VEGF Receptor Signaling in Tumor Angiogenesis

Gerald McMahon

The Oncologist 2000, 5:3-10.
doi: 10.1634/theoncologist.5-suppl_1-3

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://theoncologist.alphamedpress.org/content/5/suppl_1/3

Downloaded from http://theoncologist.alphamedpress.org/ by guest on May 7, 2014

VEGF Receptor Signaling in Tumor Angiogenesis

GERALD MCMAHON

SUGEN, Inc., South San Francisco, California, USA

Key Words. *Angiogenesis · Vascular endothelial growth factor (VEGF) · Tyrosine kinase receptors · Tyrosine kinase inhibitors*

ABSTRACT

The growth of human tumors and development of metastases depend on the de novo formation of blood vessels. The formation of new blood vessels is tightly regulated by specific growth factors that target receptor tyrosine kinases (RTKs). Vascular endothelial growth factor (VEGF) and the Flk-1/KDR RTK have been implicated as the key endothelial cell-specific factor signaling pathway required for pathological angiogenesis, including tumor neovascularization. Inhibition of the VEGF tyrosine kinase signaling pathway blocks new blood vessel formation in growing tumors, leading to stasis or regression of tumor growth. Advances in understanding the biology of angiogenesis have led to the development of several therapeutic modalities for the inhibition of the VEGF tyrosine kinase signaling pathway. A number of these modalities are under investigation in clinical studies to evaluate their potential to treat human cancers. ***The Oncologist*** *2000;5(suppl 1):3-10*

INTRODUCTION

Angiogenesis, the process by which capillaries sprout from preexisting blood vessels, is tightly regulated by a large number of proangiogenic and antiangiogenic factors. Tumor cells have an absolute requirement for a persistent supply of new blood vessels to nourish their growth and to facilitate metastasis. Thus, tumor vascularization is a vital process for the progression of a neoplasm from a small localized tumor to an enlarging tumor with the ability to metastasize [1, 2]. The angiogenic cascade leading to tumor vascularization can be divided into two general phases, the prevascular phase (referred to as the "angiogenic switch") and the vascular phase [3, 4]. Once tumor cells undergo the transformation to an angiogenic phenotype, these malignant cells are capable of inducing phenotypic changes in endothelial cells as well as in other cell types [5, 6]. At that point, avascular tumors can acquire their own blood supply, which permits a rapid rate of growth. While tumors lacking adequate vasculature become necrotic [7] or apoptotic [8], tumors that have undergone neovascularization may not only enter a phase of rapid growth but may also have increased metastatic potential.

ANGIOGENIC FACTORS

A large number of proangiogenic factors and their cognate receptors have been identified, including basic fibroblast growth factor [9], platelet-derived growth factor (PDGF) [10], platelet-derived endothelial cell growth factor [11], fibroblast growth factor [12], angiopoietin-1 [13], transforming growth factor beta-1 (TGF-β1) [14], transforming growth factor alpha (TGF-α), and epidermal growth factor (EGF) [15]. Perhaps the best characterized of the proangiogenic factors is vascular endothelial growth factor ([VEGF] also known as vascular permeability factor), which is relatively unique among growth factors in terms of its specificity for the vascular endothelium [16-18]. The VEGF family currently includes six known members: VEGF, placenta growth factor, VEGF-B, VEGF-C, VEGF-D, and VEGF-E [19-21]. These are secreted as dimeric glycoproteins, all of which contain the characteristic regularly spaced eight-cysteine residues referred to as the "cysteine knot" motif [20, 22, 23]. These glycoproteins belong to a structural superfamily of growth factors that also includes PDGF-BB and TGF-β2 [24].

VEGF, the most potent direct-acting angiogenic protein known [25, 26], is a diffusible endothelial cell-specific mitogen and angiogenic factor that also increases vascular permeability. It elicits a pronounced angiogenic response in a variety of in vivo models [27-31]. Endothelial cell survival in newly formed vessels is VEGF-dependent [32]. VEGF overproduction has been identified as a major factor underlying pathological angiogenesis in vivo in conditions such as psoriasis, macular degeneration, and tumor proliferation

Correspondence: Gerald McMahon, Ph.D., Vice President, Drug Discovery, SUGEN, Inc., 230 East Grand Avenue, South San Francisco, California 94080, USA. Telephone: 650-553-8774; Fax: 650-553-8307; e-mail: Jerry-mcmahon@SUGEN.com
Accepted for publication February 14, 2000. *©AlphaMed Press 1083-7159/2000/$5.00/0*

Downloaded from http://theoncologist.alphamedpress.org/ by guest on May 7, 2014

[33]. Malignant transformation of cultured cells often results in an induction of VEGF expression. For example, *Kieser et al.* [34] reported that mutant murine *p53* tumor-suppressor gene induced expression of VEGF mRNA in transient transfection assays. Oncogenic forms of the tumor-suppressor genes, Ras and Raf, have also been shown to upregulate VEGF expression [35]. Recently, constitutive expression of mRNA and proteins for VEGF and its cognate receptors was observed in most primary and metastatic melanoma cell lines and in SV40T-transformed melanocytes [36]. Neonatal melanocytes did not express VEGF or VEGF receptors, and VEGF expression could not be induced by exogenous growth factors [36].

Role of VEGF in Angiogenesis

Hypoxia appears to be an important stimulus for VEGF production in both malignant and normal cells [37, 38]. The induction of VEGF gene expression by hypoxia in tumor cells involves both an increase in the rate of gene transcription, mediated by the transcription factor hypoxia-inducible factor-1 [39], and an enhancement of the stability of VEGF mRNA [40]. This mechanism is discussed in more detail in a subsequent chapter on the role of VEGF in von Hippel-Lindau Syndrome by *Adrian Harris*. In addition to its effect in tumors, hypoxia-induced VEGF is capable of stimulating angiogenesis in a number of other sites, including endothelial cells [41], retinal pericytes [42], and the myocardium [43-46].

Transcription of VEGF mRNA is also induced by a variety of growth factors and cytokines, including PDGF, EGF, tumor necrosis factor alpha, TGF-β1, and interleukin 1-beta [19, 25]. In addition to its role in the paracrine stimulation of angiogenesis, VEGF may also have an autocrine stimulatory effect on tumor cells [47]. These autocrine and paracrine effects are summarized in Figure 1. The initial event of hypoxia-mediated transcription and factor secretion by the growing tumor and the stromal tissue leads to an upregulation and activation of growth factor receptors. This results in endothelial sprouting, increased vascular permeability, the expression of tissue matrix metalloproteinases (MMPs), and eventually the digestion of matrix, which is required for the endothelial cell to move. The increased endothelial cell mitogenesis and spread and activation of other factors lead to the formation and movement of endothelial cells, including other supporting cells like pericytes, and eventually lead to vessel extension, increased capillary integrity, differentiation of microvessel support cells, and formation of the vascular network. VEGF plays a role in the earliest events in this process. Recent evidence suggests that VEGF may not only play a role in inducing angiogenesis but also is important in promoting the survival of new vessels formed in tumors. *Benjamin et al.* [48] demonstrated that downregulating VEGF transgene expression using a tetracycline-regulated expression system results in the selective obliteration of immature blood vessels that have not yet recruited periendothelial cells in a human glioma xenograft model. Similar results were observed when the constitutive production of VEGF by the glandular epithelium was suppressed as a consequence of androgen-ablation therapy in human prostate cancer. These results underscore the pivotal role of VEGF in the stimulation and maintenance of newly formed vessels in tumors.

VEGF and Flk-1/KDR Signaling Pathway

Three high-affinity cognate endothelial receptors for VEGF have been identified: VEGFR-1/Flt-1, VEGFR-2/Flk-1/KDR, and VEGFR-3/Flt-4. These receptors function as signaling molecules during vascular development [49]. VEGFR-1 and VEGFR-2 are cell surface receptor tyrosine kinases (RTKs), which are localized on endothelial cells during embryogenic development. The coordinated patterns of expression of the genes for VEGF and its receptors suggest that these proteins participate in vascular development during embryogenesis [50, 51]. As shown in Figure 2, VEGF RTKs are single-pass transmembrane receptors that possess intrinsic cytoplasmic enzymatic activity, catalyzing the transfer of the gamma-phosphate of ATP to tyrosine residues in protein substrates

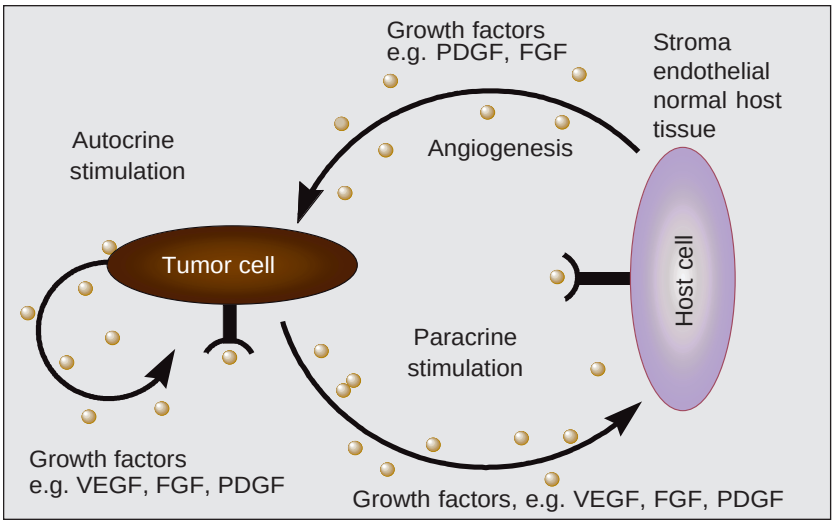

Figure 1. Paracrine and autocrine stimulation by angiogenic growth factors. *VEGF = vascular endothelial growth factor; FGF = fibroblast growth factor; PDGF = platelet-derived growth factor.*

Downloaded from http://theoncologist.alphamedpress.org/ by guest on May 7, 2014

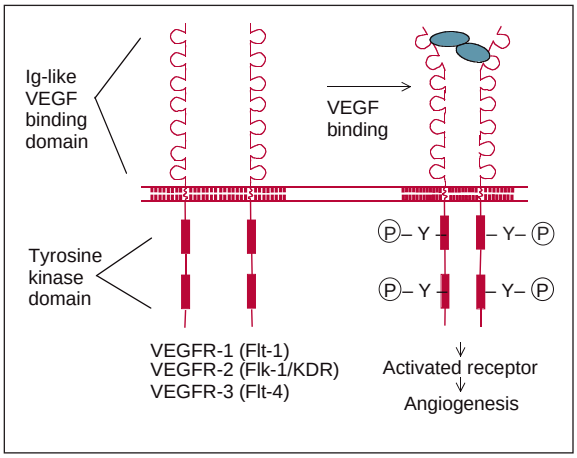

Figure 2. Representative structure of vascular endothelial growth factor (VEGF) tyrosine kinase receptors. *The VEGF receptor family is represented by seven immunoglobulin-like loops in the extracellular domain, which binds VEGF. Two VEGF receptors form a dimer to activate autophosphorylation of tyrosine residues on the cytoplasmic domain. Ig = immunoglobulin; VEGF = vascular endothelial growth factor;* Y- Ⓟ *= phosphorylated tyrosine residues.*

[52]. VEGF RTKs, members of a large family of RTKs, are essential components of signal transduction pathways that affect cell proliferation, differentiation, migration, and metabolism. Activation of VEGF RTKs occurs through ligand binding, which facilitates receptor dimerization and autophosphorylation of tyrosine residues in the cytoplasmic portion. The phosphotyrosine residues either enhance receptor catalytic activity or provide docking sites for downstream signaling proteins [52, 53].

VEGFR-2 is exclusively expressed in endothelial cells and appears to play a pivotal role in endothelial cell differentiation and vasculogenesis [54, 55]. Many studies using molecular techniques have provided evidence for the role of VEGFR-2 in tumor vascularization, growth, and metastasis. For example, the manipulation of the cloned receptor to create a "dominant negative" mutation is one experimental technique that helps establish the relevance of Flk-1 to tumor angiogenesis. *Millauer et al.* [56] used a retrovirus encoding a dominant-negative mutant of the VEGF-2 receptor to prevent the growth of a transplanted glioblastoma tumor, demonstrating the biological relevance of the VEGF-2/Flk-1/KDR receptor/ligand system for tumor-associated angiogenesis in vivo. Recently, *Bernatchez* [57] used antisense oligomers directed against Flk-1 and Flt-1 to show that VEGF stimulation of endothelial cell proliferation, migration, and platelet-activating factor synthesis is Flk-1 dependent, whereas inhibition of Flt-1 expression failed to affect VEGF ability to modulate these activities. These studies have validated targeting of the VEGFR-2 signaling pathway for the development of antiangiogenic agents.

Therapeutic Strategies for Inhbiting VEGF Pathway

VEGF and its receptors have been implicated in the angiogenesis that occurs in many solid tumors including breast cancer [58], colon cancer [59], hepatoma [60], bladder cancer [61], gastric cancer [62], and prostate cancer [63]. Since formation of solid tumors is angiogenesis dependent, several strategies have been developed for targeting the VEGF pathway as part of anticancer therapy (Table 1) [59, 64-75]. Potential approaches for blocking VEGF action include inhibiting secretion of endogenous tumor VEGF, neutralizing VEGF in the microcirculation, and preventing VEGF binding and subsequent signal transduction. A number of these strategies for inhibiting tumor angiogenesis by selectively targeting the VEGF signaling pathway are currently being tested in early phase I/II clinical trials.

Oligonucleotides

Secretion of VEGF can be inhibited by antisense oligonucleotides or expression constructs specific for VEGF, which have been successfully used in models of thyroid carcinoma [76], glioma [77], and melanoma [78]. Ribozymes are RNA molecules that possess the dual properties of RNA sequence-specific recognition and site-specific cleavage of other RNA molecules. Ribozymes that target the VEGF receptor mRNAs

Table 1. Therapeutic agents that have been developed to specifically target the VEGF pathway

Agent	Target	Reference
Angiozyme™ (Ribozyme Pharmaceuticals; Boulder, CO)	VEGFR	[64]
rhumAb-anti-VEGF Antibody (Genentech; South San Francisco, CA)	VEGF	[65-67]
Soluble Flt-1 (Genentech)	VEGF	[68]
Anti-Flk Antibody (Imclone; Somerville, NJ)	VEGFR-2	[69]
SU5416 (SUGEN; South San Francisco, CA)	VEGFR	[59, 70, 71]
ZD4190 (AstraZeneca; Macclesfield, UK)	VEGFR	[72-74]
PTK787/ZK222584/CGP 41251 (Novartis AG; Basel, Switzerland)	VEGFR	[75]

Downloaded from http://theoncologist.alphamedpress.org/ by guest on May 7, 2014

were developed, and their biological activities in cell culture and in an animal model were assessed [64]. Ribozymes targeting Flt-1 or KDR mRNA sites reduced VEGF-induced proliferation of cultured human vascular endothelial cells and specifically lowered the level of Flt-1 or KDR mRNA present in the cells. Anti-Flt-1 and KDR ribozymes also exhibited antiangiogenic activity in a rat corneal pocket assay of VEGF-induced angiogenesis [64].

Anti-VEGF Monoclonal Antibodies

Several anti-VEGF monoclonal antibodies have been developed and have demonstrated efficacy in a wide variety of human tumors in xenograft models [25, 65-67, 79-81]. Initial studies by *Kim et al.* [65] demonstrated treatment with a murine monoclonal antibody specific for VEGF (mumAb VEGF A.4.6.1) potently suppressed angiogenesis and growth in a variety of human tumor cell lines transplanted in nude mice. Subsequently, a recombinant humanized mAb VEGF version of this antibody, rhumAb VEGF, was developed for clinical evaluation in the treatment of solid tumors and other disorders [82]. A phase I clinical trial of rhumAb VEGF in 25 cancer patients showed that multiple doses of anti-VEGF were safe and well tolerated [83]. In a phase II trial of rhumAb VEGF in 15 patients with hormone refractory prostate cancer, administration of 10 mg/kg of rhumAb VEGF intravenously every two weeks resulted in three possible mixed responses [84].

Similarly, *Prewett et al.* [69] demonstrated that treatment with an anti-Flk-1 mAb significantly suppressed the growth of primary murine Lewis lung, 4T1 mammary, and B16 melanoma tumors and the growth of Lewis lung metastases. This antibody also completely inhibited the growth of established epidermoid, glioblastoma, pancreatic, and renal human tumor xenografts. Histological examination of anti-Flk-1 mAb-treated tumors showed evidence of decreased microvessel density, tumor cell apoptosis, decreased tumor cell proliferation, and extensive tumor necrosis. These findings support the conclusion that anti-Flk-1 mAb treatment inhibits tumor growth by suppressing tumor-induced neovascularization and demonstrate the potential for therapeutic application of anti-VEGF receptor antibody in the treatment of angiogenesis-dependent tumors [69]. Based on these results, the anti-Flk-1 antibody has been introduced into the clinic.

Soluble VEGF Receptor

An alternative approach would be to use a soluble recombinant Flt-1 receptor to inhibit the VEGF signaling pathway. Using this approach, a soluble full-length recombinant Flt-1 could be used to bind to circulating VEGF [68]. Another approach would be to produce tumor cells transfected with cDNA encoding the native soluble Flt-1 (sFlt-1) truncated VEGF receptor, which can function by sequestering VEGF or by forming inactive heterodimers with membrane-spanning VEGF receptors in a dominant negative fashion [85]. Both of these techniques have demonstrated preclinical efficacy. Although VEGF has been successfully neutralized using these strategies in preclinical models, their potential application may be limited by the significant amount of protein that would be needed to treat humans. Therefore, preventing RTK signal transduction may be a more appropriate means of tumor control in humans.

Small Molecule Inhibitors

The 3-substituted indolinone compound, SU5416, is a specific and potent catalytic inhibitor of VEGFR protein kinases [86] (Fig. 3). It inactivates Flk-1/KDR by binding in the adenine-binding pocket (Fig. 4). It is a specific VEGFR inhibitor that has virtually no inhibitory activity against serine threonine protein kinases and tyrosine kinases, such as Src, FGF receptor, Met, and Abl and has little activity against PDGF receptor [70]. At present, SU5416 is the most clinically advanced VEGF RTK-selective tyrosine kinase inhibitor being developed for antiangiogenic treatment of cancer [87]. As would be expected by its mechanism of action, SU5416 inhibits tumor growth in vivo in a dose-dependent manner, whereas it has no effect on tumor cells in vitro [70]. SU5416 has shown activity in a large number of tumor xenografts in nude mice including melanoma, glioma, fibrosarcoma, and lung, epidermoid, mammary, and prostate carcinomas [70] as well as in neurogenic sarcoma xenografts [71]. Recently, *Shaheen et al.* [59] evaluated the effect of SU5416 on tumor angiogenesis and metastasis in a human colon cancer xenograft model. In this study, SU5416 inhibited tumor metastases, microvessel formation, and cell

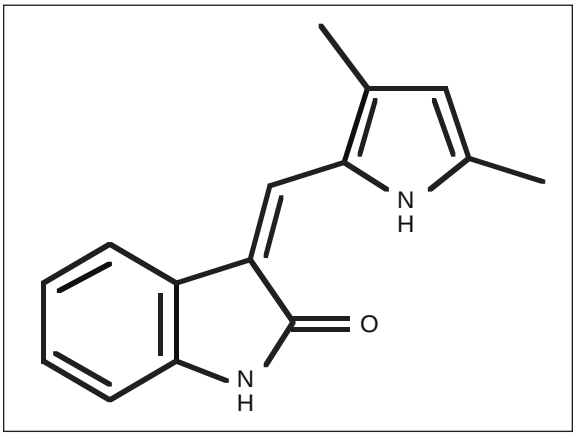

Figure 3. Structural formula of SU5416. *The chemical name of the agent is 3-[(2,4-dimethylpyrrol-5-yl) methylidene]-indoline-2-one.*

Downloaded from http://theoncologist.alphamedpress.org/ by guest on May 7, 2014

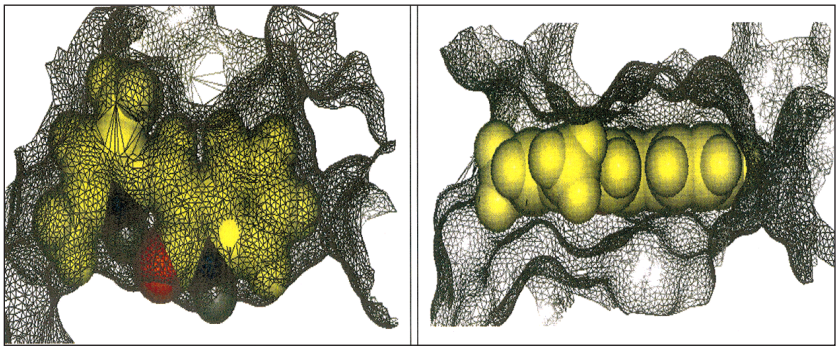

Figure 4. Binding site of SU5416 in the adenine-binding pocket of VEGFR. *SU5416 is shown modeled in the adenosine triphosphate binding site of the Flk-1/KDR intracellular catalytic core. (Two views are shown.)*

proliferation [59]. These findings indicate that targeting the VEGF receptor/ligand system with SU5416 decreases tumor vascularity and vessel density and increases tumor cell apoptosis and is a rational approach to inhibiting tumor growth. In a phase I clinical trial with SU5416 after enrollment of 69 patients, the drug was well tolerated at dose levels of 4.4-145 mg/m^2/day, and stable disease was seen in patients with Kaposi's sarcoma and in patients with non-small cell lung, colorectal, and basal cell cancers [88, 89].

Another substituted indolin-2-one inhibitor of RTKs, SU6668, inhibits the signaling of the VEGF receptor (Flk-1/KDR) and also targets the PDGF and fibroblast growth factor receptors [86, 90, 91]. This novel drug is believed to inhibit tumor growth by preventing angiogenesis and by its direct effects on the tumor cells and the surrounding stromal cells, which support tumor cell growth. SU6668 has recently entered phase I human trials for the treatment of solid tumors.

Several other VEGF RTK inhibitors have shown preclinical efficacy. AstraZeneca Pharmaceuticals (Macclesfield, UK) have identified a series of substituted 4-anilinoquinazolines that are potent inhibitors of VEGFR-1 and VEGFR-2. Of these, ZD4190 is an orally active inhibitor of Flt-1 and Flk-1/KDR that prevented VEGF-mediated proliferation of endothelial cells in vitro [72]. Following chronic oral administration, this compound significantly inhibited the growth of various human tumor xenografts in vivo including breast, colon, lung, ovarian, and prostate carcinomas [73]. ZD4190 has also been shown to reduce significantly vascular endothelial permeability in experimental models [74]. Two additional oral inhibitors of VEGF RTK activity, ZK222584 and CGP 41251, are under development by Novartis Pharma (Basel, Switzerland). Originally identified as an inhibitor of protein kinase C, CGP 41251 has been shown to inhibit the ligand-induced autophosphorylation of VEGF-R2/Flk-1/KDR without affecting the activity of other RTKs such as VEGF-1/Flt-1 and FGF [92]. CGP 41251 has been shown to have a broad antiproliferative effect in vitro and inhibits the angiogenic response to VEGF in vivo [92]. Similarly, ZK222584 has been shown to inhibit angiogenesis and growth of human ovarian carcinomas in vivo in a dose-dependent manner [75]. In mice, this compound was associated with increased survival time, decreased tumor weight, and decreased ascites volume when administered orally [75].

Conclusion

The future of RTK inhibitors as therapeutic agents will be clearer over the next several years as results of the current clinical trials become available and as the factors regulating angiogenesis and the interactions between these factors are more fully elucidated. Drug resistance is a major problem with chemotherapy agents because many tumors are genetically unstable. Since cytotoxic and antiangiogenic therapies are aimed at different cellular targets, clinical strategies to combine antiangiogenic agents with cytotoxic therapy are being devised. It is hoped that combining these agents will be more effective in controlling tumor growth; cytotoxic agents will reduce the tumor burden of already vascularized tumors, while antiangiogenic agents will prevent neovascularization and growth of small and occult metastatic foci as well as the formation of new metastatic lesions.

References

1 Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182-1186.

2 Liotta LA, Kleinerman J, Saidel GM. Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. Cancer Res 1974;34:997-1004.

3 Pepper MS, Montesano R, Mandriota SJ et al. Angiogenesis: a paradigm for balanced extracellular proteolysis during cell migration and morphogenesis. Enzyme Protein 1996;49:138-162.

4 Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86:353-364.

5 Norrby K. Angiogenesis: new aspects relating to its initiation and control. APMIS 1997;105:417-437.

Downloaded from http://theoncologist.alphamedpress.org/ by guest on May 7, 2014

6 Polverini PJ. How the extracellular matrix and macrophages contribute to angiogenesis-dependent diseases. Eur J Cancer 1996;32A:2430-2437.

7 Brem S, Brem H, Folkman J et al. Prolonged tumor dormancy by prevention of neovascularization in the vitreous. Cancer Res 1976;36:2807-2812.

8 Holmgren L, O'Reilly MS, Folkman J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nat Med 1995;1:149-153.

9 Rifkin DB, Moscatelli D. Recent developments in the cell biology of basic fibroblast growth factor. J Cell Biol 1989;109:1-6.

10 Nicosia RF, Nicosia SV, Smith M. Vascular endothelial growth factor, platelet-derived growth factor, and insulin-like growth factor-1 promote rat aortic angiogenesis in vitro. Am J Pathol 1994;145:1023-1029.

11 Takahashi Y, Bucana CD, Liu W et al. Platelet-derived endothelial cell growth factor in human colon cancer angiogenesis: role of infiltrating cells. J Natl Cancer Inst 1996;88:1146-1151.

12 Jouanneau J, Moens G, Montesano R et al. FGF-1 but not FGF-4 secreted by carcinoma cells promotes in vitro and in vivo angiogenesis and rapid tumor proliferation. Growth Factors 1995;12:37-47.

13 Suri C, McClain J, Thurston G et al. Increased vascularization in mice overexpressing angiopoietin-1. Science 1998;282:468-471.

14 Pepper MS, Vassalli JD, Orci L et al. Biphasic effect of transforming growth factor-beta 1 on in vitro angiogenesis. Exp Cell Res 1993;204:356-363.

15 Gleave ME, Hsieh JT, Wu HC et al. Epidermal growth factor receptor-mediated autocrine and paracrine stimulation of human transitional cell carcinoma. Cancer Res 1993;53:5300-5307.

16 Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 1989;161:851-858.

17 Leung DW, Cachianes G, Kuang WJ et al. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989;246:1306-1309.

18 Ferrara N, Alitalo K. Clinical applications of angiogenic growth factors and their inhibitors. Nat Med 1999;5:1359-1364.

19 Neufeld G, Cohen T, Gengrinovitch S et al. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 1999;13:9-22.

20 Meyer M, Clauss M, Lepple-Wienhues A et al. A novel vascular endothelial growth factor encoded by Orf virus, VEGF-E, mediates angiogenesis via signalling through VEGFR-2 (KDR) but not VEGFR-1 (Flt-1) receptor tyrosine kinases. EMBO J 1999;18:363-374.

21 Ogawa S, Oku A, Sawano A et al. A novel type of vascular endothelial growth factor, VEGF-E (NZ-7 VEGF), preferentially utilizes KDR/Flk-1 receptor and carries a potent mitotic activity without heparin-binding domain. J Biol Chem 1998;273:31273-31282.

22 Potgens AJ, Lubsen NH, van Altena MC et al. Covalent dimerization of vascular permeability factor/vascular endothelial growth factor is essential for its biological activity. Evidence from Cys to Ser mutations. J Biol Chem 1994;269:32879-32885.

23 Tischer E, Gospodarowicz D, Mitchell R et al. Vascular endothelial growth factor: a new member of the platelet-derived growth factor gene family. Biochem Biophys Res Commun 1989;165:1198-1206.

24 McDonald NQ, Hendrickson WA. A structural superfamily of growth factors containing a cystine knot motif. Cell 1993;73:421-424.

25 Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997;18:4-25.

26 Torimura T, Sata M, Ueno T et al. Increased expression of vascular endothelial growth factor is associated with tumor progression in hepatocellular carcinoma. Hum Pathol 1998;29:986-991.

27 Connolly DT, Heuvelman DM, Nelson R et al. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 1989;84:1470-1478.

28 Plate KH, Breier G, Weich HA et al. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 1992;359:845-848.

29 Phillips GD, Stone AM, Jones BD et al. Vascular endothelial growth factor (rhVEGF165) stimulates direct angiogenesis in the rabbit cornea. In Vivo 1994;8:961-965.

30 Tolentino MJ, Miller JW, Gragoudas ES et al. Vascular endothelial growth factor is sufficient to produce iris neovascularization and neovascular glaucoma in a nonhuman primate. Arch Ophthalmol 1996;114:964-970.

31 Cao Y, Linden P, Farnebo J et al. Vascular endothelial growth factor C induces angiogenesis in vivo. Proc Natl Acad Sci USA 1998;95:14389-14394.

32 Alon T, Hemo I, Itin A et al. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat Med 1995;1:1024-1028.

33 Bell C, Lynam E, Landfair DJ et al. Oligonucleotide NX1838 inhibits VEGF165-mediated cellular responses in vitro. In Vitro Cell Dev Biol Anim 1999;35:533-542.

34 Kieser A, Weich HA, Brandner G et al. Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. Oncogene 1994;9:963-969.

35 Grugel S, Finkenzeller G, Weindel K et al. Both v-Ha-Ras and v-Raf stimulate expression of the vascular endothelial growth factor in NIH 3T3 cells. J Biol Chem 1995;270:25915-25919.

36 Graeven U, Fiedler W, Karpinski S et al. Melanoma-associated expression of vascular endothelial growth factor and its receptors FLT-1 and KDR. J Cancer Res Clin Oncol 1999;125:621-629.

37 Shweiki D, Itin A, Soffer D et al. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992;359:843-845.

38 Minchenko A, Bauer T, Salceda S et al. Hypoxic stimulation of vascular endothelial growth factor expression in vitro and in vivo. Lab Invest 1994;71:374-379.

Downloaded from http://theoncologist.alphamedpress.org/ by guest on May 7, 2014

39 Forsythe JA, Jiang BH, Iyer NV et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 1996;16:4604-4613.

40 Ikeda E, Achen MG, Breier G et al. Hypoxia-induced transcriptional activation and increased mRNA stability of vascular endothelial growth factor in C6 glioma cells. J Biol Chem 1995;270:19761-19766.

41 Liu Y, Cox SR, Morita T et al. Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5′ enhancer. Circ Res 1995;77:638-643.

42 Takagi H, King GL, Robinson GS et al. Adenosine mediates hypoxic induction of vascular endothelial growth factor in retinal pericytes and endothelial cells. Invest Ophthalmol Vis Sci 1996;37:2165-2176.

43 Hashimoto E, Ogita T, Nakaoka T et al. Rapid induction of vascular endothelial growth factor expression by transient ischemia in rat heart. Am J Physiol 1994;267:H1948-H1954.

44 Gu JW, Adair TH. Hypoxia-induced expression of VEGF is reversible in myocardial vascular smooth muscle cells. Am J Physiol 1997;273:H628-H633.

45 Partovian C, Adnot S, Eddahibi S et al. Heart and lung VEGF mRNA expression in rats with monocrotaline- or hypoxia-induced pulmonary hypertension. Am J Physiol 1998;275:H1948-H1956.

46 Ware JA, Simons M. Angiogenesis in ischemic heart disease. Nat Med 1997;3:158-164.

47 Liu B, Earl HM, Baban D et al. Melanoma cell lines express VEGF receptor KDR and respond to exogenously added VEGF. Biochem Biophys Res Commun 1995;217:721-727.

48 Benjamin LE, Keshet E. Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal. Proc Natl Acad Sci USA 1997;94:8761-8766.

49 Mustonen T, Alitalo K. Endothelial receptor tyrosine kinases involved in angiogenesis. J Cell Biol 1995;129:895-898.

50 Breier G, Albrecht U, Sterrer S et al. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development 1992;114:521-532.

51 Jakeman LB, Armanini M, Phillips HS et al. Developmental expression of binding sites and messenger ribonucleic acid for vascular endothelial growth factor suggests a role for this protein in vasculogenesis and angiogenesis. Endocrinology 1993;133:848-859.

52 Hubbard SR. Structural analysis of receptor tyrosine kinases. Prog Biophys Mol Biol 1999;71:343-358.

53 Strawn LM, Shawver LK. Tyrosine kinases in disease: overview of kinase inhibitors as therapeutic agents and current drugs in clinical trials. Expert Opinion on Investigational Drugs 1998;7:553-573.

54 Millauer B, Wizigmann-Voos S, Schnurch H et al. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell 1993;72:835-846.

55 Quinn TP, Peters KG, De Vries C et al. Fetal liver kinase 1 is a receptor for vascular endothelial growth factor and is selectively expressed in vascular endothelium. Proc Natl Acad Sci USA 1993;90:7533-7537.

56 Millauer B, Shawver LK, Plate KH et al. Glioblastoma growth inhibited in vivo by a dominant-negative Flk-1 mutant. Nature 1994;367:576-579.

57 Bernatchez PN, Soker S, Sirois MG. Vascular endothelial growth factor effect on endothelial cell proliferation, migration, and platelet-activating factor synthesis is Flk-1-dependent. J Biol Chem 1999;274:31047-31054.

58 Kurebayashi J, Otsuki T, Kunisue H et al. Expression of vascular endothelial growth factor (VEGF) family members in breast cancer. Jpn J Cancer Res 1999;90:977-981.

59 Shaheen RM, Davis DW, Liu W et al. Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis. Cancer Res 1999;59:5412-5416.

60 Yoshiji H, Kuriyama S, Hicklin DJ et al. KDR/Flk-1 is a major regulator of vascular endothelial growth factor-induced tumor development and angiogenesis in murine hepatocellular carcinoma cells. Hepatology 1999;30:1179-1186.

61 Droller MJ. Vascular endothelial growth factor is a predictor of relapse and stage progression in superficial bladder cancer. J Urol 1998;160:1932.

62 Kitamura M, Toi M, Arai K et al. Concentrations of vascular endothelial growth factor in the sera of gastric cancer patients. Oncol Rep 1998;5:1419-1424.

63 Balbay MD, Pettaway CA, Kuniyasu H et al. Highly metastatic human prostate cancer growing within the prostate of athymic mice overexpresses vascular endothelial growth factor. Clin Cancer Res 1999;5:783-789.

64 Parry TJ, Cushman C, Gallegos AM et al. Bioactivity of anti-angiogenic ribozymes targeting Flt-1 and KDR mRNA. Nucleic Acids Res 1999;27:2569-2577.

65 Kim KJ, Li B, Winer J et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 1993;362:841-844.

66 Borgstrom P, Hillan KJ, Sriramarao P et al. Complete inhibition of angiogenesis and growth of microtumors by anti-vascular endothelial growth factor neutralizing antibody: novel concepts of angiostatic therapy from intravital videomicroscopy. Cancer Res 1996;56:4032-4039.

67 Melnyk O, Shuman MA, Kim KJ. Vascular endothelial growth factor promotes tumor dissemination by a mechanism distinct from its effect on primary tumor growth. Cancer Res 1996;56:921-924.

68 Lin P, Sankar S, Shan S et al. Inhibition of tumor growth by targeting tumor endothelium using a soluble vascular endothelial growth factor receptor. Cell Growth Differ 1998;9:49-58.

69 Prewett M, Huber J, Li Y et al. Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors. Cancer Res 1999;59:5209-5218.

Downloaded from http://theoncologist.alphamedpress.org/ by guest on May 7, 2014

70 Fong TAT, Shawver LK, Sun L 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 Res 1999;59:99-106.

71 Angelov L, Salhia B, Roncari L et al. Inhibition of angiogenesis by blocking activation of the vascular endothelial growth factor receptor 2 leads to decreased growth of neurogenic sarcomas. Cancer Res 1999;59:5536-5541.

72 Hennequin LF, Thomas AP, Johnstone C et al. The design and synthesis of a novel, orally active VEGF receptor tyrosine kinase inhibitor. Proc Am Assoc Cancer Res 1999;40:69a.

73 Ogilvie DJ, Wedge SR, Dukes M et al. ZD4190: an orally administered inhibitor of VEGF signaling with pan-xenograft anti-tumor activity. Proc Am Assoc Cancer Res 1999;40:69a.

74 Wedge SR, Waterton JC, Tessier JJ et al. Effect of the VEGF receptor tyrosine kinase inhibitor ZD4190 on vascular endothelial permeability. Proc Am Assoc Cancer Res 1999;40:415a.

75 Xu L, Herrera CA, Yoneda J et al. Therapy of VEGF-dependent human ovarian carcinoma by oral administration of CGP 79787/ZK222584, an inhibitor of the VEGF receptor tyrosine kinase. Proc Am Assoc Cancer Res 1999;40:457a.

76 Belletti B, Ferraro P, Arra C et al. Modulation of in vivo growth of thyroid tumor-derived cell lines by sense and antisense vascular endothelial growth factor gene. Oncogene 1999;18:4860-4869.

77 Shih SC, Mullen A, Abrams K et al. Role of protein kinase C isoforms in phorbol ester-induced vascular endothelial growth factor expression in human glioblastoma cells. J Biol Chem 1999;274:15407-15414.

78 Oku T, Tjuvajev JG, Miyagawa T et al. Tumor growth modulation by sense and antisense vascular endothelial growth factor gene expression: effects on angiogenesis, vascular permeability, blood volume, blood flow, fluorodeoxyglucose uptake, and proliferation of human melanoma intracerebral xenografts. Cancer Res 1998;58:4185-4192.

79 Warren RS, Yuan H, Matli MR et al. Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis. J Clin Invest 1995;95:1789-1797.

80 Asano M, Yukita A, Suzuki H. Wide spectrum of antitumor activity of a neutralizing monoclonal antibody to human vascular endothelial growth factor. Jpn J Cancer Res 1999;90:93-100.

81 Wang G, Dong Z, Xu G et al. The effect of antibody against vascular endothelial growth factor on tumor growth and metastasis. J Cancer Res Clin Oncol 1998;124:615-620.

82 Presta LG, Chen H, O'Connor SJ et al. 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.

83 Gordon M, Talpaz M, Margolin K et al. Phase I trial of recombinant humanized monoclonal anti-vascular endothelial growth factor (anti-VEGF MAB) in patients (pts) with metastatic cancer. Proc Am Soc Clin Oncol 1998;17:211a.

84 Reese D, Frohlich M, Bok R et al. A phase II trial of humanized monoclonal anti-vascular endothelial growth factor antibody (rhumAb) in hormone refractory prostate cancer. Proc Am Soc Clin Oncol 1999;18:351a.

85 Goldman CK, Kendall RL, Cabrera G et al. Paracrine expression of a native soluble vascular endothelial growth factor receptor inhibits tumor growth, metastasis, and mortality rate. Proc Natl Acad Sci USA 1998;95:8795-8800.

86 Sun L, Tran N, Liang C et al. Design, synthesis, and evaluations of substituted 3-[(3- or 4- carboxyethylpyrrol-2-yl)methylidenyl]indolin-2-ones as inhibitors of VEGF, FGF, and PDGF receptor tyrosine kinases. J Med Chem 1999;42:5120-5130.

87 Hamby JM, Showalter HDH. Small molecule inhibitors of tumor-promoted angiogenesis, including protein tyrosine kinase inhibitors. Pharmacol Ther 1999;82:169-193.

88 Rosen L, Mulay M, Mayers A et al. Phase 1 dose-escalating trial of SU5416, a novel angiogenesis inhibitor in patients with advanced malignancies. J Clin Oncol 1999;18:161a.

89 Scigalla P, Hannah A, Langecker P et al. First preclinical and clinical results with the antiangiogenetic substance SU5416 in malignancies. Eur J Cancer 1999;35(suppl 5):S62a.

90 Shawver LK, Strawn LM, Fong TAT et al. SU6668 is a potent, broad spectrum angiogenesis inhibitor that exhibits anti-tumor properties. Proc Am Assoc Cancer Res 1999;40:723a.

91 Liang C, Sun L, Tran N et al. Discovery and design of angiogenesis inhibitors that inhibit tyrosine kinase activities associated with VEGF, FGF, and PDGF receptors. Proc Am Assoc Cancer Res 1999;40:68a.

92 Fabbro D, Buchdunger E, Wood J et al. Inhibitors of protein kinases: CGP 41251, a protein kinase inhibitor with potential as an anticancer agent. Pharmacol Ther 1999;82:293-301.

Downloaded from http://theoncologist.alphamedpress.org/ by guest on May 7, 2014

Citations

This article has been cited by 33 HighWire-hosted articles:
http://theoncologist.alphamedpress.org/content/5/suppl_1/3#otherarticles

Downloaded from http://theoncologist.alphamedpress.org/ by guest on May 7, 2014