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Review

Transcriptional regulation of the Vascular Endothelial Growth Factor gene—a concert of activating factors☆

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Abstract

The vascular endothelial growth factor A (VEGF-A) is essential during embryonic development as inactivation of only one allele of its gene results in embryonic lethality. Up-regulation of VEGF under physiological situations allows for adaptation to hypoxic stress, to transient inflammatory processes, and to wounding. Its expression also increases all along the process of neovascularization of solid and hematological tumors. The object of this article is to focus on the transcriptional regulation of its gene. The major *cis*-acting sequences and *trans*-activating factors will be described as well as the physiological and pathological situations leading to the intervention of such sequences and factors. We will also focus on two transcription factors essential to VEGF gene transcription: the hypoxia-inducible factor-1, which is responsible for its increased by hypoxia, as well as Sp1, which is implicated in the response to various extracellular stimuli.

Keywords: VEGF; Transcription; Promoter; Sp1; HIF

1. Introduction

The vascular endothelial growth factor (VEGF) was initially characterized and molecularly cloned by three independent teams in 1989 as a new angiogenic factor specific for endothelial cells. It was described as a specific growth factor for endothelial cells and a permeability factor that acts through specific cell surface receptors. Several VEGF isoforms with different properties are produced from a single gene by alternative splicing to form active disulfide-linked homodimers. At least five isoforms are produced: VEGF 121, 145, 165, 189, and 206. VEGF 165 and 121 are the more abundant forms. The mRNA of VEGF 189 is also present in all the tissues, whereas VEGF 206 is only found in embryonic tissues. VEGF 145 is composed of exons 1 to 6 and exon 8. It is mitogenic for endothelial cells but less than VEGF 165. VEGF 115 was cloned from mouse immortalized fibroblasts. It is equivalent to VEGF 121 in its N terminus but 37 amino acids are different in the C-terminal part. It was demonstrated to be mitogenic for

Abbreviations: Abl, abelson; AP-2, activating protein 2; EGF, epidermal growth factor; Egr 1, early gene response protein; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; HIF, hypoxia inducible factor; IGF, insulin-like growth factor; IL, interleukin; PDGF, platelet derived growth factor; PDK-1, phospholipid dependent kinase; PKC, protein kinase C; Sp1, specificity protein 1; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; VEGF, vascular endothelial growth factor; VHL, von Hippel Lindau

☆ During the completion of our manuscript, Chang et al. have demonstrated that the NFAT transcription factor binds to a site at −2400 of the VEGF promoter region (GGAAA). NFAT inhibits the VEGF promoter activity in the myocardium. This repression is essential to mediate a transformation of myocardial cells into mesenchymal cells. Chang CP, Neilson JR, Bayle JH, Gestwicki JE, Kuo A, Stankunas K, et al. A field of myocardial-endothelial NFAT signaling underlies heart valve morphogenesis. Cell 2004;118:649–63.

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endothelial cells. Whereas the regulation of expression of the more abundant forms was intensively investigated, recent experiments have demonstrated that each isoform is differentially regulated depending on the stimulus or the patho-physiological situation [1–3]. After this first characterization, the importance of VEGF in normal and pathological angiogenesis processes [4], as well as protection against motor neurons degeneration [5,6], has been demonstrated. VEGF is a model of gene regulation as its expression is controlled at many levels including transcription (the purpose of this article), mRNA stability through the binding of regulatory proteins to the 3′ untranslated region [7–10], and mRNA translation via IRES sequences present in the 5′ untranslated region [11,12]. VEGF expression is regulated by a plethora of external factors. One of the best characterized actor of VEGF secretion is hypoxia. Hypoxia-induced VEGF production serves as a "driving force" for the development of neovessels during embryonic development and for the vascularization of solid tumors. This review proposes an exhaustive overview of the mechanisms leading to regulation of VEGF levels via transcriptional control of *vegf* gene.

As the literature concerning the VEGF regulation is "enormous," we apologize for not being able to cite every work due to space limitations.

2. The VEGF gene: organization and promoter region

The VEGF gene is organized in 8 exons separated by 7 introns. The coding region encompasses approximately 14 kb [13]. The human gene is located on chromosome 6 at 6p21.3 [14]. The VEGF gene has been cloned in many species including the mouse, rat, and human. The importance of a region of 1.2 kb was demonstrated for the mouse and the rat gene [15,16], while a bigger region of 2.362 kb was investigated in the human gene [17]. The VEGF promoter does not contain a consensus TATA box. The promoters from the different species share a lot of homology including consensus sites for Sp1/Sp3, AP-2, Egr-1, STAT-3, and HIF-1. The mouse promoter is the only one to contain an additional NF-kB consensus site between −90 and −185 [15] but its functionality has not been proven. Recently, a cryptic promoter present in a domain originally described as a part of the 5′ noncoding region of the VEGF mRNA was described [18]. The new transcription initiation site is located at +632 downstream of the classical start site. This promoter was not further investigated but is apparently activated independently of the classical promoter region. One particular feature is its insensitivity to hypoxia. The promoter has been found to contain numerous putative binding sites for various transcription factors. We will discuss the experimental evidence that demonstrates the implication of such factors as well as the stimuli that directly induce the transcriptional activity.

2.1. The proximal promoter region integrates multiple signals (Fig. 1)

VEGF expression was shown to be up-regulated through the activation of tyrosine kinase receptors including ones of the EGF family [19], the insulin [20] and IGF [21] receptors, the HGF receptor [22], the PDGF receptor [23] and FGF receptors [24]. Two common denominators between all these signals are the activation of the Ras>Raf>MEK>Erk as well as the PI3-kinase/Akt pathways. Hence, increased VEGF expression was shown

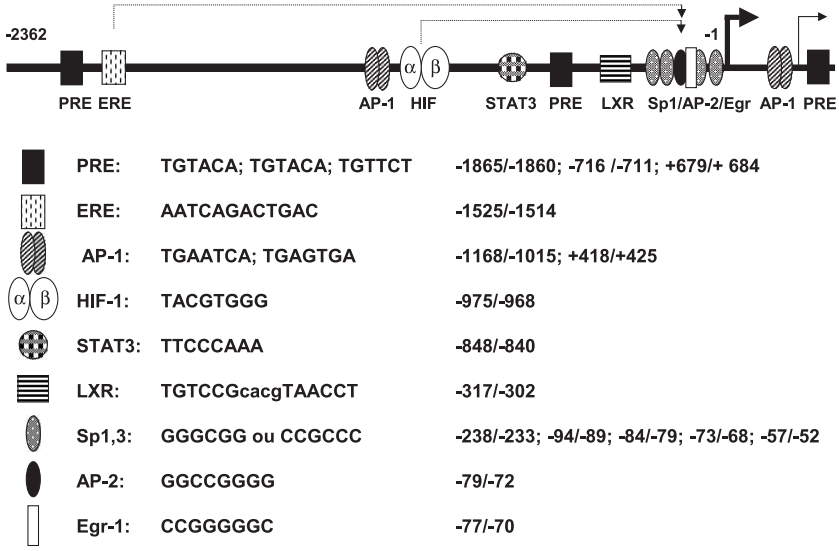

Fig. 1. The VEGF promoter: response elements and *trans*-activating factors. The entire region of the promoter is represented. The heavy arrow indicates the classical initiation of transcription, whereas the light arrow indicates the position of the cryptic promoter present in a domain of the gene described as the 5′ untranslated region of the VEGF mRNA. The exact positions and sequences of the *cis*-regulatory elements, the functionality of which has been demonstrated, have been mentioned. The long arrows above the classical promoter indicate a relationship between different *cis*-acting sequences and *trans*-activating factors.

in cells transformed by an oncogenic form of Ras, Raf, and MEK [25]. Moreover, the expression of a constitutively active form of PI3-kinase in ovarian cells results in increased expression of VEGF, thus facilitating their expansion by up-regulating angiogenesis [26]. PKC zeta is the major kinase downstream of PI3-kinase that regulates VEGF expression in a PDK-1-dependent manner [27]. Finally, as the Ras>MEK>Erk pathway appears essential for VEGF up-regulation in fibroblasts, the PI3-kinase pathway is more implicated in such up-regulation in epithelial cells [28]. Abnormal angiogenesis was also demonstrated in the case of hematological cancers including myelodysplasia (MD), acute myeloid leukemia (AML) [29], and chronic myeloid leukemia (CML) [29]. Such myeloid disorders are associated with increased medullar vessel density and VEGF up-regulation [30]. CML is characterized by a chromosomic translocation, which results in the fusion of the Bcr and Abl genes [31]. This fusion is accompanied by activation of the Ras>Raf>MEK>Erk pathway, the JAK-STAT pathway and the PI3-kinase pathway resulting in increased transcription of the VEGF gene (for a review, see Ref. [32]). The Erk pathway is also activated in two others situations, which include the stimulation by oxidative stress [33] and infection by *Helicobacter pylori* of gastric cells, such infection results in cancer development in many cases [34]. In all the cases cited above, transcriptional regulation of the VEGF gene is mediated through the proximal region of the promoter (−88 base pairs upstream of the initiation of transcription), which contains a high proportion of GC domains. Such regions bind factors of the Sp family that interact with the sequence GGCGGG; the AP-2 transcription factor which binds to the consensus sequence (GGCCGGGG), Egr-1 which binds to the consensus (GCGGGGGCG). All these factors are implicated in increased transcription following exposure to different stimuli (see Table 1). However, the Sp1 and Sp3 factors seem to be principally involved in VEGF regulation. In the following sections, we will focus in particular on the implication of Sp1 in the regulation of VEGF gene transcription.

Table 1
Factors and stimuli implicated in the regulation of the proximal VEGF promoter

Factor	Stimulus	Reference
Sp1	TNF-α	[90]
Sp1	PDGF	[91]
AP-2	TGF-α	[56]
Sp1/AP-2	Ras/Erk	[25]
Sp1	IL-1β	[92]
Sp1	Endotoxin	[93]
Sp1/Sp3	All *trans* retinoic acid	[94]
Sp1	Retinoic acid	[95]
Sp1	Oxidant	[96]
Sp1/AP-2	ErbB tyrosine kinase receptors	[19]
Sp1	HGF	[97]
Sp1	TGF-β1	[98]
Sp1/Sp3	Interferon α	[99]
Sp1/Sp3	Oxidative stress	[33]
Sp1/Sp3	*Helicobacter pylori*	[34]
Sp1	IRS/PKC zeta	[100]
Sp1/Sp3 ER α	Estrogen	[74]
Sp1/Sp3	Gonadotrophin	[101]
Sp1/Sp3	Bcr/Abl	[30]

2.1.1. Sp proteins; transcription factors that are more complicated than expected

Sp1, one of the first transcription factor cloned in 1987 by the group of Robert Tjian [35], is finely regulated and implicated in the transcription of numerous genes. Invalidation of its gene results in early embryonic lethality [36]. However, Sp1−/− embryonic stem cells are viable, which implicates the presence of redundant factors that bind to equivalent sequence. Hence, Sp1 belongs to a multigene family which include Sp2, Sp3, and Sp4, the "Krüppel-like factor" (XKLFs) and the "TGF-β Inducible Early Gene" (TIEGs). XKLFs and TIEGs are implicated in the differentiation and cell growth [37].

As Sp1 is crucial to the expression of many genes following induction by various stimuli, we will focus on the situations where regulation of Sp1 by protein/protein interaction or post-translational modification are implicated in VEGF expression.

2.1.2. Sp1 associated proteins and the modulation of VEGF expression

2.1.2.1. Sp1 and pVHL interaction. Sp1 directly interacts with the E3 ligase pVHL, a tumor suppressor mutated in patients with the von Hippel–Lindau Syndrome. Such patients develop vascularized tumors characterized by high VEGF expression. The interaction between Sp1 and pVHL occurs in a domain of pVHL comprised between amino acids 96 and 122 (a zone frequently mutated) and in the zinc-finger domain of Sp1. This interaction results in an inhibition of the Sp1 activity by preventing its interaction with its target sequence on the VEGF promoter [38]. These results support the notion that pVHL is a permanent inhibitor of the positive action of Sp1 in normal cells. The loss of Sp1 inhibition plays a key role in the pathogenesis of the VHL disease.

2.1.2.2. Sp1 and p53/p73 interaction. p53 is an ubiquitous transcription factor responsible for G1 arrest when DNA is damaged. It is mutated in 50% of human cancers and possesses a tissue-specific homologue, p73, also mutated in numerous human cancers. p53 mutation induces VEGF expression in NIH3T3 fibroblasts and potentiates its induction by TPA. Moreover, p73 inhibition by hypermethylation in leukemias or lymphomas is concomitant to increased VEGF expression. Overexpression of p53 [39] and p73 [40] in cancer cells represses VEGF transcription via the proximal region of the promoter that is a target of

Sp1. The mechanism of action is unknown but, as p53 and Sp1 can interact, p53 and p73 could prevent the interaction of Sp1 with its target sequence like in the case of pVHL.

2.1.2.3. Sp1 phosphorylation is required for full VEGF promoter induction. Sp1 is a highly phosphorylated protein on serine and threonine residues [41]. Numerous kinases can phosphorylate Sp1 in vitro including DNA PK [41], casein kinase II [42], and PKA [43]. Sp1 is phosphorylated in response to serum [44], HGF, EGF [45–47], Neu differentiation factors (NDFs) [48], TGF-β1 [49], and activation of the Erk pathway by the Raf:ER chimera or constitutive active members of the Ras/Erk pathway [50]. Both binding and *trans*-activation capacities are stimulated following exposure to these different stimuli. One explanation for this phenomenon depends on direct phosphorylation of Sp1 by Erk on Threonine 453 and 739, two residues shown to be phosphorylated in vivo by Erks [50]. Indeed, inducible expression of Sp1 mutated in the two phosphorylation sites strongly compromise the VEGF expression in response to Erk stimulation [50]. This finding strongly establishes that Erk phosphorylation of Sp1 is a major determinant in VEGF expression in response to Ras activation.

2.1.2.4. Sp1 and Sp3 compete for the same DNA element. Sp3 is also an ubiquitous factor that is highly homologous to Sp1 [37]. Both the transcriptional activator and repressor potential have been attributed to Sp3 [51,52]. This discrepancy is due to the fact that a previous incomplete cDNA was cloned in 1992 [53]. This cDNA does not contain the N-terminal extremity that is crucial for Sp3 activity. Moreover, Sp3 also contains two more internal initiation sites of translation that generate shorter forms of Sp3 with a repressor potential [52]. As Sp3 could have antagonist activities conflicting results have been published. The Sp1/Sp3 ratio is the most important mode of regulation. For example, this ratio is more important in endothelial cells than in non-endothelial cells. Hence, the transcription of the VEGF receptor 2 gene, which contains in its promoter GC-rich sequences, is more abundant in endothelial cells because Sp3 cannot exert its repressor activity [54]. Under hypoxic conditions, the amount of Sp3 decreases, whereas Sp1 is unchanged. A concomitant induction of transcription is observed in the absence of the hypoxia-inducible factor-1 (HIF-1) response element [55]. In this case, the Sp1/Sp3 ratio represents a putative HIF-1 independent regulatory mechanism in hypoxia.

2.1.2.5. Antagonist effects of activator protein-2 alpha (AP-2) on Sp3. AP-2 binding sites have been described on different elements of the VEGF promoter but the functionality of only one site was demonstrated in the proximal region of the promoter. This region contains the classical AP-2 consensus element 5′-GCCNNNGGC-3′ [56], which has been shown to mediate induction of VEGF expression induced by serum, UVA exposure [57], transforming growth factor α [56], hepatocyte growth factor [58], and epidermal growth factor [59] stimulation. In these different cellular models (breast epithelial cells, keratinocytes, a glioma cell line), AP-2 was demonstrated to be an activator of VEGF transcription. However, whereas AP-2 is highly expressed in normal prostatic epithelium, its expression is lost in the case of prostate cancer cells. Forced AP-2 expression decreased the capacity of tumor formation induced by prostatic tumor cells in nude mice, which coincides with a 15-fold reduction in VEGF expression. In that case, AP-2 acts as a tumor suppressor by competing with the binding of Sp3 to its target sequence located between −88 and −66 [60]. Hence, even if this region generally exerts a basal or regulated activity of the promoter, such a phenomenon is highly cell specific.

2.1.3. Down-regulation of Sp1 results in decreased tumor vascularization

Sp1 is a key modulator of VEGF expression and, as a consequence, is a potent regulator of angiogenesis. Hence, oligonucleotides, corresponding to the Sp1 consensus binding site, have been used to antagonize Sp1 action on the expression of VEGF. Sp1 decoy oligonucleotides transferred to cultured cancer cells (A549 and U251 cells) can efficiently block VEGF, TGF-β1, and tissue factor produced in response to TNF-α, whereas mutated Sp1 decoy is inefficient [61]. In vitro invasiveness, production of urokinase-type plasminogen (Upa) activator, and cell proliferation were also inhibited. Sp1 decoy oligonucleotides were also directly injected in melanomas tumors produced in mice. This led to significant tumor necrosis, whereas mutated oligonuleotides have no effect [62]. Tumor necrosis correlated with a decrease in microvascular density as well as a reduction in VEGF 188 and 164 production. Both experiments suggested that the Sp1 decoy oligonucleotide strategy could be really efficient for cancer therapy by decreasing gene expression implicated in cell proliferation (cyclin D1, p21, p27), invasiveness (Upa), and angiogenesis (VEGF).

Down-regulation of Sp1 binding to, and activity on, the VEGF promoter was also observed following treatment with celecoxib (Celebrex), a selective cyclooxygenase-2 inhibitor. In this case, anti-tumor activity measured on pancreatic cancer cells correlated with reduced Sp1 protein and phosphorylation [63]. A strong inhibition of angiogenesis evaluated by microvessel formation in tumor xenografts was associated with a reduction in VEGF production. Down-regulation of VEGF production in CML cells following GLEEVEC (Imatinib mesylate) treatment was also correlated with a decrease in Sp1 binding and *trans*-activation capacities [30]. Sp1 appears to be a target protein for the action of many drugs affecting solid and hematological tumor development.

2.1.4. Use of an engineered zinc-finger transcription factor for in vivo regulation of VEGF

As seen above Sp1 and its relative have a strong impact on VEGF transcription and belong to the family of transcription factors with "zinc finger domains". Hence, artificial zinc-finger proteins (ZFP) containing cassettes encoding each three- or six-finger ZFP, the genes encoding the nuclear translocation signal from SV40 large T antigen, the herpes simplex virus VP16 *trans*-activation domain spanning amino acids 413–490 and a FLAG peptide have been produced. In vivo expression of these artificial ZFP led to induced expression of the VEGF mRNA and protein, stimulation of angiogenesis, and acceleration of experimental wound healing [64].

In another assay, ZFP was fused to either the ligand binding domain of thyroid hormone receptor alpha or its viral relative, vErbA. ZFP-ErbA acts as a transcriptional repressor by mediating the deacetylation of histones H3 and H4. It induced a strong decrease in VEGF expression in the highly tumorigenic glioblastoma cell line U87MG [65].

These two approaches have shown that artificial ZFP, by regulating the expression of VEGF, could favor or counteract in vivo angiogenesis depending on the initial goal, and are promising therapeutic agents.

2.2. Regulation of the VEGF promoter by hypoxia

In response to hypoxia (a decrease in pO_2), higher eukaryotes have developed coordinated mechanisms, at both the transcriptional and translational levels, to cope with this stress. Transcription of genes controlling glycolysis, glucose transport, cell survival and death, angiogenesis and erythropoiesis, are activated by the hypoxia-inducible factor (HIF) to facilitate cell survival and restore O_2 homeostasis [66]. Interestingly, hypoxia, via the activation of HIF-1, is a major actor on VEGF expression, therefore VEGF gradients, reflecting the nutrient needs of the cells, attract and guide the sprouting neo-vessels to the most oxygen-depleted areas in the tissue. Forsythe et al. [67] were the first to identify a functional hypoxia response element (HRE) in the 5′ flanking region of the VEGF-A human promoter where later the heterodimer HIF-1β/HIF-1α was found to bind. However, an additional region, upstream of HIF binding site, is essential to mediate a complete transcriptional activation in response to hypoxia in C6 glioabastoma cells. This domain comprised between −1168 and −1015, which binds the AP-1 transcription factor, cannot by itself drive a response to hypoxia [68]. The relevance of this domain seems cell-specific since, in NIH3T3 cells, hypoxia-inducible VEGF expression is independent on functional AP-1 transcription factor [69]. All conditions known to activate HIF-1 will have an impact on the expression of VEGF. Hypoxia activates HIF-1 by stabilising the limiting subunit, HIF-1α This action is triggered by inhibiting HIF prolyl-4 hydrolysase 2 or PHD2 [70], the key enzyme involved in the instability of HIF-1α. Besides this major stimulus, a variety of growth factors and cytokines including EGF, heregulin, FGF2, insulin, IGF1 and 2, and IL-1 increase HIF-1α protein levels and induce HIF-1 dependent genes under non-hypoxic conditions [71].

We have seen above that the Ras>Raf>Erk pathway strongly activates the VEGF promoter by targeting the proximal region (−88/−66) where Sp1/AP2 transcription factors bind. When we inactivate this Sp1/AP2 proximal binding site by appropriate mutations, we totally abrogate promoter activation by Erk. However, using this construct in cells expressing HIF-1 (co-expression of HIF-1α and β), we recovered Erk activation of the VEGF promoter [72]. This experiment led us to conclude that Erk, in addition to targeting Sp1, must also target the HIF-1 transcription factor. Indeed, we established that Erk specifically phosphorylates HIF-1α in vivo, an action essential for the *trans*-activation of the VEGF promoter [72]. However, the sites of phosphorylation and the mechanism of activation remain to be determined.

In conclusion, HIF-1, a master transcription factor activated by environmental stresses like hypoxia and acidic pH but also by several growth factors and cytokines, is a major determinant in the expression and secretion of VEGF by cells.

2.3. Regulation of the VEGF promoter by female hormones

VEGF is the major inducer of neo-vascularization occurring in normal endometrium, ovary and mammary glands. Hence, 17β estradiol induces VEGF transcriptional activity through an imperfect estrogen response element (ERE) situated at −1520 upstream of the initiation of the transcription start site. ERE can bind the estrogen receptors α and β (ER α, β) [17]. However, the capacity of both receptors to activate the VEGF promoter in response to E2 or tamoxifen depends on the cell type. The AF-2 domain of ER α is sufficient to induce VEGF transcription activation similar to that obtained with the full -length protein. ER α can associate with BRCA1, preventing transcriptional activation of the VEGF promoter [73]. However, in the presence of estrogen, ER α dissociates from BRCA1 and interacts with its target ERE. Mutated forms of BRCA1 found in human tumors failed to interact with ER α. Hence, BRCA1 serves as an angiogenic inhibitor. ER α was also shown to directly interact with the Sp1 and Sp3 transcription factors. These results demonstrate that the estrogen receptor can modulate the activity of the VEGF promoter without directly interacting with DNA [74]. Progesterone also induced VEGF transcription via progesterone receptors A and B (PRA,B) [75]. Three progesterone responsive elements were identified in the promoter PRE1 (TGTACA) from −1865 to −1860, PRE2 (TGTACA) from −716 to −711, and PRE3 (TGTTCT) from +679 to +684. This last site is downstream of the cryptic promoter present in the region corresponding to the 5′ UTR (+632).

Mutation or deletion of one PRE is sufficient to reduce the progestin effect without abolishing it. Moreover, site-directed mutagenesis of all three PREs does not fully abrogate VEGF transcription [76]. Therefore, other response elements within the VEGF gene promoter sequence appear to play an important contributory role in the regulation of VEGF gene transcription by progestins. PRB preferentially regulates VEGF expression in cancer cells and as a consequence PRB-enriched tumor cells produce highly vascularized tumors.

2.4. Regulation of the VEGF promoter by liver X receptors

Liver X receptors α and β (LXRα and LXRβ) are members of the nuclear hormone receptor family that hetero dimerize with the retinoid X receptor (RXR) and bind to the DR4 type sequence also known as the LXR response element (LXRRE). Oxidized derivatives of cholesterol are natural ligands of LXR. LXRα is highly expressed in the liver, whereas LXRβ is ubiquitous. The VEGF promoter is a direct target of LXR on a region comprised between −302 and −317 in the human promoter (TGTCCGcacg-TAACCT) and −272 and −257 in the mouse promoter (TGTCCGcataTAACCT). VEGF expression is induced in response to synthetic LXR agonists in murine and human primary macrophages as well as in murine adipose tissue in mice [77]. This phenomenon is independent of HIF-1 and can be induced by both LXRα and LXRβ. The physiological relevance of the role of LXR is not clear. LXR does not seem to be involved in basal VEGF expression as LXR−/− mice show no obvious vascular problems but rather, in response to inflammation, wound healing or an excess of oxidized lipids or cholesterol responsible for the formation of atherosclerotic lesions. However, this unsuspected role of LXR in vascular biology is far from being totally understood.

2.5. Regulation of the VEGF promoter by retinoids

Retinoids are both natural and synthetic vitamin A derivatives. They are frequently used for the treatment of skin disorders [78] that are characterized by abnormal angiogenesis such as psoriasis [79], delayed-type skin hypersensitivity reactions, bullous diseases [80], and Kaposi's sarcoma [81]. Their biological effects are mediated through two families of nuclear receptors belonging to the super-family of steroid/thyroid hormone nuclear receptors. These receptors comprise the retinoic acid receptors (RAR) and the retinoid X receptors (RXR) that are ligand-dependent *trans*-activating factors. They can also down-regulate the expression of many genes by antagonizing the positive effect of the AP-1 *trans*-activation factor formed by c-Jun and c-Fos heterodimers. It is via this antagonizing effect that the retinoic acid derivative, CD 2409, which exhibits a strong anti AP-1 activity, inhibits VEGF expression and VEGF promoter activity induced by TPA. Even if the VEGF promoter contains four potential AP1 consensus sites, only one is functional. It is situated in a region corresponding to the 5′UTR (+418/+425) but upstream of the cryptic promoter present in this region (+632). A treatment with CD 2409 inhibits AP1 binding to this consensus site and strongly reduced the VEGF promoter. Mutation of this site totally suppresses the effect of the retinoic acid derivative demonstrating the specificity of action, which is independent of any association of RXR or RAR to the DNA.

2.6. Regulation of the VEGF promoter by signal transducer and activator of transcription 3 (STAT-3)

STAT proteins comprise a family of latent, cytoplasmic, transcription factors that relay to the nucleus cytokine, growth factor, and hormone signaling through tyrosine site-specific phosphorylation of proteins [82]. They play a crucial role in differentiation, proliferation, cell survival, apoptosis, and angiogenesis. In recent years, the constitutive or elevated expression of STAT has been found in cancer cells and oncogene transfected cells, and has been shown to be involved in the immune rejection of allografts and the inflammatory processes of auto-immune diseases. Among the STAT family, STAT3 is a convergence point of signaling pathways implicating receptor and nonreceptor tyrosine kinases and was shown to be constitutively activated in a wide range of cancer cells. A DNA response element for STAT3 was described between −848 and −840 of the human VEGF promoter (TTCCCAAA) and its relevance was confirmed by chromatin immunoprecipitation [83]. Inhibition of STAT3 activity by dominant negative forms down-regulates promoter activity, whereas a constitutive active form of STAT3 activates it [84]. Oncostatin (OSM), abundantly expressed in gliobastomas [85], and interleukin-6 (IL-6) [86], which is required in the pathogenesis of cervical cancers, were shown to induce VEGF expression. STAT3 is indeed essential for such induction since a dominant negative form of STAT3 block OSM or IL-6 induced VEGF expression. In the case of IL-6, VEGF induction is not dependent on the Erk or PI3-kinase pathways. The physiological importance of STAT3-mediated VEGF regulation was also demonstrated in cardiomyocytes. Adenoviral transfer of a constitutively active form of STAT3 [87] promotes secretion of functional VEGF in the culture medium [88], whereas induction of VEGF by LIF or cardiotrophin is inhibited by a dominant negative form of STAT3 [89]. Transgenic mice expressing caSTAT3 in the heart showed increased capillary density accompanied by an increase in VE-cadherin, whereas overexpression of a dominant negative form is associated with reduced expression of VEGF mRNA. Hence, STAT3, through its effect on VEGF expression, is essential for heart integrity and reparation after ischemic injury.

3. Conclusion

VEGF is a key growth factor essential for normal and pathological angiogenesis. Many stimuli including growth factors, hormones, cytokines, and cellular stress regulate its expression, particularly at the transcriptional level. A recapitulative figure (Fig. 1) integrates all the domains of the promoter region that have been identified in mediating the above regulatory mechanisms. However, the implication of different zones of the promoter has not been elucidated. Moreover, the interplay between different regulatory regions [proximal Sp1 binding domains and the HIF-1 binding domain, for example (see Sp1 and pVHL interaction)] needs to be further investigated. We can imagine that interaction between HIF-1 and Sp1 in hypoxic conditions would probably boost the VEGF gene transcription by a better accessibility of the promoter to RNA polymerase. If VHL is present in such complex, it would permanently lower VEGF transcription even in hypoxic conditions. In VHL disease, this transcriptional "break" is lost inducing accumulation of VEGF mRNA. In addition, particular attention should be given to the role of the cryptic promoter described by Akiri et al. [18]. Different forms of VEGF mRNA initiated from this promoter have a potential different pattern of expression and could be differentially induced in response to different signals or repressed in response to anti-angiogenic drugs. Considering the myriad of modes of regulation of the VEGF promoters, an integrated view of VEGF regulation at the transcriptional level is far to be resolved, however two major avenues for interfering with VEGF expression should focus on the action of the two transcription factors Sp1 and HIF-1.

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