Vascular endothelial growth inhibitor in human cancer (Review)

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Abstract. Vascular endothelial growth inhibitor (VEGI), also known as tumour necrosis factor superfamily member 15 (TNFSF15) and TNF ligand related molecule 1 (TL1), is a recently identified anti-angiogenic cytokine that belongs to the TNF superfamily. Three isoforms of VEGI, VEGI 174, 192, and 251 have been documented, all sharing 151 common C-terminal amino acids but differing in their N-terminal regions. The investigations into the biological functions of VEGI have pointed to a potential cancer inhibitory role for the cytokine. The inhibitory effects of VEGI on cancer are manifested in three main areas, the direct effect on cancer cells, the anti-angiogenic effects on endothelial cells, and stimulation of maturation of dendric cells. The clinical aspect of VEGI in cancer is also being explored in recent years. The present article overviews the recent progress on this molecule and discusses the value of VEGI as a potential therapeutic target in cancer therapy.

Contents

1. Introduction

Vascular endothelial growth inhibitor (VEGI) was first reported in 1999 from human umbilical vein endothelial cells (1,2) and was found to be identical to another gene known as tumour necrosis factor superfamily member 15 (TNFSF15) and TNF ligand related molecule 1 (TL1). The prime function of VEGI was thought to be anti-angiogenic (1,2). Originally, it was reported to be expressed exclusively in endothelial cells, but more recently VEGI 251 was found to be highly expressed in dendritic cells (DCs) after *in vitro* activation and in inflammatory bowel disease (IBD), particularly Crohn's disease (CD), rheumatoid arthritis, as well as renal inflammation (3-6). It was believed to be one of the evolutionarily earliest members of the TNF superfamily (7). There is evidence showing that VEGI is involved in atherogenesis. Thus, VEGI is a multipotential cytokine and plays a role in inflammation, septic shock, fever, and growth modulation through the functions of inducing apoptosis, regulating immunity, and anti-angiogenesis.

Moreover, other studies demonstrated an intricate relationship between VEGI and carcinoma *in vitro* and *in vivo* (8-11). It is a potent inhibitor of endothelial cell proliferation, angiogenesis, and tumour growth (12). The present review will briefly discuss the VEGI family and focus on the impact of VEGI in cancer.

2. VEGI and the TNF superfamily

Three decades ago, lymphotoxin (LT) and tumour necrosis factor-α (TNF) were identified as products of lymphocytes and macrophages that cause lysis of certain types of cells, especially tumour cells (13,14). The two molecules are members of a gene superfamily (15,16). There are at least 19 members so far identified within this family (Table I), which is generally referred to as tumour necrosis factor superfamily (TNFSF). These proteins generally have an intracellular N-terminal domain, a short transmembrane segment, an extracellular stalk, and a globular TNF-like extracellular domain of about 150 residues. These cytokines are either type II transmembrane proteins (N-terminal inside the cell and C-terminal outside the cell) or soluble proteins (17). TNFSF and TNFSF receptor (TNFSFR) have a diverse array of biological functions, including the growth regulation of normal cells by inducing apoptosis or enhancing cell survival and proliferation. In addition, TNF family members are involved in regulating the immunity, inflammation, bone metabolism, and organogenesis. As a late identified member of the TNF family, VEGI (TNFSF15) shares certain homology in amino acid sequence with other members of the same family, and some of its functions are similar to other members,

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Table I. Members of TNF superfamily.

TNF family ligands	Human chromosome	Full name	Other names	Reference
TNF-α	6p21.3	Tumor Necrosis factor-α		(16,35,36)
TNF-ß	6p21.3	Tumor Necrosis factor-ß	Lymphotoxin-a, LTA	(15,37)
LTB	6p21.3	Lymphotoxin-ß	TNFC	(38)
CD27L	19p13	CD27 antigen ligand	TNFSF7	(39)
CD30L,	1P36	CD30 antigen ligand	TNFSF8, Ki-1	(40,41)
CD40L	Xq26	CD40 antigen ligand	CD154, TNFSF5,GP39	(42)
FasL	1q23	Fas ligand	TNFSF6, CD95L, CD178	(43,44)
4-1BBL	19p13.3		TNFSF9	(45)
OX40L (TNFSF4)	1q25	OX40 antigen ligand	GP34, TNFSF4, CD134L	(46)
TRAIL	3q26	TNF-related apoptosis-inducing ligand	APO2L, TNFSF10	(47)
VEGI(TNFSF15)	9q32	Vascular endothelial growth inhibtor	TL1, TNFSF15	(1)
ODF	13q14	Osteoclast differentiation factor	OPGL, RANKL, TRANCE, TNFSF11	(48)
TNFSF13 (APRIL)		TNF- and apol-related leukocyte expressed ligand 2	TALL2, TNFSF13	(49)
TNFSF12	17p13.3		TWEAK, TWEPRIL,INCLUDED	(50,51)
BAFF	13q32-q34	B cell activating factor	TNFSF13B, BLYS, TALL1, THANK	(52)
LITAF	16p13.3-p12	LPS-induced TNF-α factor	SIMPLE	(53)
TNFSF18	1q23		AITRL, GITRL	(54,55)
TNFSF14	19p13.3		HVEML	(56,57)
C1QTNF5	11q23.3	C1q and tumor necrosis factor-related protein 5	CTRP5	(58)

such as inducing apoptosis of epithelia cells. However, most recent studies indicated potential implications of this molecule in cancer and tumour related angiogenesis.

3. Structure of VEGI and VEGI isoforms

In 1997, Tan *et al* identified a novel TNF-like molecule by searching a cDNA database and named it TL1 (later known as VEGI, TNFSF15 or TL1A) (1). These molecules are abundant in arterial endothelial cells. Hydrophobicity analysis of the protein revealed a 13 amino acid hydrophobic region that follows the amino-terminal segment of 12 residues. VEGI protein has a carboxyl terminus on the exterior cell surface (residues 26-174), a single transmembrane domain, and a short cytoplasmic tail. These features are consistent with characteristics of type II transmembrane proteins.

VEGI protein has 20-30% sequence identity to other TNF family members, except TNF-ß (2). The full gene of VEGI is ~17 kb, which consists of four exons and three introns and mapped to human chromosome 9q32. To date, three splicing variants of VEGI have been reported. The initially reported VEGI protein is composed of 174 amino acids, of which the 1-25 AA residues at the N-terminus are the predicted intracellular and transmembrane domain and the 26-174 residues at the C-terminus form an extracellular domain. The intracellular domain is released after a cleavage (8).

Two other isoforms, VEGI 251 and 192, were discovered subsequently (10,18,19). VEGI 251 is encoded by exons I, II, III b and IV, and VEGI 174 by exons IV and IV b. All three isoforms share a common region of 453 bp that encodes a domain of 24-174 amino acids at the C-terminus of VEGI 174. However, the three isoforms differ in their N-terminal regions due to alternative exons (Fig. 1) (18). In the three isoforms, the longest and most abundant form of VEGI protein contains 251 amino acids and is also known as TL1A (20). Similar to most tumour necrosis factor family members, VEGI 251 is a type II membrane-bound protein with a hydrophobic transmembrane region near the N-terminus and an extracellular carboxyl domain (containing 180 amino acid residues 72-251). Its extracellular domain is cleaved off from the cell membrane by unidentified protease(s) and exists in soluble form (7). The cleavage at different sites generates other soluble forms of VEGI 251 which have different functions, such as VEGI 72-251, 101-251, and 106-251. In 2007, Jin *et al* crystallized recombinant human VEGI 251, which belongs to the tetragonal space group P41212. It has self-rotation functions with three molecules in the asymmetry unit. The three VEGI 251 monomers in the asymmetric unit form a homotrimer that resembles the trimer structure of other TNF ligand family members (7,20). It is now known that only the solubilised extracellular domain of the three isoforms of VEGI is responsible for its biological activity (8,10,18,19,21). For example, full-length VEGI 174 was found to have no effect on tumour growth when over-expressed in cancer cells, whereas a secretable fusion protein (sVEGI) comprising a secretion signal peptide and the putative extracellular domain of VEGI 174 inhibited tumour growth when over-expressed in cancer cells (9,22,23).

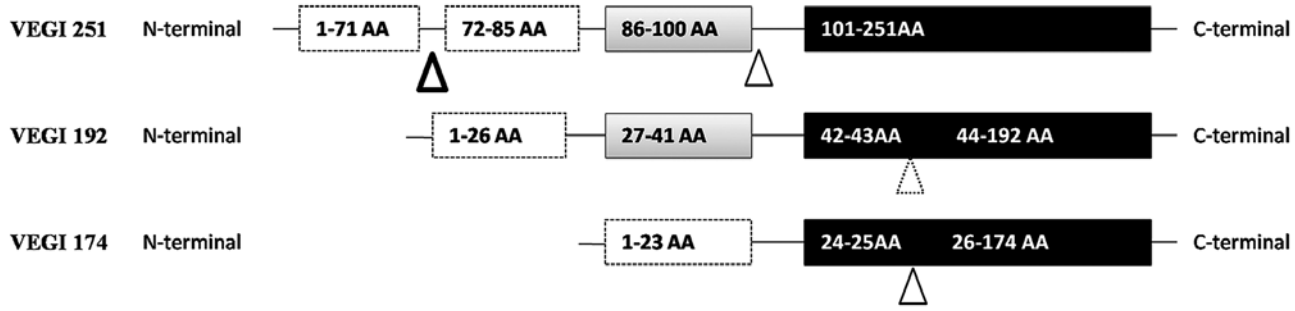

Figure 1. The amino acid structure of the three isoforms of VEGI. VEGI 251, 192, and 174 share a common region of 151 amino acids at the C-terminus (in black boxes). VEGI 251 and 192 share another identical 15 amino acids next to the common region (in gray boxes). However, the three isoforms differ in their N-terminal regions (in blank boxes). The molecular weight of VEGI 251, 192, and 174 is 28,087, 21,857, and 20,131 Da, respectively. In VEGI 251, the extracellular carboxyl domain contains 72-251AA. In VEGI 174, the extracellular carboxyl domain contains 26-174AA. The exact extracellular carboxyl domain of VEGI 192 was not reported previously. In VEGI 251, the cleavage site was in 71 and 72 amino acids. Another cleavage site (XVVR) which was verified in VEGI 174 and 251 also exists in VEGI 192. △Cleavage site.

4. Expression and cell/tissue distribution of VEGI

VEGI was originally thought to be exclusively expressed in endothelial cells (24). VEGI 251 and 192 were also detected in the same cell types including HCAE, HUVE, and HMVE cells, but are not seen in HCASM and ABAE. It should also be noted that more than one isoform is expressed in the same cell type, in which VEGI 251 is the most abundant. It is interesting to note that VEGI is highly expressed in some vascularised tissues, such as the kidney but not in other vascularised tissues, such as the heart. This indicates that only a subset of endothelial cells in the tissues expresses VEGI (2). It was found that VEGI is highly expressed in dendritic cells (DCs) after *in vitro* activation and also up-regulated in Crohn's disease, rheumatoid arthritis, and mouse models of inflammatory bowel disease (3,25,26). Furthermore, VEGI expression was also found in activated lymphocytes, plasma cells, and monocytes (24,25,27).

On the other hand, the tissue distribution of VEGI and its isoforms were also examined. Using Northern blotting, the VEGI transcript was found to be expressed in placenta, lung, kidney, skeletal muscle, pancreas, spleen, prostate, small intestine, and colon. VEGI signal was only rarely detected in heart, brain, liver, thymus, testis, ovary, and peripheral blood lymphocytes (2). VEGI isoforms also have different tissue expression patterns. A 7.5 kb VEGI 251 transcript was detected in placenta, kidney, lung, and liver, whereas the 2 kb VEGI 174 transcript was observed in liver, kidney, skeletal muscle, and heart. VEGI 251 and 174 were both expressed in prostate, salivary gland, and placenta. However, VEGI 251 is more abundant than VEGI 174 in fetal kidney and lung, whereas VEGI 174 is more abundant in heart, skeletal muscle, pancreas, adrenal gland, and liver (18). VEGI 192 RNA is generally low in tissues and little information is available on this isoform.

5. Regulation of VEGI expression

It was reported that TNF-α upregulates the expression of VEGI transcripts in endothelial cells (18). On the other hand, IFN-γ showed a suppressive effect on the expression of both basal levels of VEGI, as well as TNF-stimulated VEGI expression.

VEGI utilizes death receptor 3 (DR3, also known as TNFRSF25) for its biological functions. VEGI activation results in activation of nuclear factor-κB (NF-κB), a central transcription factor that controls expression of numerous genes in the immune system. Recombinant soluble VEGI induces NF-κB activation in DR3-expressing cells and increases ectopic expression of VEGI in human endothelial cells (18). This suggests VEGI regulates its own expression in an autocrine manner. In a mouse model, it was found that a full-length mouse VEGI gene is transcribed from a major transcription initiation site. Critical control of VEGI expression resides in a segment within the promoter that carries NF-κB and specificity protein-1 (SP1) binding sites, and binding of NF-κB to this site is important for basal and TNF-α induced VEGI expression (22).

6. VEGI and angiogenesis

Angiogenesis is essential for many physiological (as seen in uterus and in wound healing) and pathological (such as cancer, rheumatoid arthritis and diabetes) processes (28,29). The process of angiogenesis involves cell proliferation, migration, tubule formation of endothelial cells, remodelling of the extracellular matrix, and invasion to surrounding tissue (30), and is tightly regulated by a balance of pro- and anti-angiogenic molecules (9,30). A number of endogenous angiogenesis inhibitors, such as angiostatin, endostatin, restin, canstatin, and tumstatin were reported and have been shown to suppress the growth of primary and metastatic tumours without affecting the normal vasculature (31).

VEGI induces apoptosis in endothelial cells via an autocrine pathway (2,18,32). Its mRNA was also found in many normal and tumour tissues, and tumour cell lines, suggesting a physiological and pathological role for this unique molecule in the regulation of new vasculature. Over-expression of VEGI was shown to inhibit tumour neovascularisation and progression in cellular and animal models (8,32).

Using recombinant VEGI protein consisting of the putative extracellular domain of VEGI, it was found that the protein inhibited the growth of capillary-like structures regardless of the cause of the angiogenic process *in vitro* and markedly inhibits the growth of breast cancer and colon cancer xenograft

tumours (2,8). The antitumour effect of VEGI is likely to result from the ability of VEGI to suppress neovascularisation because recombinant VEGI has no inhibitory activity on the growth of cancer cells *in vitro*. Furthermore, over-expression of VEGI 251 induced apoptosis in endothelial cells (18) and inhibited the growth of xenograft tumours and corresponding microvessel density (18). However, over-expression of full-length VEGI 174 by cancer cells has little effect on the growth of the xenograft tumours. Further investigation has shown that the density of endothelial cells exhibited an 88% decrease within 1 week of treatment with recombinant human VEGI 192 and a further decrease within 3 weeks (10). Interestingly, the number of the smooth muscle cells remained relatively unchanged. However, it was unclear whether the residual vascular structures would supply enough blood circulation in the tumours. Nonetheless, additional evidence indicates that the antitumour activity of sVEGI is not due to a direct effect on tumour cells but rather to an interference with the development of tumour-associated vasculature (22,28).

A few mechanisms underlying the inhibitory effect of VEGI on endothelial cells were revealed. First, VEGI prevents G0/G1 cells from re-entering the cell cycle in response to growth stimuli. Second, there are two members of the TNF receptor superfamily which VEGI interacts with, namely death receptor 3 (DR3) and decoy receptor 3 (DcR3). DR3 is the functional receptor of VEGI 251. It contains a death domain in its cytoplasmic tail and induces apoptosis in death receptor-3-expressing cell lines, such as in human umbilical vein endothelial cells (HUVECs). On the contrary, previous studies indicated that DcR3 is over-expressed in malignant tumours arising from oesophagus, stomach, glioma, lung, colon, and rectum (20,22,25-27). DcR3 enhances angiogenesis by blocking the autocrine angiostatic function of VEGI in human umbilical vein endothelial (27). Meanwhile, anti-VEGI and -DR3 antibodies lead to increases of both cell proliferation and motility, and an induction of the formation of tube network (28). The angiogenic effect of VEGI antibody and DR3 antibody is similar to that induced by DcR3. Furthermore, VEGI induces apoptosis in actively proliferating cells, via activation of the stress protein kinases, SAPK/JNK and p38 MAPK (SAPK, stress-activated protein kinase, JNK, c-Jun N-terminal protein kinase, p38 MAPK, p38 mitogen-activated protein kinase), and the caspases, mainly caspase-3-like protease. Additionally, VEGI-induced apoptosis is attenuated by the caspase inhibitor.

In contrast, no obvious inhibitory effect on proliferation of the blood vessels of chorioallantoic membrane was seen after treatment with VEGI 72-251 in another study (19). The authors suggested that although the 151 residues at the C-terminal of VEGI 72-251 is identical with that of VEGI 24-174, the assay of biological activity found that VEGI 72-251 did not possess the biological activities of VEGI 24-174, such as inhibiting the growth of endothelial cells. The results indicated that soluble VEGI 72-251 is not able to bind with endothelial cells, which is due to a change of protein structure. Another investigation reported that truncated VEGI 251 proteins, which correspond to amino acids 101-251 and 106-251 of VEGI 251, both have an antiangiogenic effect (24).

The C-terminus 151 amino acids from all known forms of VEGI are the same and this part of the protein, when expressed alone, induced apoptosis of endothelial cells within 36 h. Over-expression of the full-length VEGI did not give rise to a VEGI peptide in cell conditioned media and did not show anti-angiogenic activity *in vivo*. This suggests the soluble domain of VEGI is critical for this negative regulator of angiogenesis produced predominantly by endothelial cells, but not when it is associated with the amino-terminal cytoplasmic and trans-membrane domains of VEGI (residues 1-23). Endothelial cell-secreted VEGI functions as an autocrine inhibitor of angiogenesis and a naturally existing modulator of vascular homeostasis. Thus, this novel gene is not only vital for future study of vascular biology but also is a potential target in the development of angiogenesis-based cancer therapy.

7. Implication of VEGI in solid tumours

The expression and effect of VEGI were investigated in a wide variety of human cancer cell lines, including breast, prostate, bladder, colorectal and liver, and also expressed in human epithelial cells (9). Moreover, recent studies revealed a profound implication of VEGI in clinical cancer.

VEGI is able to inhibit the growth of various human tumour cell lines including human histiocytic lymphoma (U-937), human breast carcinoma (MCF-7), human epithelial carcinoma (HeLa) and human myeloid lymphoma ML-1a (11). VEGI was also shown to inhibit tumour growth *in vivo*. VEGI also suppresses the growth of colon carcinoma cells (murine colon cancer cells, MC-38) both *in vitro* and *in vivo* (2,8,22). Systemic administration of VEGI 192 remarkably inhibited tumour growth and increased survival time of the treated animals in a Lewis lung cancer (LLC) murine tumour model. As much as 50% inhibition of the tumour growth rate was achieved with treatment from which no obvious liver and kidney toxicity was found (10). In 2006, Parr *et al* reported that patients with breast tumours expressing reduced levels of VEGI had a higher local recurrence, shorter survival time and an overall poorer prognosis than those patients expressing high levels of VEGI. In addition, VEGI levels tended to be lower in lobular tumours compared to tumours of ductal origin. However, no significant correlations were observed between VEGI expression and tumour grade, TNM classification, or nodal involvement (9). It is plausible that the pro-apoptotic effect of soluble VEGI in endothelial cells is critical for its anti-tumour activity (10,19-22,28,32,33). However, it is unclear whether other mechanisms, such as activation of tumour-specific or non-specific B or T lymphocytes or induction of cytokines (18), also operate in the soluble VEGI-mediated tumour suppression.

8. Other functions of VEGI involved in carcinoma

VEGI is a T cell costimulator. It directly stimulates DC maturation, and induces nuclear factor-κB activation and apoptosis in death receptor-3-expressing cell lines. However, it is unclear whether these mechanisms are involved in the VEGI-mediated tumour suppression.

The structure of VEGI itself is of interest when considering its functions. While VEGI 24-174 is anti-angiogenic, VEGI 72-251 serves as an anti-cancer factor through its activation of T lymphocytes (19,20). VEGI 72-251, not VEGI 24-174, binds

T cell receptors. The functional receptor for VEGI 251 is DR3 (6), which is predominantly expressed on activated lymphocytes. Ligand-receptor binding creates co-stimulatory signals for T cells, increases IL-2 responsiveness and secretion of IFN-γ and GM-CSF, both *in vitro* and *in vivo*. There are suggestions that the anti-cancer activities of VEGI 72-251 mainly depend on the stimulation of T cells but not on anti-angiogenesis (19,20,24). Moreover, VEGI 251 binds to DR3 and activates NF-κB, caspase, induces c-IAP2 production and regulates DR3 mediated apoptosis in the tumour cell line TF-1 (20,24,34). However, this interaction fails to induce significant caspase activation or apoptosis in T cells. These results suggest that VEGI 251 and DR3 are involved in multiple diseases where immunity plays a major role. VEGI 251 mediates cancer rejection through tumour-specific or non-specific B or T lymphocytes or induction of cytokines. However, these functions are inhibited in the presence of a decoy receptor (DcR3, also known as TR6, or TNFRSF21), which competes for the binding of VEGI 251 (20,24,34). Upon binding to DR3, VEGI 251 directly stimulates DC maturation, which is an essential component of host immunity against cancer development (33). Collectively, it suggests that VEGI 251 plays a central role in the interaction between the endothelium and the immune system to modulate angiogenesis and inflammation toward the suppression of tumour development and progression.

9. Perspective

Further investigation of VEGI and the signal pathways will expand the understanding of its role in cancer and tumour related angiogenesis. For example, the exact membrane receptor responsible for VEGI induced endothelial cell apoptosis is yet to be clarified. Second, underlying mechanisms, such as activation of tumour- or non-specific B or T lymphocytes or induction of cytokines and soluble VEGI-mediated tumour rejection are yet to be established (2). Third, the up-regulation of VEGI transcripts by TNF-α indicates that VEGI-mediated activity is potentially a target of TNF-α action. Fourth, the clinical impact of circulating VEGI in cancer should be studied in detail. It was reported that VEGI is not only expressed in HUVECs but also secreted to the culture medium (28). In certain inflammatory diseases, VEGI also has a remarkable increase in peripheral blood. Little is known about the serum level of VEGI in cancer. Synthetic chimeric protein VEGI-CTT was demonstrated to be an effective inhibitor of lymphoma tumour growth *in vivo*, and it has a more potent antitumour activity than VEGI, CTT, or the combination of both (28). Further investigation will highlight the regulation of VEGI functions by other anti-angiogenesis or -cancer factors.

VEGI serves as a potential target in the development of angiogenesis-based cancer therapy. In contrast to the function of DR3, a soluble DcR3 is generated to interfere with the autocrine function of VEGI (28). Increasing expression of DcR3 was indicated in various solid tumours, which protects vascular endothelial cells from induced apoptosis by VEGI and contributes to tumour related angiogenesis. Targeting at DcR3 has provided a potential approach to block tumour associated angiogenesis.

In summary, the aberrance of VEGI expression and function was implicated in carcinoma and related angiogenesis. Future study will elucidate mechanisms of VEGI regulation and its role in angiogenesis under both physiological and pathological conditions. It will also provide novel therapeutic approaches for targeting carcinoma and angiogenesis.

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