Targ Oncol (2012) 7:87–90
DOI 10.1007/s11523-012-0206-0

REVIEW

Advancement in the research on vascular endothelial growth inhibitor (VEGI)

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Received: 15 April 2011 / Accepted: 11 January 2012 / Published online: 3 March 2012
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Abstract Vascular endothelial growth inhibitor (VEGI), also known as tumor necrosis factor superfamily member 15 or TNF ligand-related molecule 1, is identified as one kind of antiangiogenic cytokine that belongs to the tumor necrosis factor superfamily. VEGI includes three isoforms: VEGI-174, VEGI-192, and VEGI-251. VEGI can activate multiple signaling pathways including nuclear factor-kappaB, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase. Moreover, it suppresses endothelial cell proliferation, angiopoiesis, and tumor growth. Genetic engineering techniques have been used to produce recombinant human vascular endothelial growth inhibitor, and great progress has been made in its application for curing cancer. VEGI could serve as a potential target in the development of angiogenesis-based cancer therapy, and this paper briefly summarizes the progress of the research on VEGI.

Keywords Vascular endothelial growth inhibitor · Apoptosis · Angiogenesis · Solid tumor

Introduction

In 1997, Tan et al. first reported vascular endothelial growth inhibitor (VEGI) by screening the cDNA library of human umbilical vein endothelial cells to search homologous molecules of tumor necrosis factor (TNF) and Fas ligand in the expressed sequence tag library and named it TNF ligand-related molecule 1 (TL1) or tumor necrosis factor superfamily member 15 [1]. Further research confirmed that it had 20–30% sequence identity to other TNF family members except for TNF-ß [2]. *VEGI*, ~17 kb long and mapping to human chromosome 9q32, consists of four exons and three introns. It contains an open reading frame of 575 nucleotides and long untranslated 5′-terminus and 3′-terminus regions. The initially reported VEGI protein is composed of 174 amino acids. The N-terminal region of 1–25 amino acid residues encodes intracellular and transmembrane domains, and the C-terminal region of 26–174 amino acid residues encodes an extracellular domain. The amino acid residues in the intracellular domain are released via cell lysis [3]. These features are consistent with characteristics of type II transmembrane proteins. Three isoforms of VEGI, VEGI-174, VEGI-192, and VEGI-251, share a common region which contains the 24–174 amino acid residues at C-terminal regions. However, the three isoforms differ in N-terminal regions due to their different exons [4]. In exploring the impact of the deletion of N-terminus of VEGI on its bioactivity, it was found that the 1–43 amino acid residues at the N-terminus of VEGI had no obvious effect on its bioactivity, but the 44–51 amino acid residues showed a great one [5, 6]. It is well known that VEGI can suppress endothelial cell proliferation, angiopoiesis, and tumor growth. Hence, VEGI is a promising candidate for cancer treatment.

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VEGI and endothelial cell apoptosis

It was recently reported that VEGI-251 was highly expressed in dendritic cells and was activated in vitro and in inflammatory

organs, such as the colon, the rheumatoid knuckle, and the kidney [7]. VEGI could regulate immunity and induce maturation and osteoclastogenesis of dendritic cells by activating the TNF receptor family member known as death receptor 3 [8]. Meanwhile, expression of decoy receptor 3 (DcR3) was increased by VEGI in various solid tumors, and DcR3 could suppress the autocrine function of VEGI, which protected the vascular endothelial cell from VEGI-induced apoptosis [9].

Xiao et al. revealed that the promoter of VEGI contains the binding site of nuclear factor-kappaB (NF-κB) and that NF-κB could significantly enhance the mRNA level of VEGI [10]. On the other hand, VEGI can activate the transcription factor NF-κB, and VEGI-induced activation of NF-κB has been determined to be a pro-survival factor in many cell types [11, 12]. Interestingly, Sammy et al. found that the ability of VEGI to induce endothelial cell apoptosis was sharply increased when using the NF-κB inhibitors curcumin or BMS345541 to treat endothelial cells [13]. Therefore, it is possible that NF-κB activation may have a role in determining the specificity of VEGI towards endothelial cells.

Yue et al. showed that VEGI-induced apoptosis of bovine pulmonary artery endothelial cells (BPAEC) was suppressed when BPAEC were transfected with *c-Jun* defect forms or mutants and treated with the p38 mitogen-activated protein kinase (MAPK)-specific inhibitor SB203580, whereas caspase-3 was activated and the expression of Fas receptor was increased after VEGI was added into BPAEC in vitro [14]. The results above showed that VEGI could promote apoptosis by activating stress-activated protein kinase (SAPK) in BPAEC, such as p38 MAPK, SAPK/JNK, and some caspase family members [15]. It was also proven that VEGI-induced apoptosis might be associated with Fas and bcl-2 expression in BPAEC [14].

Yu et al. [5] demonstrated that when cells were subjected to variable growth conditions and treated simultaneously with VEGI, early G1 growth arrest occurred, the retinoblastoma gene product was hyperphosphorylated, and the expression of c-myc gene was suppressed for late G1 cells. Additionally, VEGI could play a role through inhibiting the activity of cyclin-dependent kinases, such as CDK2, CDK4, and CDK6. VEGI could induce apoptosis in proliferating endothelial cells but not in nonproliferating endothelial cells. It was obvious that VEGI could not only keep cells in G0/G1 but also induce the apoptosis of S-phase cells.

VEGI and solid tumors therapy

Previous studies showed that VEGI could inhibit the growth of epithelial cells and various human tumor cells, such as human histiocytic lymphoma U-937, human breast carcinoma MCF-7, human epithelial carcinoma, murine colon cancer cells MC-38, and human myeloid lymphoma ML-1a [10, 16, 17]. Recent studies revealed that VEGI had an inhibitory effect on the motility and adhesion of bladder cancer cells and prostate cancer cells [18, 19]. Parr et al. found that breast cancer patients with reduced levels of VEGI had higher local recurrence, shorter survival time, and poorer prognosis than those with high levels of VEGI [16].

VEGI can induce endothelial cell apoptosis via an autocrine pathway [14]. Recombinant VEGI had no inhibitory activity on the growth of cancer cells in vitro. In animal models, recombinant VEGI inhibited the growth of cancer cells via an interference with the development of tumor-associated vasculature [9, 10]. Therefore, the antitumor effect of VEGI is likely to be attributable to its ability to suppress neovascularization. It was recently found that VEGI inhibited the differentiation of endothelial progenitor cells (EPCs) from mouse bone marrow cells and prevented EPCs from being incorporated into Lewis lung cancer tumors by inducing EPC apoptosis, which meant that VEGI could suppress postnatal vasculogenesis by inhibiting EPC differentiation signals in early-stage EPCs [20, 21]. Therefore, VEGI could serve as an ideal therapeutic agent in the development of angiogenesis-based cancer therapy.

Genetic engineering production of VEGI

The source of natural VEGI is limited, so the research on recombinant VEGI is imperative. Nowadays, recombinant human vascular endothelial growth inhibitor (rhVEGI) expressed by *Escherichia coli* (*E. coli*) accounts for about 40% of the total proteins of *E. coli* and can inhibit endothelial cell proliferation after purification [22]. The proliferation of 18% endothelial cells was inhibited when the concentration of rhVEGI was 40 ng/ml, and the proliferation of 30% endothelial cells was inhibited when the concentration of rhVEGI increased up to 160 ng/ml [22]. The research on the correlation between different strains, induction methods, and rhVEGI production revealed that a combination of Origami B (DE3) strain and autoinduction expression system gave rise to a high yield of rhVEGI-192 at 105.38 mg/l [23]. Synthetic peptide CTT (CTTHWGFTLC) was found to suppress the invasion and migration of both tumor and endothelial cells, so the recombinant expression vector pET–VEGI–CTT was constructed and the fusion protein VEGI–CTT was expressed in *E. coli* BL21 (DE3). As a result, chimeric protein VEGI–CTT was found to have better antitumor activity than VEGI and/or CTT peptide against CA46 human lymphoma xenografts in nude mice in vivo [24].

Pichia pastoris was also used to express rhVEGI. *VEGI* was connected to the expression vector pPICZα and then the latter was transfected into *P. pastoris* GSI15 via

electrotransfection. Finally, about 5 mg/l rhVEGI was harvested after the selected positive recombinant strain was induced to express rhVEGI through methanol [25].

VEGI could also be obtained from mammalian cells. VEGI-251 was inserted into an adenovirus with E1B 55 kDa gene deletion; then, VEGI-251 was secreted when adenovirus infected the cancer cells, and secretory VEGI-251 was proven to effectively suppress proliferation of endothelial cells, angiogenesis, and growth of tumors [26]. In addition, the recombinant adenovirus vector which carries *hENDO-sVEGI* had been constructed, which laid a foundation basis for the further study of cancer gene therapy [27].

Perspective

Up to now, many proangiogenic factors as well as antiangiogenic factors have been discovered. The studies that used destructive drugs specifically to destroy the formed tumor blood vessel according to the differences between normal blood vessel and tumor blood vessel are on the rise, and many antiangiogenic drugs have been applied in clinical trials. Antiangiogenic gene therapy strategy, targeted drug delivery system, immunotherapy, antiangiogenic drugs, etc. are being explored, and they will provide effective approaches for cancer therapy.

Acknowledgment The work was supported by the National Basic Research 973 Pre-research Program of China (2010CB534905).

Conflicts of interest The authors have no conflicts of interest in writing this paper.

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