accumulation of macrophages. 2. vascular malformations (VM), thought to represent MV

that have reacquired a smooth muscle cell coat. and 3. typical capillaries that form as MV sprout inwards instead of outwards, projecting cytoplasmic processes into their lumens that form interconnecting intraluminal bridges that subsequently separate MV into smaller vessels by intussusception. VM and capillaries, unlike MV and GMP, are stable vascular structures that persist indefinitely. Arterio-venogenesis proceeds in parallel with angiogenesis as arterioles, arteries and veins undergo extensive remodeling and enlargement with proliferation of EC and smooth muscle cells (13, 14) (Fig. 1).

What does anti-VEGF/VEGFR therapy do to tumor (and normal) blood vessels?

Folkman conceived of anti-angiogenesis primarily as a means of preventing angiogenesis and thereby limiting further tumor growth. However, it was soon found that anti-VEGF/VEGFR therapies not only prevented angiogenesis but additionally caused considerable pruning of preexisting vessels (5, 17). All of the drugs used, whether targeting VEGF-A or its receptors, had similar effects. Endothelial fenestrations, when present, disappeared, vascular sprouting and permeability were suppressed, and blood flow and vessel patency were extensively reduced. Overall vascular density decreased (depending on the tumor type by as much as 70%). Changes were also noted in the normal vasculature, particularly a loss of EC fenestrae in renal glomeruli and several endocrine organs (5, 17). These findings suggested an explanation for at least some of the side effects that have been found in patients receiving anti-VEGF-A/VEGFR drugs (9); they also provided strong evidence that VEGF-A was necessary for the maintenance of the normal adult vasculature. Some tumor cells, as well as tumor infiltrating macrophages, express VEGF receptors, particularly VEGFR-1, and so anti-VEGF/VEGFR agents may act on these cells as well (18). This is more likely to be the case for small molecule RTKs, because large molecules such as antibodies are unlikely to penetrate the vascular barrier efficiently.

Explanations commonly offered for the failure of anti-VEGF/VEGFR therapy to work better in cancer patients

A number of explanations have been offered to explain the modest effectiveness of anti-VEGF-A/VEGFR therapy in cancer patients vis-à-vis their more potent effects in tumor-bearing mice (19). All of these explanations are likely to have some validity. One obvious possibility is that cancer patients are often elderly and very ill, in contrast to the young, relatively healthy tumor-bearing mice treated in the laboratory. Further, higher dosing is possible in mice who cannot complain about side effects. A second likely reason for the limited effectiveness of anti-VEGF/VEGFR therapy is that it does not result in the killing of all tumor cells; residual tumor cells rendered hypoxic by a compromised blood supply are stimulated to make increased amounts of VEGF-A that may overwhelm anti-VEGF/VEGFR therapy, especially when accompanied by increased expression of matrix components that bind and sequester VEGF-A, protecting it from anti-VEGF drugs (20). Hypoxic tumor cells also begin to make a plethora of other growth factors and cytokines, which have the capacity to replace VEGF and stimulate new blood vessel growth. Included among these are FGF, PDGFB, HGF, EGF, IL-8, IL-6, Ang-2, SDF1 α , PDGF-C, CXCL6, etc., as well as their receptors. Other possibilities are recruitment from bone marrow of vascular progenitor cells and pro-angiogenic myelocytes that can serve as a rich source of growth factors (19, 21).

Another explanation is that of “vascular normalization”, a limited period of time during which tumors treated with anti-VEGF/VEGFR therapy exhibit a reduction in tumor vessel tortuosity, hyperpermeability, interstitial tissue pressure and edema (6). The mechanisms responsible for vascular normalization are not yet fully understood and could reflect a return

to normality of abnormal tumor blood vessels and/or pruning of the most abnormal vessels, leaving behind those that are less abnormal. Whatever the mechanism, vascular normalization persists for only a short time in both mouse and human cancers, and could have Janus-like effects on tumor growth. On the one hand, more normal blood vessels provide improved blood flow, and so might be expected to provide a window of opportunity for improved drug delivery; however, a better blood supply could also favor tumor growth (for a review of this issue, see (6, 10)).

Another possible explanation for the limitations of anti-VEGF/VEGFR therapy: differential sensitivity of tumor blood vessel subtypes to anti-VEGF/VEGFR therapy

Many of the early, highly successful studies targeting VEGF or its receptors in mice followed protocols in which therapy was initiated concurrently with or soon after the transplant of tumor xenografts. Obviously, however, this treatment pattern does not mimic the clinical situation in which tumors have been present for months or years prior to discovery and onset of therapy. Bergers and Hanahan (19, 22) recognized this distinction, raising an important red flag that called attention to the limitations of anti-VEGF/VEGFR therapy in treating established tumors. They found that VEGF receptor inhibitors were highly effective in preventing the development of the spontaneous Rip-Tag tumor and in inhibiting its early growth, but that they were of much less benefit in regressing tumors with an established vasculature (19, 22). Thus, in mice as in patients, anti-VEGF/VEGFR therapy was found to be less effective in advanced disease. Bergers and Hanahan (19) attributed the failure of late therapy to the maturing of the vasculature with increased pericyte coverage and found that addition of an RTK inhibitor that targeted PDGFR- β (highly expressed on pericytes) improved anti-VEGFR therapy, though in other systems this has not been the case. Many other reports indicate that “immature” vessels, variously defined, are preferentially susceptible to anti-VEGF/VEGFR therapy (19, 22-24). However, there are exceptions to this pattern of differential sensitivity. For example, in some tumors nearly all blood vessels are pericyte- or smooth muscle cell-coated and yet undergo extensive pruning in response to anti-VEGF/VEGFR therapy (5).

Effects of rapamycin and Aflibercept[™] (VEGF Trap) on tumor surrogate blood vessels induced by Ad-VEGF-A¹⁶⁴

In two recent papers we investigated the sensitivity of tumor surrogate blood vessels to anti-VEGF-A drugs (23, 25). When injected into the skin of immunodeficient mice, Ad-VEGF-A¹⁶⁴ replicates at successive intervals large numbers of each of the six types of blood vessels that we had identified in human cancers. Therefore, by initiating treatment at different intervals before or after Ad-VEGF-A¹⁶⁴ injection, we were able to evaluate the effects of drugs that targeted VEGF-A indirectly (rapamycin) (25) or directly (VEGF Trap) (23). Rapamycin, acting on mTOR downstream of the VEGF-A-VEGFR-2 signaling pathway, prevented both angiogenesis and arterio-venogenesis and regressed early blood vessels (MV, GMP). VEGF Trap, a human soluble decoy receptor protein with high affinity for all of the VEGF-A isoforms, as well as for VEGF-B and placental growth factor (26), had similar effects (Fig. 1). Up to about one month MV, GMP, early VM and developing FA and DV regressed, at least to some extent, and vascular volume and vascular leakage were strikingly reduced. However, like rapamycin, VEGF Trap became progressively less effective when administered at later times after Ad-VEGF-A¹⁶⁴ injection, when the great majority of blood vessels induced by angiogenesis (VM) and arterio-venogenesis (FA,DV) had developed fully. Thus, although all six types of tumor surrogate blood vessels were induced by VEGF-A¹⁶⁴, not all remained sensitive to anti-VEGF-A therapy. Results

consistent with ours were recently reported in a model of angiogenesis induced by HIF-1 α overexpressing keratinocytes; antibodies against VEGFR-2 were initially successful in preventing new blood vessel formation but were largely ineffective when administered two weeks later (27).

In an effort to explain our results mechanistically, we immunostained Ad-VEGF-A¹⁶⁴-induced blood vessels for VEGFR-2. Whereas early susceptible vessels (MV, GMP) expressed high levels of VEGFR-2, late, rapamycin- and VEGF Trap-resistant vessels (VM, FA, DV) expressed extremely low and sometimes undetectable levels of this receptor as determined by immunohistochemistry (23), and by RT-PCR (unpublished data). Thus, a possible explanation for the insensitivity of VM, FA and DV to VEGF Trap is that their lining EC have lost dependence on exogenous VEGF-A, and that any remaining requirement for VEGF-A as a survival factor had been met by the pericytes or smooth muscle cells that closely enveloped them (28).

Our findings with rapamycin and VEGF-Trap on Ad-VEGF-A¹⁶⁴-induced tumor surrogate blood vessels are likely to be relevant to anti-VEGF/VEGFR therapy of mouse tumors and human cancer. Personal review of many different tumors and of the published photomicrographs of others indicate that vessels with the structure and functional properties of MV predominate during early stages of the growth of most transplantable mouse tumors and tumor xenografts and also in such spontaneously arising tumors as the Rip-Tag2 model. Additional evidence for this conclusion comes from the finding that anti-VEGF/VEGFR drugs potently reverse tumor vascular hyperpermeability (7, 23, 29, 30). In the pathological angiogenesis induced by tumors or Ad-VEGF-A¹⁶⁴, MV (and to a lesser extent GMP) are the *only* hyperpermeable blood vessels that have thus far been identified (13, 14). The same reasoning suggests that the “vascular normalization” following anti-VEGF-A/VEGFR treatment results, at least in part, from the pruning of hyperpermeable MV. Left behind are relatively unaffected pericyte- and smooth muscle-coated VM, FA and DV; i.e., vessels that by MRI and histology appear relatively normal in that they are not hyperpermeable and are smooth muscle cell coated.

Looking back and moving forward

The work reviewed here invites some conclusions but also raises new questions. On the plus side, Folkman's postulates have been affirmed. Targeting VEGF and its receptors offers benefit to patients with at least some types of cancer and provides proof of principle that attacking the vasculature is a valid approach to cancer therapy. At present, however, these benefits are limited and may have untoward consequences. Just as inadequately treated bacterial infections can result in mutations that lead to antibiotic resistance, so residual tumor cells, rendered hypoxic but not killed by anti-VEGF/VEGFR therapy, can reprogram their genomes to express increased amounts of VEGF-A as well as other growth factors that can overwhelm or bypass the VEGF/VEGFR axis and stimulate new blood vessel growth. To mix metaphors, wounding a bear can make him very angry!

A further conclusion is that tumor blood vessels are heterogeneous and that some are susceptible, whereas others are highly resistant, to anti-VEGF/VEGFR therapy. Resistant surrogate vessels such as VM, FA, and DV develop gradually over time after Ad-VEGF-A¹⁶⁴ administration; they are relatively large vessels, coated by one or more layers of smooth muscle cells, and are lined by EC that express very low levels of VEGFR-2. Late-appearing vessels of this type are likely to predominate in human cancers, which are often present for months-years before they are discovered.

The mechanisms by which tumor blood vessels are actually destroyed by anti-VEGF/VEGFR therapy requires further investigation (18). An obvious possibility is that vessels

lined by EC expressing high levels of VEGFR-2 and coated with few pericytes require substantial amounts of VEGF-A for survival and therefore undergo apoptosis when the VEGF-A/VEGFR axis is disrupted. However, VEGFR-2 expression levels and pericytes may not provide the entire explanation for resistance to anti-VEGF/VEGFR therapy. Pericyte-coated capillaries are not always spared by anti-VEGF/VEGFR therapy (5) and other mechanisms can be envisioned for their destruction (18). In some cases, blood vessel pruning could result from tumor cell killing, e.g., dying tumor cells could release cytokines or toxic products that damage blood vessels. A possibility that has received little attention is that anti-VEGF-A/VEGFR therapy could not only act on angiogenic capillaries but upstream on the larger FA that supply the tumor microcirculation. The VEGF-A-VEGFR-2-eNOS-NO pathway has an important role in maintaining normal arterial tone, and, when interrupted, could cause constriction of the FA supplying tumors, leading to reduced downstream blood flow and necrosis of both tumor blood vessels and the tumor cells they supply. Consistent with this possibility is the finding that patients receiving anti-VEGF-A/VEGFR therapy commonly develop systemic hypertension (18). It would be ironic if treating this hypertension, as is commonly done, actually interfered with therapeutic benefit.

Finally, going forward, it is important to think beyond VEGF and its receptors as the sole vascular targets. We need to identify new molecular targets on the EC lining the large tumor blood vessels that supply and drain the tumor microvasculature (VM, FA and DV) and that have proved resistant to anti-VEGF/VEGFR therapy. Attacking these large vessels would cut off the blood supply to the entire tumor and therefore have a larger effect than pruning the innumerable VEGF-A-sensitive microvessels. As any plumber knows, shutting off the water supply at its source makes more sense than trying to turn off every faucet in the house. Evidence for the potential of this concept comes from studies in which photodynamic occlusion of FA and DV led to eradication of mouse ear tumors (31) and from findings that uterine artery embolization or myolysis can ablate uterine fibroids in patients (32). Progress is being made in this regard and a number of tumor vessel markers worthy of further investigation have been identified (33, 34). We anticipate bright days ahead for targeting the vasculature as a mode of cancer therapy.

Acknowledgments

Grant support: This work was supported by U.S. Public Health Service grants P01 CA92644, CA-142262, and by a contract from the National Foundation for Cancer Research (Dvorak), and by The Swedish Research Council and The Swedish Society of Medicine (Sitohy)

References

1. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med.* 1971; 285:1182–6. [PubMed: 4938153]
2. Kim ES, Serur A, Huang J, Manley CA, McCrudden KW, Frischer JS, et al. Potent VEGF blockade causes regression of coopted vessels in a model of neuroblastoma. *Proc Natl Acad Sci U S A.* 2002; 99:11399–404. [PubMed: 12177446]
3. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science.* 1983; 219:983–5. [PubMed: 6823562]
4. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science.* 1989; 246:1306–9. [PubMed: 2479986]
5. Inai T, Mancuso M, Hashizume H, Baffert F, Haskell A, Baluk P, et al. Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts. *Am J Pathol.* 2004; 165:35–52. [PubMed: 15215160]

6. Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nature reviews Drug discovery*. 2011; 10:417–27.
7. Zhu AX, Sahani DV, Duda DG, di Tomaso E, Ancukiewicz M, Catalano OA, et al. Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. *J Clin Oncol*. 2009; 27:3027–35. [PubMed: 19470923]
8. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*. 2004; 350:2335–42. [PubMed: 15175435]
9. Hayes DF. Bevacizumab treatment for solid tumors: boon or bust? *JAMA*. 2011; 305:506–8. [PubMed: 21285431]
10. Jain RK, Duda DG, Clark JW, Loeffler JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. *Nat Clin Pract Oncol*. 2006; 3:24–40. [PubMed: 16407877]
11. Warren, B. The vascular morphology of tumors. In: Peterson, HI., editor. *Tumor blood circulation: angiogenesis, vascular morphology and blood flow of experimental and human tumors*. Boca Raton: CRC Press; 1979. p. 1-47.
12. Fu, Y.; Nagy, JA.; Dvorak, AM.; Dvorak, HF. Tumor Blood Vessels Structure, Function and Classification. In: Teicher, BA.; Ellis, LM., editors. *Cancer Drug Discovery and Development Antiangiogenic Agents in Cancer Therapy*. Totowa, NJ: Humana Press; 2007. p. 205-24.
13. Nagy JA, Chang SH, Shih SC, Dvorak AM, Dvorak HF. Heterogeneity of the tumor vasculature. *Semin Thromb Hemost*. 2010; 36:321–31. [PubMed: 20490982]
14. Nagy JA, Dvorak AM, Dvorak HF. VEGF-A and the induction of pathological angiogenesis. *Annu Rev Pathol*. 2007; 2:251–75. [PubMed: 18039100]
15. Chang SH, Kanasaki K, Gocheva V, Blum G, Harper J, Moses MA, et al. VEGF-A induces angiogenesis by perturbing the cathepsin-cysteine protease inhibitor balance in venules, causing basement membrane degradation and mother vessel formation. *Cancer Res*. 2009; 69:4537–44. [PubMed: 19435903]
16. Dvorak AM, Kohn S, Morgan ES, Fox P, Nagy JA, Dvorak HF. The vesiculo-vacuolar organelle (VVO): a distinct endothelial cell structure that provides a transcellular pathway for macromolecular extravasation. *J Leukoc Biol*. 1996; 59:100–15. [PubMed: 8558058]
17. Mancuso MR, Davis R, Norberg SM, O'Brien S, Sennino B, Nakahara T, et al. Rapid vascular regrowth in tumors after reversal of VEGF inhibition. *J Clin Invest*. 2006; 116:2610–21. [PubMed: 17016557]
18. Ellis LM, Hicklin DJ. VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer*. 2008; 8:579–91. [PubMed: 18596824]
19. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nature reviews Cancer*. 2008; 8:592–603.
20. Kadenhe-Chiweshe A, Papa J, McCrudden KW, Frischer J, Bae JO, Huang J, et al. Sustained VEGF blockade results in microenvironmental sequestration of VEGF by tumors and persistent VEGF receptor-2 activation. *Mol Cancer Res*. 2008; 6:1–9. [PubMed: 18234958]
21. Ferrara N. Role of myeloid cells in vascular endothelial growth factor-independent tumor angiogenesis. *Current opinion in hematology*. 2010; 17:219–24. [PubMed: 20308892]
22. 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; 111:1287–95. [PubMed: 12727920]
23. Sitohy B, Nagy JA, Jaminet SC, Dvorak HF. Tumor-Surrogate Blood Vessel Subtypes Exhibit Differential Susceptibility to Anti-VEGF Therapy. *Cancer research*. 2011; 71:7021–8. [PubMed: 21937680]
24. Helfrich I, Schadendorf D. Blood vessel maturation, vascular phenotype and angiogenic potential in malignant melanoma: one step forward for overcoming anti-angiogenic drug resistance? *Molecular oncology*. 2011; 5:137–49. [PubMed: 21345752]
25. Xue Q, Nagy JA, Manseau EJ, Phung TL, Dvorak HF, Benjamin LE. Rapamycin inhibition of the Akt/mTOR pathway blocks select stages of VEGF-A164-driven angiogenesis, in part by blocking S6Kinase. *Arterioscler Thromb Vasc Biol*. 2009; 29:1172–8. [PubMed: 19443844]

26. Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M, et al. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci U S A*. 2002; 99:11393–8. [PubMed: 12177445]
27. Oladipupo SS, Hu S, Santeford AC, Yao J, Kovalski JR, Shohet RV, et al. Conditional HIF-1 induction produces multistage neovascularization with stage-specific sensitivity to VEGFR inhibitors and myeloid cell independence. *Blood*. 2011; 117:4142–53. [PubMed: 21307392]
28. Evensen L, Micklem DR, Blois A, Berge SV, Aarsaether N, Littlewood-Evans A, et al. Mural cell associated VEGF is required for organotypic vessel formation. *PloS one*. 2009; 4:e5798. [PubMed: 19495422]
29. Verheul HM, Hammers H, van Erp K, Wei Y, Sanni T, Salumbides B, et al. Vascular endothelial growth factor trap blocks tumor growth, metastasis formation, and vascular leakage in an orthotopic murine renal cell cancer model. *Clin Cancer Res*. 2007; 13:4201–8. [PubMed: 17634549]
30. Yuan F, Chen Y, Dellian M, Safabakhsh N, Ferrara N, Jain RK. Time-dependent vascular regression and permeability changes in established human tumor xenografts induced by an anti-vascular endothelial growth factor/vascular permeability factor antibody. *Proc Natl Acad Sci U S A*. 1996; 93:14765–70. [PubMed: 8962129]
31. Madar-Balakirski N, Tempel-Brami C, Kalchenko V, Brenner O, Varon D, Scherz A, et al. Permanent occlusion of feeding arteries and draining veins in solid mouse tumors by vascular targeted photodynamic therapy (VTP) with Tookad. *PloS one*. 2010; 5:e10282. [PubMed: 20421983]
32. Levy BS. Modern management of uterine fibroids. *Acta Obs Gyn Scand*. 2008; 87:812–23.
33. Seaman S, Stevens J, Yang MY, Logsdon D, Graff-Cherry C, St Croix B. Genes that distinguish physiological and pathological angiogenesis. *Cancer Cell*. 2007; 11:539–54. [PubMed: 17560335]
34. Shih SC, Zukauskas A, Li D, Liu G, Ang LH, Nagy JA, et al. The L6 protein TM4SF1 is critical for endothelial cell function and tumor angiogenesis. *Cancer Res*. 2009; 69:3272–7. [PubMed: 19351819]

Figure 1. Schematic diagram of the angiogenic and arterio-venogenic blood vessels that are induced by Ad-VEGF-A¹⁶⁴ in mouse tissues, identifying those that are (enclosed within the dash-lined box) or are not, susceptible to anti-VEGF therapy with rapamycin and VEGF Trap.