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VEGF-VEGFR Signals in Health and Disease

Masabumi Shibuya[1,2,3,*]

[1]*Jobu University, Director, Institute of Physiology and Medicine, Gunma 372-8588,* [2]*Tokyo Medical and Dental University, Department of Molecular Oncology, Tokyo 113-8519,* [3]*University of Tokyo, Tokyo 108-8639, Japan*

Abstract

Vascular endothelial growth factor (VEGF)-VEGF receptor (VEGFR) system has been shown to play central roles not only in physiological angiogenesis, but also in pathological angiogenesis in diseases such as cancer. Based on these findings, a variety of anti-angiogenic drugs, including anti-VEGF antibodies and VEGFR/multi-receptor kinase inhibitors have been developed and approved for the clinical use. While the clinical efficacy of these drugs has been clearly demonstrated in cancer patients, they have not been shown to be effective in curing cancer, suggesting that further improvement in their design is necessary. Abnormal expression of an endogenous VEGF-inhibitor sFlt-1 has been shown to be involved in a variety of diseases, such as preeclampsia and aged macular degeneration. In addition, various factors modulating angiogenic processes have been recently isolated. Given this complexity then, extensive studies on the interrelationship between VEGF signals and other angiogenesis-regulatory systems will be important for developing future strategies to suppress diseases with an angiogenic component.

Key Words: VEGF, VEGF receptor, Angiogenic signals, Anti-Angiogenic therapy

INTRODUCTION

Vertebrates, including humans, have a closed circulatory system for supplying oxygen and nutrients to various tissues in the body, and for removing CO_2 and waste materials from peripheral tissues (Risau, 1997). In addition to the blood circulatory system, vertebrates have a similar tubular system, the lymph vessel system, which is essential for the absorption and delivery of fluids, lipids and immune cells from peripheral tissues to lymph nodes and circulating blood.

The circulatory system plays a crucial role in the etiology of many diseases in humans. The hypothesis that suppressing tumor angiogenesis was a potentially novel anti-cancer strategy was first suggested by J. Folkman in 1970 (for review, Hanahan and Folkman, 1996), although the molecular basis of the regulation of angiogenesis was not clearly characterized prior the late 1980s.

Around 1990th, the genes encoding vascular endothelial growth factor (VEGF) and its receptor (VEGFR) were isolated and characterized. Based on extensive studies of these molecules, signals mediated by members of the VEGF and VEGFR families were shown to play central roles in angiogenesis and lymphangiogenesis (Leung *et al*., 1989; Shibuya *et al*., 1990; Alitalo and Carmeliet, 2002; Ferrara, 2004; Shibuya and Claesson-Welsh, 2006; Shibuya, 2011).

In parallel to the VEGF-VEGFR axis, other regulatory systems, including angiopoietin (Ang)-Tie, Delta-Notch and Ephrin-Eph, have been shown to play a role in angiogenesis (Suri *et al*., 1996; Wang *et al*., 1998; Noguera-Troise *et al*., 2006). Furthermore, studies have demonstrated the existence of a variety of endogenous anti-angiogenic factors, such as thrombospondin-1 (TSP-1), as well as factors involved in negative feedback loops that act to suppress angiogenesis (Watnick *et al*., 2003).

Based on the knowledge that VEGF signals are key players in tumor angiogenesis, a variety of antibodies and kinase inhibitors which suppress VEGF-VEGFR signaling have been developed that are now widely used in the treatment of cancer. The clinical efficacy of such therapeutics is limited however, and many future studies will focus on improving their performance in the clinic (Hurwitz *et al*., 2004).

Recently, a soluble form of Flt-1/VEGFR-1, sFlt-1, has been shown to be associated with preeclampsia (PE) (Maynard *et al*., 2003; Koga *et al*., 2003; Levine *et al.*, 2004; Young *et al*., 2010), aged macular degeneration (AMD) (Luo *et al*., 2013) and nephrotic syndrome (Jin *et al*., 2012). Furthermore, VEGF

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***Corresponding Author**
E-mail: shibuya@ims.u-tokyo.ac.jp
Tel: +81-270-32-1010, Fax: +81-270-32-1021(Jobu University)

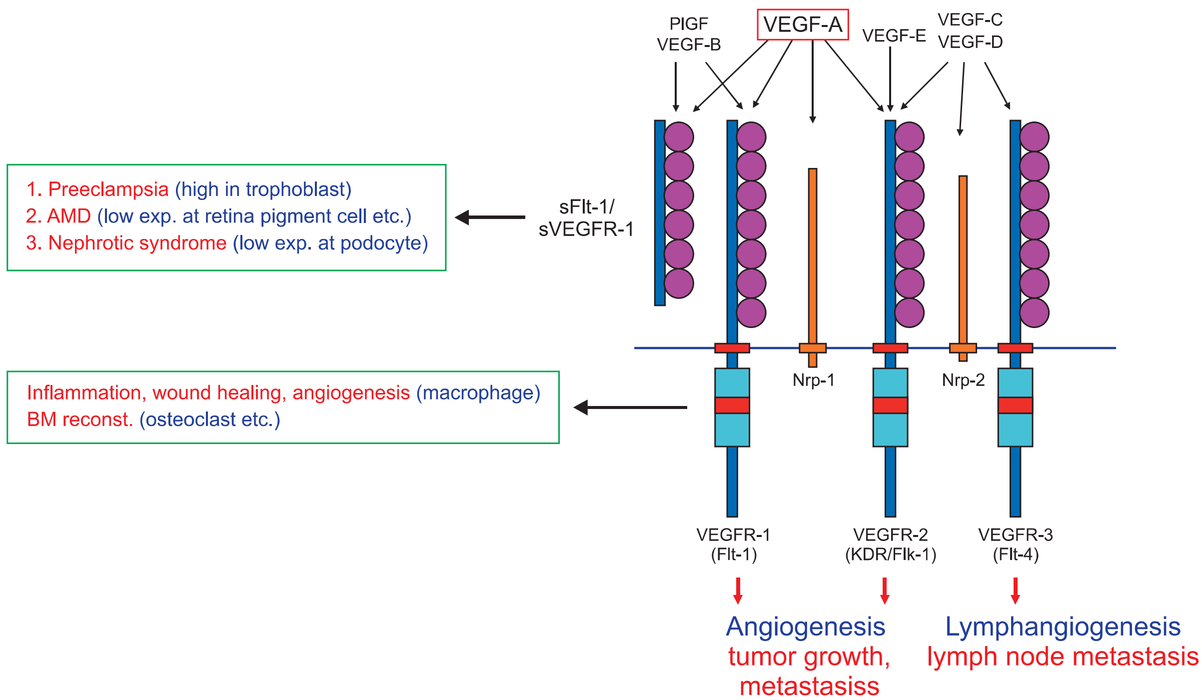

Fig. 1. VEGF-VEGFR system: a crucial regulator of angiogenesis and lymphangiogenesis. Genes encoding all VEGF family members except VEGF-E are present in the mammalian genome. Abnormally high or low expression of sFlt-1 correlates with a variety of diseases.

signals have been reported to directly regulate neuronal function and survival under certain conditions (Oosthuyse *et al*., 2001). These results are discussed in this review.

STRUCTURAL AND BIOLOGICAL CHARACTERISTICS OF VEGFS

In 1989, two research groups independently isolated a cDNA, one encoding for vascular permeability factor (VPF) and another for VEGF, which proved to be identical, and encoded a single protein, now widely known as VEGF (for review, Dvorak, 2002; Ferrara, 2004). The human genome contains five genes encoding five distinct VEGF family members, namely VEGF-A (also called VEGF), placenta growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D (Fig. 1). Structurally, VEGF family proteins are homodimeric, with two subunits of about 120 to 200 amino acids in length. Given their overall structural resemblance to members of the platelet-derived growth factor (PDGF)/macrophage colony-stimulating factor (M-CSF)/stem cell factor (SCF) ligand family, the VEGF and PDGF families are considered to constitute a supergene family, the VEGF-PDGF superfamily. With regard to interactions between VEGF ligands and their receptors, VEGF-A, which contains subtypes such as VEGF-121, -165 and -189, binds VEGFR-1/Flt-1 and VEGFR-2 (De Vries *et al*., 1992), whereas PlGF and VEGF-B bind only VEGFR-1/Flt-1. VEGF-C and VEGF-D bind tightly to VEGFR-3 and more weakly to VEGFR-2. Interestingly, a possibly suppressive ligand, VEGF(xxx)b, has recently been reported (Pritchard-Jones *et al*., 2007). Although it does not exist in mammalian genome, another VEGF family member, VEGF-E encoded in the Orf-viral genome, has been shown to be a VEGFR-2 specific ligand (Shibuya and Claesson-Welsh, 2006).

VEGF-A (+/-) mice, in which a single allele of *VEGF-A* has been deleted, exhibit embryonic lethality due to immature angiogenesis and cardiovascular insufficiency. Lethality resulting from loss of a single allele of a gene is rare in mammals, and the phenotype of these mice indicates a strict relationship between VEGF dosage and angiogenic homeostasis (Carmellet *et al*., 1996; Ferrara *et al*., 1996). Expression of *VEGF-A* is known to be upregulated under hypoxic conditions, as well as by growth factor signaling, and by hormones such as estrogen (Ferrara, 2004).

In contrast to VEGF-A, PlGF and VEGF-B appear to have a relatively minor role in the regulation of angiogenesis, and have been shown to play a role in cardiac muscle function (Bellomo *et al*., 2000; Bry *et al*., 2010). VEGF-C and VEGF-D are initially synthesized as precursor forms that subsequently undergo post-translational processing, and are involved in the regulation of lymphangiogenesis (Alitalo and Carmeliet, 2002). Moreover, they have been demonstrated to be involved in angiogenesis at early stage of embryogenesis, approximately E10.5, as well as in tumor angiogenesis (Dumont *et al*., 1998; Tammela *et al*., 2008).

UNIQUE STRUCTURE AND SIGNALING OF VEGFRS

In 1990, our group isolated a cDNA from human placenta that encoded a novel receptor-type tyrosine kinase containing seven extracellular immunoglobulin (Ig)-domains and a kinase insert sequence of approximately 70 amino acids in length. Based on its structural similarity to the Fms receptor, we named it Fms-like tyrosine kinase-1 (Flt-1) (Shibuya *et al*., 1990). In addition to full length Flt-1, we found that human placenta also expressed a truncated mRNA encoding a protein

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named sFlt-1, which contained only the ligand-binding region of Flt-1 (Shibuya *et al*., 1990; Kendall and Thomas, 1993) (Fig. 1). Based on a subsequent study demonstrating its high affinity for VEGF (De Vries *et al*., 1992), Flt-1 is also referred to as VEGFR-1. Soon after the isolation of Flt-1, VEGFR-2/KDR (Flk-1 in mouse) and VEGFR-3/Flt-4 were isolated, indicating the existence of three distinct *VEGFR* genes in the mammalian genome.

VEGF-A binds to VEGFR-1/Flt-1 with high affinity (Kd=1-10 pM) and less strongly to VEGFR-2 (Kd=10-100 pM), although the tyrosine kinase (TK) activity of VEGFR-1/Flt-1 is about 10 fold weaker than VEGFR-2 (Keyt *et al*., 1996; Sawano *et al*., 1996). Along with the results of gene knockout studies, these data indicate that the major signal transducer in angiogenesis is VEGFR-2 (Shalaby *et al*., 1995). The VEGF-VEGFR-2 axis regulates angiogenesis in a number of different physiological contexts, including hormone-dependent angiogenesis (Ferrara, 2004; Kim *et al*., 2013).

Members of the PDGFR/Fms (M-CSF receptor)/Kit family are distantly related to VEGFRs, and are known to signal through PI3K activation domains with tyrosine (Y)-containing motifs such as Y-x-x-methionine (M) and Y-M-x-M, at the TK-insert domain. After autophosphorylation of PDGFR family members, the p85 subunit of PI3K binds the Y-x-x-M and Y-M-x-M motifs via SH2 domain, resulting in activation of downstream PI3K-Akt and Ras pathways and strong signaling for cell proliferation (Heldin and Westermark, 1999). Very interestingly however, VEGFRs, including VEGFR-2, lack Y-M-x-M and Y-x-x-M motifs in the TK-insert and other regions. In contrast, we found that VEGFR-2 Y1175, a major autophosphorylation site of this receptor, binds the SH2 domain of PLCγ, activating the PLCγ-C kinase-Raf-MEK-MAP kinase pathway to mediate endothelial cell proliferation (Takahashi *et al*., 1999; Takahashi *et al*., 2001). Similar to *flk-1* (*VEGFR-2*)-/- mice, VEGFR-2 Y1173F homozygous knock-in mice, in which this tyrosine residue is mutated to phenylalanine (F) (the mouse VEGFR2 protein is two amino acid shorter than the human form), exhibit embryonic lethality due to poor vasculogenesis and angiogenesis (Sakurai *et al*., 2005). Furthermore, Sase *et al*. (2009) reported that, using an *in vitro* ES cell differentiation system into vascular endothelial cells, Y1175F-mutant VEGFR-2 fails to induce endothelial differentiation. Collectively, these reports indicate that VEGF-VEGFR-2 mediated signal for vasculogenesis and angiogenesis is highly dependent on the Phospho (P)Y1175-PLCγ-C kinase pathway.

VEGFR-2 Y1175 has also been shown to be involved in von Willebrand factor release from endothelial cells (Xiong *et al*., 2009), while VEGFR-2 Y951 is important for vascular permeability and cell migration (Matsumoto *et al*., 2005). In addition, the VEGFR-2-Hdac6-Hsp90-Bcl2 pathway has been reported to transduce cell survival signals (Dias *et al*., 2002).

While most TK family kinases engage the Ras activation pathway to stimulate cell proliferation, this pathway appears to be relatively unimportant in VEGFR-2 signaling. The dependence of VEGFR-2 on PLCγ-C kinase in signaling for the MAP kinase activation is therefore unique among TK family members.

VEGFR-1/FLT-1 AND VEGFR-3

The biological characteristics of VEGFR-1/Flt-1 are distinct from those of VEGFR-2. Fong *et al*. (1995) reported that *flt-1 -/-* mice are embryonic lethal due to overgrowth of endothelial cells and dysfunction of blood vessels. These results strongly suggest that VEGFR-1/Flt-1 has a negative role in angiogenesis at an early stage of embryogenesis, possibly by maintaining an appropriate level of activation of VEGFR-2 via partial suppression of VEGF. To clarify whether the VEGF-trapping with the binding domain of VEGFR-1/Flt-1 or the TK-dependent negative signaling is crucial for this biological role of VEGFR-1/Flt-1 in embryogenesis, we generated Flt-1 TK-deficient (*flt-1 TK-/-*) mice. To our surprise, *flt-1 TK-/-* mice were viable and showed basically normal blood vessel formation (Hiratsuka *et al*., 1998). These mice, however, exhibited a deficiency in VEGF-dependent migration of macrophages which is in agreement with expression of VEGFR-1/Flt-1 in macrophages and its role in VEGF-dependent macrophage migration (Barleon *et al*., 1996; Clauss *et al*., 1996).

Since the *flt-1 TK-/-* mice lack only signals mediated by VEGFR-1/Flt-1, they are useful for elucidating the importance of VEGFR-1 signals under physiological conditions (Niida *et al*., 2005), as well as in diseases such as cancer. Studies using these mice by our own group and others have demonstrated that VEGFR-1 TK stimulates angiogenesis in various carcinomas and glioblastomas (Kerber *et al*., 2008; Muramatsu *et al*., 2010; Schwartz *et al*., 2010; Laurent *et al*., 2011), tumor metastasis (Hiratsuka *et al*., 2002; Kaplan *et al*., 2005), inflammatory disease similar to rheumatoid arthritis (Murakami *et al*., 2006), stroke (Beck *et al*., 2010), as well as liver repair (Kato *et al*., 2011) and gastric ulcer healing (Sato *et al*., 2013). These results indicate that although its TK activity itself is 10-fold weaker than that of VEGFR-2, VEGFR-1/Flt-1 nonetheless represents a potentially important therapeutic target in a variety of diseases, particularly cancer (Shibuya, 2006).

VEGFR-3 is highly expressed in lymphatic endothelial cells, and VEGF-C/D-VEGFR-3 signals have been shown to stimulate lymphangiogenesis and lymph-node metastasis in cancer. In addition, VEGFR-3 has been shown to play a role in tumor angiogenesis (Tammela *et al*., 2008; Sallinen *et al*., 2011).

ANGIOGENESIS-REGULATORY SYSTEMS OTHER THAN VEGFS, AND ENDOGENOUS ANTI-ANGIOGENIC MOLECULES

Recently a variety of angiogenesis-regulatory systems have been elucidated, including: the Ang-Tie receptor axis, which mediates stabilization or destabilization of blood vessels; the Ephrin-Eph receptor pathway, which plays a role in arterio-venous differentiation of vessels; the Delta-Notch pathway, which is involved in regulation of vascular morphology and tip cell-stalk cell communication; and the Netrins-UNC5R pathway, which plays a role in guidance during angiogenesis (Suri *et al*., 1996; Wang *et al*., 1998; Noguera-Troise *et al*., 2006; Freitas *et al*., 2008). Of these, the Delta-Notch receptor system, particularly that involving Dll4, has attracted attention given its crucial role in sprouting and morphogenesis during angiogenesis. Anti-Dll4 neutralizing antibodies were shown to suppress tumor growth in mice, initially suggesting that this might represent an alternative strategy for blocking tumor angiogenesis (Noguera-Troise *et al*., 2006). However, although Dll4 blockade using these antibodies suppressed tubular formation by endothelial cells, these endothelial cells survived

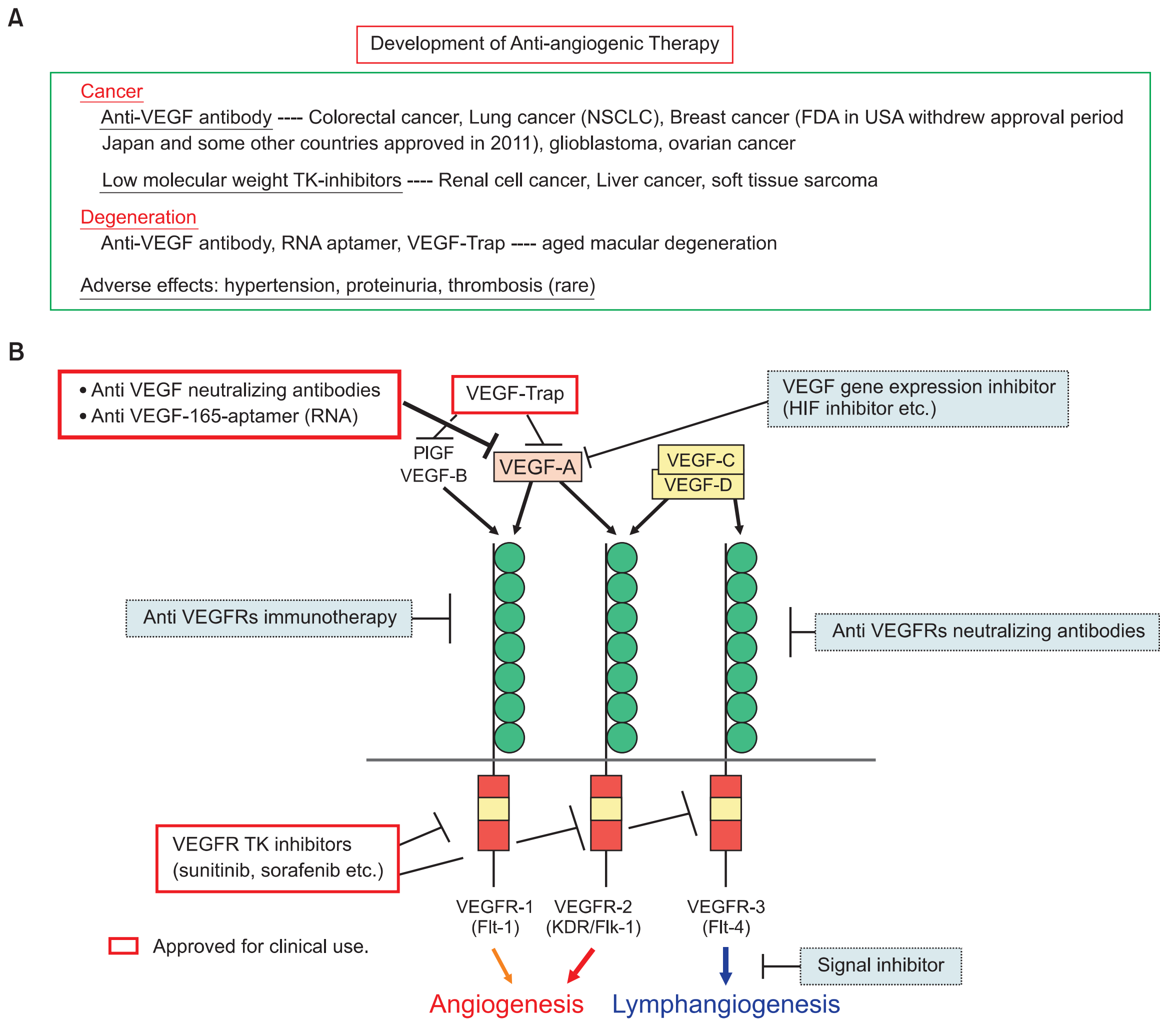

Fig. 2. A summary of the clinical use of anti-angiogenic therapy. Anti VEGF neutralizing antibodies (Bevacizumab, Ranibizumab), $VEGF_{165}$-neutralizing RNA aptamer (Pegaptanib), VEGFR1-R2 fusion peptide (VEGF-A-Trap) and VEGFR/multi TK inhibitors (Sorafenib, Sunitinib, etc.) were approved for clinical use. Bevacizumab and VEGFR/multi TK inhibitors are for the treatment of cancer. Others are for the treatment of AMD.

in tissues and gave rise to angiomas (Yan *et al*., 2010). This finding resulted in the termination or significant suppression of development of anti-tumor drugs based on Dll4 blockade.

In addition to these angiogenesis-regulatory systems, a variety of endogenous angio-suppressive factors have been identified. TSP-1, for example, has been shown to suppress angiogenesis by inducing apoptosis or cell cycle arrest of vascular endothelial cells via upregulation of p21 (Watnick *et al*., 2003; Yamauchi *et al*., 2007). Down syndrome patients show lower incidence of cancer. A gene *DSCR-1* responsible for anti-cancer was recently identified (Minami *et al*., 2004). *DSCR-1* protein has an anti-angiogenic activity, and an increase in the gene copy number of *DSCR-1* gene in Down syndrome patients appear to partly suppress tumor angiogenesis. In addition, VASH1, which is induced in endothelial cells subsequent to activation of VEGF-VEGFR, have been shown to possess anti-angiogenic activity (Sato, 2013). Moreover, angiostatin and endostatin have been characterized as endogenous tumor suppressors in animal models, although it is not clear whether they possess similar roles in humans.

Future studies will be required to clarify which of these factors and signaling pathways are involved in suppressing tumor angiogenesis and which, if any, are suitable for development of therapeutics for clinical use.

VEGF-VEGFR INHIBITORS: DEVELOPMENT OF ANTI-ANGIOGENIC THERAPY

Based on the evidence that VEGF-VEGFR signals play central roles in angiogenic processes in a variety of diseases such as cancer, various VEGF signal inhibitors, including anti-VEGF neutralizing antibodies and VEGFR kinase/multi kinase inhibitors, have been successfully developed and now widely used in the clinic (Kim *et al*., 1993; Hurwitz *et al*., 2004) (Fig. 2, 3). These drugs are effective in prolongation of PFS (progres-

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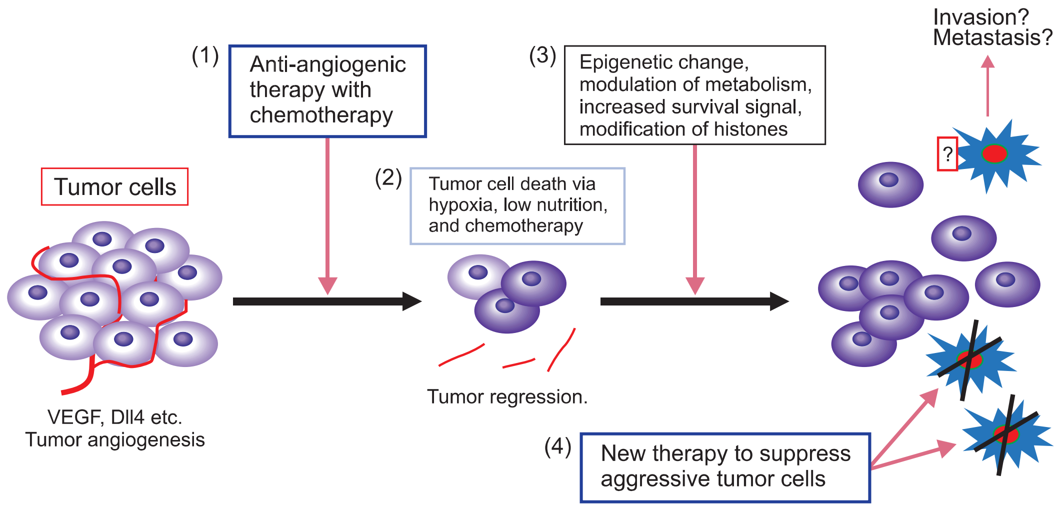

Fig. 3. Hypothetical response of tumors to anti-angiogenic therapy. The double stresses of hypoxia and low nutrition in tumor cells after anti-angiogenic therapy, might result in the acquisition of resistance to drugs.

sion-free survival) as well as OS (overall survival) in the treatment of cancer, and have been approved for a variety of solid tumors such as colorectal cancer, lung cancer (nonepithelial, NSCLC), breast cancer, glioblastoma, liver cancer and renal cell carcinoma (Hurwitz *et al*., 2004; Cohen *et al*., 2007). The efficacy of anti-VEGF antibody in breast cancer is complex however, in that unlike previous relatively small size phase III studies, recent large scale phase III studies have indicated that a combination of chemotherapy plus anti-VEGF antibody treatment was better than chemotherapy-only treatment with respect to PFS, but not OS. Based on this result, the United States Food and Drug Administration withdrew its approval for the use of anti-VEGF antibody in breast cancer treatment. In contrast however, citing its improvement of PFS in patients, Japan and other countries approved anti-VEGF antibodies for the treatment of breast cancer in 2011. More recently an anti-VEGF antibody has been approved for the treatment of ovarian cancer based on the successful clinical trials.

Given the potential of adverse clinical effects of small molecules kinase inhibitors, novel compounds are currently undergoing clinical trials to identify drugs with fewer side effects.

To date, there have been no successful results in the use of anti-VEGF signaling drugs in the treatment of pancreatic or gastric cancer.

While the clinical efficacy of anti-VEGFs has been demonstrated in solid tumors, it is not clear whether this benefit continues for long periods. OS curves in some phase III trials indicate that over time their efficacy decreases, and the potential for resistance or refractoriness exists in later periods. Refractoriness may be due to several possible mechanisms as follows: (1) other angiogenic factors such as HGF and FGF compensate for the loss of the VEGF proangigenic signal (Pàez-Ribes *et al*., 2009), (2) vascular endothelial cells in tumor tissue acquire increased resistance to anti VEGF-VEGFR drugs (Hida *et al*., 2013), or (3) in response to the conditions of hypoxia and low nutrition which result from anti-angiogenic therapy, cancer cells may become more invasive and/or resistant to apoptosis by induction of phospho-Akt and upregulation of histone-demetylases such as JHJM1A (Huveldt *et al*., 2013; Osawa *et al*., 2013) (Fig. 4). Extensive studies are required to elucidate the actual mechanism of resistance in this context.

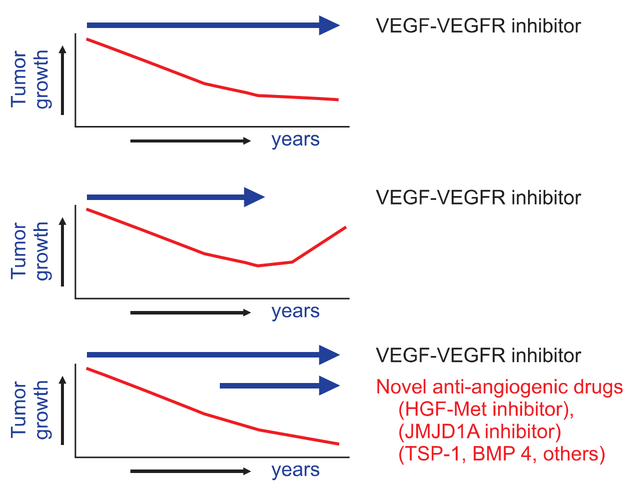

Fig. 4. Anti-tumor angiogenesis therapy: is targeting VEGF signals effective in later stages of cancer? The clinical efficacy of anti VEGF signal drugs may decrease in later stages of cancer, prompting the search for novel anti-angiogenic drugs.

Anti-VEGF signal inhibitors have been shown to be effective in suppressing symptoms of AMD. A recovery of visual acuity was demonstrated in response to intraocular injection of an anti-VEGF neutralizing antibody.

NEW INFORMATION ON AN INTIMATE RELATIONSHIP BETWEEN VEGF SIGNALING AND DISEASES OTHER THAN CANCER

PE, which occurs in 5-7% of pregnancies, causes hypertension and proteinuria in the mother and growth retardation in the fetus, and Cesarean section is often required to save the fetus. sFlt-1 is present at high levels in the serum of PE patients, and the degree of sFlt-1 overexpression has been

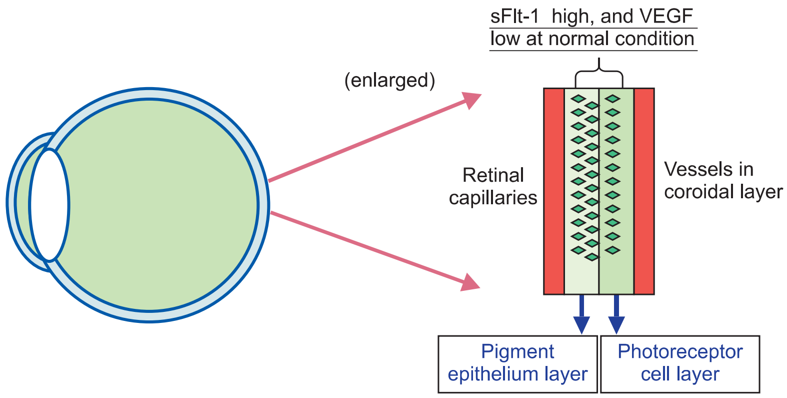

Fig. 5. sFlt-1 (soluble VEGF receptor-1) is important for the maintenance of the photoreceptor avascular area in the eye. AMD (aged macular degeneration) is characterized by a decrease in sFlt-1 levels, resulting in increased in VEGF levels and inappropriate angiogenesis (Luo *et al*., 2013).

shown to correlate with the severity of PE (Koga *et al*., 2003; Maynard *et al*., 2003) (Fig. 1). Furthermore, Levine *et al*. (2004) found that elevated sFlt-1 in the serum in asymptomatic women at early stages of pregnancy predisposed to the development of PE in the later stages of pregnancy. In animal models, inoculation of sFlt-1 expression vector into pregnant rats induced hypertension and proteinuria, similar to PE in humans (Maynard *et al*., 2003).

Since sFlt-1 is derived from the ligand-binding region of VEGFR-1/Flt-1, its major biochemical function is thought to be trapping of VEGF for suppression of VEGF signals (Tanaka *et al*., 1997; Shibuya, 2006). To our surprise, cancer patients treated with anti-VEGF signal drugs often develop side effects such as hypertension and proteinurea that are similar to the symptoms of PE (Hurwitz *et al*., 2004). Taken together, these results indicate that the abnormally high levels of sFlt-1 secreted from trophoblasts in placenta contribute to the development of PE, and that strategies targeting this protein have considerable potential in the treatment of this disease (Mezquita *et al*., 2003; Nagamatsu *et al*., 2004; Gilbert *et al*., 2007; Foidart *et al*., 2009; Kumasawa *et al*., 2011; Thadhani *et al*., 2011).

Furthermore, sFlt-1 is expressed in lens epithelial cells as well as pigment epithelial and photoreceptor cells in the eye, where it plays a role in maintaining avascularity in the cornea and in tissues outside of the retina (Ambati *et al*., 2006; Luo *et al*., 2013) (Fig. 5). Decreased expression of sFlt-1 in photo-receptor and pigment epithelial cells has been reported in AMD patients, suggesting that an increase in free VEGF may stimulate pathological angiogenesis in the retinas of these individuals.

sFlt-1 is also expressed in podocytes in the kidney, where it binds to the cell membrane lipid to maintain the physiological functions of podocytes and vascular endothelial cells in renal glomeruli (Jin *et al*., 2012). Interestingly, sFlt-1 does not necessarily sequester VEGF in podocytes, and sFlt-1 localized in lipid rafts in the cell surface of podocytes is sufficient to support physiological secretion of primary urine from glomerular capillaries. Indeed, podocyte-specific knock-out of the *flt-1* gene in mice has been shown to induce chronic proteinuria, a condition characteristic of nephrotic syndrome in humans (Jin *et al*., 2012).

Another exciting advance in the VEGF-VEGFR field in past 12 years is the discovery of an intimate relationship between VEGF signaling and the neuronal system. Although VEGF signaling in neuronal tissues was thought to function directly upon blood vessels rather than neuronal cells, an extensive study clearly showed that this is not the case (Oosthuyse *et al*., 2001). A hypoxia response element (HRE) sequence in the transcriptional regulatory region of *VEGF* gene mediates hypoxia-inducible factor (HIF) binding, and is essential for hypoxia-responsive upregulation of VEGF. Deletion of this HRE sequence in mice (*VEGF*δ/δ mice) resulted in motor neuron degeneration several months after birth, whose pathological alterations in neurons are comparable to amyotrophic lateral sclerosis (ALS) in humans. They also showed that although decreased angiogenesis due to reduced VEGF levels contributes to this phenomenon in part, a direct effect of VEGF on motor neuron also appears to be important. *In vitro* studies have shown that purified motor neurons express VEGFR-2, and that VEGF signals via VEGFR-2 to stimulate cell survival. Furthermore, treatment of *VEGF*δ/δ mice with VEGF results in partial suppression of their motor neuron degeneration, strongly suggesting that stimulation of VEGF signaling is an attractive new strategy for the treatment of ALS patients.

Sensory nerve cells in the dorsal root ganglion of mice also express VEGFR-2, and VEGF signals are required to maintain the healthy condition of these cells (Verheyen *et al*., 2012). Blockade of this signal by drugs such as anti-VEGF neutralizing antibodies results in painful sensory neuropathy, an adverse effect of anti-VEGF signaling therapy. Further studies on this problem are required to improve PFS in cancer patients undergoing anti-VEGF therapy.

On the other hand, olfactory sensory neurons express VEGFR-1, and the VEGF-VEGFR-1 pathway has been shown to be important for physiological function of these neurons (Wittko *et al*., 2009; Dhondt *et al*., 2011). Various neuronal diseases should be carefully characterized in the VEGF signal point of view.

CONCLUSION

VEGF signaling plays a crucial role not only in cancer but also in a variety of other diseases, including neuronal degeneration and nephrotic syndrome. We anticipate that further studies on VEGF signaling and its modulators will herald a new era in which the severe diseases whose etiology is associated with abnormal VEGF signaling can be brought under control.

CONFLICT OF INTEREST

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