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Chapter

Vascular Endothelial Growth Factor Expression in the Pathological Angiogenesis in Oral Squamous Cell Carcinoma

Biagio Rapone and Elisabetta Ferrara

Abstract

Tumor angiogenesis and tumor progression to late oral squamous cell carcinoma are closely related. Vascular endothelial growth factor (VEGF), a heparin-binding growth factor with mitogenic activity specific for vascular endothelial cells, regulates key events of the pathological angiogenesis involved in the metabolic functions of malignant tissues. The level of high-affinity tyrosine kinase receptor for VEGF, *flt*, in tumor endothelial cells *in vivo* is seen upregulated, supporting the role of VEGF as a potential signaling tumor angiogenesis axis *in vivo* and sustaining the notion that paracrine mechanisms are responsible for the regulation of tumor angiogenesis. The expression of VEGFs is increased in the processes of oral squamous cell carcinoma (OSCC) progression and proliferation. Vascular endothelial growth factor C (VEGF-C)/VEGF3 expression induced by chemokine CCL4 is connected to lymph node metastasis in OSCC. This chapter was aimed to summarize and analyze the findings on the role of vascular endothelial growth factor in oral squamous cell carcinoma and briefly discuss the potential of vascular endothelial growth factor that targets this pathway as treatment for OSCC.

Keywords: angiogenesis, vascular endothelial growth factor, oral cancer, overexpression, VEGF polymorphism

1. Introduction

The angiogenesis process involves approximately twenty factors, such as basic fibroblast growth factor, placenta growth factor (PIGF-1), epidermal growth factor (EP), as platelet-derived GF (PDGF), and the most important angiogenic factor: vascular endothelial growth factor (VEGF). Studies of restricted expression patterns and functional roles have implicated VEGFs in the generation of new blood vessels from pre-existing vasculature complex genetic pathways. VEGF has since been documented as being a potent stimulator of endothelial cells proliferation and migration and to induce the expression of interstitial collagenases. Specifically, VEGFs regulate physiological angiogenesis, including the vessel and the organ development, the lymphogenesis, and the differentiation during embryogenesis, as well the pathogenesis of a multiplicity of disorders. The hypothesis that VEGF action is required for tumor angiogenesis has been first provided by the findings

of the vascular development of tumor xenografts in mice. These results were confirmed through in situ hybridization studies, showing a correlation between the degree of defective angiogenesis and VEGF mRNA upregulation. These studies uniformly concur that VEGF expression enhances tumor growth. In this chapter, we provide a brief historical overview of the discovery of VEGF, structural characterization of the other members of VEGF family and their receptor, and to summarize the main features of the role of vascular endothelial growth factor in angiogenesis of oral cancer development.

2. Discovery of VEGF

VEGF was first described in 1983 as a factor secreted by hepatocarcinoma cell lines [1] that increased microvascular permeability to plasma proteins in the skin of guinea-pigs. It was highly purified to homogeneity from pituitary folliculostellate cells and characterized in 1989 [2]. Other authors supported evidence that this protein potently stimulated endothelial cell migration [3–5]. It was named "vascular permeability factor" or VPF, potent inducers of vascular hyperpermeability (especially venular endothelium) to fibrinogen and other plasma proteins [6], which upon secretion by tumor cells, promotes vascular leakage [7]. More years later, it was demonstrated that VPF has potent mitogenic activity in a diversity of cell types, and also versus endothelial cells [8]. Vascular endothelial growth factors (VEGFs) are predominantly produced by endothelial, hematopoietic, and stromal cells in response to hypoxia and upon stimulation by growth factors such as transforming growth factor β (TGFβ), interleukins, or platelet-derived growth factors (PDGFs). VEGFs specifically interact with one or several receptor tyrosine kinases (RTKs), VEGF receptor −1, −2, and −3 (VEGFR-1, −2, −3), and with distinct coreceptors such as neuropilins or heparan sulfate glycosaminoglycans. VEGF receptors are classified as type V RTKs whose extracellular domains consist of seven immunoglobulin-like (Ig-like) domains [9, 10]. The intracellular domain consists of seven immunoglobulin-like domains (I–VII), a single transmembrane (TM) region, and a tyrosine kinase consensus sequence (TK) interrupted by a cytoplasmatic kinase domain [11, 12].

3. The VEGF gene and isoforms

The VEGFs term identifies a large and heterogeneous family of secreted polypeptides, named VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placenta growth factor (PGF), characterized by a highly conserved receptor-binding cystine-knot structure similar to that of the platelet-derived growth factors [13, 14]. Of those six members, VEGF-A plays a key role in vasculogenesis and angiogenesis [15]. PGF is mainly expressed in placenta, heart and lungs; it is a ligand for VEGFR-2 and it is involved in angiogenesis regulation. VEGF-B binds VEGFR-1 and neuropilin-1, mostly expressed in the extracellular matrix and abundantly expressed in brown fat, in the myocardium and skeletal muscle; it is implicated in high cellular energy metabolism. VEGF-C is produced as a precursor protein [16, 17]. VEGF-C promotes mitogenesis, migration and survival of ECs [18], and regulates the lymphatic vessel growth by binding to VEGF-receptor-3 (VEGFR-3, Flt-4) [19, 20]. VEGF-D, binds and activates VEGFR-2 and VEGFR-3, because is mitogenic for EC, angiogenic, and lymphangiogenic. Its expression was demonstrated mainly in the lung and skin during embryogenesis. VEGF-E expression, binding the VEGFR-2, promotes the release of tissue factors, proliferation, chemotaxis and

sprouting of cultured vascular ECs in vitro and angiogenesis in vivo [6, 21]. The major function of VEGF-C consist in regulation VEGF exerts its effect on tissues at several levels; mechanisms range from a plethora of physiological processes which regulate blood vessel growth, such as during pregnancy and in tissue repair to pathological conditions, including chronic inflammation, would healing process, and cancer [6, 22]. It has been proven that VEGF causes a pronounced angiogenic response in a variety of in vivo models, including the chick chorioallantoic membrane [23, 24]. Native VEGF is a basic, heparin-binding, homodimeric glycoprotein of 45,000 daltons. Interestingly, the VEGF gene has been mapped to chromosome 6 at position p21.3 [25] and consists of eight exons and seven introns in the coding sequence, which covers a region of 14 kilobases. Among these, there is evidence that the $-634G/C$, $-1154G/A$, and $-2578C/A$ *VEGF* polymorphisms have been shown to be associated with increased VEGF production. Alternative splicing of the mRNA from the gene of VEGF, VEGF-B, and PIGF results in the expression of five known human isoforms with differential diffusibility and heparin-binding properties containing 121, 145, 165, 183, 189 and 206 amino acids with different biological properties: $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$ [26]. The $VEGF_{121}$ is a weakly acidic polypeptide with a 44 *amino-acid insertion* encoded by exon 7a*,* that fails to bind to heparin [27]; *VEGF_{189} has* a further *insertion* of 24 *amino acids, highly enriched in basic residues* encoded by exon 6a. $VEGF_{121}$ and $VEGF_{165}$ have been detected predominantly in normal tissue, but VEGF121 isoform is both more angiogenic and tumorigenic than being the 165 and 189 isoforms [27, 28]. In particular, the isoform 121 has been shown to predominate in primary human breast carcinomas (Relf M) $VEGF_{189}$ expression has been shown to be dominant in normal lung [29] and the 183 isoform predominates in heart [30, 31]. Several studies have reported that the expression of $VEGF_{206}$ occurs mainly in fetal liver.

3.1 Biological effects of VEGF expression

The VEGF and the "fibroblastic growth factor" (bFGF) demonstrate a powerful synergism in the promotion of angiogenesis in vitro, as shown by models using microvascular endothelial cells invading the *three-dimensional collagen* gel system [32, 33]. Also, VEGF exerts its effect by coordinating of angiopoietins, another class of angiogenic factors. Specifically, VEGF is involved in the early sequences of events leading to the vessel development, whereas angiopoietin 1 (Ang1), an agonist ligand for the endothelial-specific Tie2 receptor, binds and activates Tie2 to promote vessel maturation, vascular stability and leakiness [34]; Ang2 acts as a Tie2 agonist in lymphatic endothelial cells generating an important vascular signaling pathway involved in angiogenesis, vascular stability and quiescence. VEGF achieves its functions of endothelial cell differentiation and proliferation by binding a family of tyrosine kinases receptors (VEGFRs), known as Flt-1 receptor (VEGF receptor-1) and VEGF receptor-2 (VEGF-2 or KDR/Flk-1). VEGFR-1 binds VEGF, VEGF-B and PlGF with high affinity and induces weak mitogenic signals in ECs. [23, 35] VEGFR-1 expression is up-regulated by hypoxia via (transcription hypoxia inducible factor) HIF-dependent mechanism [36, 37]. In the lung, VEGFR-1 provokes secretion of Matrix Metalloproteinase 9 (MMP9) at the vascular bed, thus empowering metastasis. Also, it has been discovered the role of VEGFR-1 in releasing tissue specific factors in a perivascular specific pattern at the level of vascular endothelium [38]. VEGFR-2, the major mediator of endothelial cell mitogenesis, proliferation and survival [39, 40] binds VEGF, VEGF-C, VEGF-D, VEGF-E and PlGF [22, 39]. VEGFR-2 expression is down-regulated in the adult blood vascular ECs, and is again up-regulated in the endothelium of angiogenic process. VEGF, VEGF-C and VEGF-D are bound VEGFR-3 and are involved in regulation of lymphangiogenesis,

the growth of new lymphatic vessels [41–45]. The expression of VEGFR-3 (or Flt-4) is relevant in lymphatic vessels [44] and in hematopoietic cells of monocytic lineage [6, 42], and is also expressed in a subset of capillary endothelia [19]. Studies on animal models showed that VEGF-C/VEGFR-3 axis plays a crucial role in cancer metastasis by inducing lymphangiogenesis [46–48], but further investigations would be necessary. Furthermore, it has been documented the link between mutations in VEGFR-3 with hereditary lymphedema, an autosomal dominant disorder of the lymphatic system that can lead to lymphangiosarcomas [13, 49]. Neuropilins-1 and -2 are more important in immunology and neuronal development, but they are also involved in angiogenesis [19, 44]. Neuropilins, bind especially class 3 semaphorins but the Neuropilin-1 also binds VEGF, VEGF-B and PlGF, while Neuropilin-2 binds VEGF, VEGF-C and PlGF [50]. When is coexpressed in cells together with VEGFR-2, Neuropilin-1 enhances the binding of VEGF165 to VEGFR-2 and augments tumor angiogenesis in vivo [51, 52]. Nrp-2 is expressed also on lymphatic ECs, and mutated Nrp-2 forms induce abnormalities in the formation of small lymphatic vessels and lymphatic capillaries in mice [53]. In addition, some isoforms bind to known as non-tyrosine kinase receptors, known as neuropilins (NRPs) (neuropilin-1 and neuropilin-2 (22–44)). Neuropilins-1 and -2 are also involved in immunology and neuronal development [50, 54]. Neuropilins bind, especially class 3 semaphorins but the Neuropilin-1 also binds VEGF, VEGF-B and PlGF, while Neuropilin-2 binds VEGF, VEGF-C and PlGF [50]. Neuropilin-1 is capable to improve the binding of VEGF165 to VEGFR-2 and increase tumor angiogenesis in vivo [51, 53]. Nrp-2 is expressed also on lymphatic ECs, and it has been shown in vivo that mutated Nrp-2 form alters the formation of small lymphatic vessels and lymphatic capillaries in mice [54]. The binding results in stimulation of cell-signaling pathways that act to increase cell nucleus division, and contributing to angiogenesis through extracellular matrix dissolution, and endothelial cell movement.

4. Role of VEGF in oral squamous cell carcinoma

4.1 The role of VEGF in invasion

Angiogenesis, the formation of new blood vessels, is not only a normal physiological process, but it is closely linked to both tumor growth and metastasis, providing the principal pathway through tumor cells exit the primary tumor site and enter the circulation. A multiplicity of molecular determinants is involved in these different mechanisms of vascular growth, and VEGFs play a significant role in pathological angiogenesis. In particular, the role of VEGF-A in tumor angiogenesis are clarified by several studies [23, 55], but the results concerning VEGF-expression in normal and dysplastic oral epithelium, as well as about the potential role of VEGF in oral squamous cell carcinoma progression shown contradictions. VEGF-A overexpression has been reported in most types of cancer, including oral squamous cell carcinoma, and it is thought to be a prognostic factor for survival. Some studies revealed no altered VEGF expression in the normal and mildly dysplastic oral epithelium, or the expression was significantly lower than neoplastic epithelium [16–18]. A considerable upregulation of VEGF expression during the transition from normal oral epithelium through dysplasia to OSCC, but no correlation was found between VEGF expression and the grade of dysplasia. Many researchers demonstrated an upregulation of VEGF expression in cancerous tissues in comparison to the normal oral mucosa. These results suggest that VEGF could be implicated in tumor progression by expanding the vascularity during the process of transition from the normal oral mucosa to invasive carcinoma [18, 51–56].

Immunohistochemical study of VEGF expression in oral squamous cell carcinomas clarify the relationship between vascular endothelial growth factor (VEGF) expression and clinicopathological factors in oral squamous cell carcinoma (OSCC), showing that serum VEGF levels were significantly higher in oral cancer patients as compared to normal controls that further showed an increasing trend with clinical stage and lymph node involvement, suggesting that VEGF level may be a reliable biomarker and may be a potential target for development of chemotherapeutic strategies for patients with oral carcinoma.

4.2 The role of VEGF in metastasis

Lymphangiogenesis plays a vital role in tumor growth and systemic dissemination of different carcinomas [2]. Although vascular endothelial growth factor C (VEGF-C) is well known to be associated with lymph node metastasis in patients with OSCC, inducing the production of urokinase by cancer and playing a crucial role in the extracellular matrix remodeling and tumor cell invasion, the specific mechanisms of lymphangiogenesis in OSCC are largely unknown. Further, it has been implicated in inducing the growth of both blood and lymphatic vessels and in regulation of proliferation, differentiation and migration of lymphatic endothelial cells in many physiological and pathological conditions [19, 20]. Overexpression of VEGF-C has been detected in various malignancies and is frequently associated with lymphatic invasion, nodal and distant metastasis and consequently poor survival [23, 33]. VEGF-C signaling is involved in the progression of several malignancies that put forward VEGF-C as a potential target for the development of new anticancer therapies to prevent local invasion and metastatic spread of disease. The progression of several malignancies involves VEGF-C signaling. The immunohistochemical analysis and real-time quantitative RT-PCR studies showed a significant correlation between the levels of VEGF-C mRNA expression in a human OSCC cell line, through the phosphoinositide 3-kinase-Akt pathway. The angiogenic action of this growth factor is achievable by binding to two receptor tyrosine kinases (RTK), VEGFR-1 (Flt-1) and VEGFR-2 (KDR, Flk-1) [17, 22]. VEGFR-2 is the major mediator of the mitogenic, angiogenic and permeability-enhancing effects of VEGF-A, while the role of VEGFR-1 in the regulation of angiogenesis is controversial. It is implicated in a paracrine release of growth factors, but the central role of this receptor is to prevent the interaction of VEGF with VEGFR-2. Also, it may promote angiogenesis by recruitment in tumor vasculature of monocytes and other bone marrow-derived cells [48, 52, 57]. Moreover, VEGFR-1 is involved in the induction of matrix metalloproteinases [49] that retrovirus-mediated expression of a dominant negative VEGFR-2, because of inhibition of signal transduction through wild-type VEGFR-2, suppression of the growth of glioblastoma multiforme and other tumor cell lines in vivo. Correlation of VEGF expression with clinical stage of OSCC is controversial. Clinicopathologic findings suggest that evaluation of VEGF expression is of prognostic value in patients with OSCC. VEGF expression was found to increase significantly with advancing stage of the tumor. Elevated expression of VEGF was associated with aggressive phenotype and advanced stage of the tumor [58], as well as demonstrated by Yu Hong Li et al. which found a higher VEGF expression in stage 3 and 4 tumors as compared to stage 1 and 2 [59]. An explanation could be attributed to the correlation with the intensity of VEGF expression and the degree of differentiation or invasiveness of carcinoma. The results support the findings of previous *results* on the average density of immunohistochemistry (IHC), indicating that in normal oral squamous cells, VEGF expression was detected only in the vascular endothelial cells in the mesenchymal tissues [60, 61]. According to these results, to Kim's study on the up-regulation of vascular

endothelial growth factor (VEGF) expression in oral squamous cell carcinoma, immunohistochemical staining with VEGF, found a very little expression of VEGF in normal oral squamous cell tissues and a significant positive relationship between VEGF expression and the degree of differentiation or invasiveness of carcinoma [62]. In addition, histopathological findings showed that the VEGF had *higher expression* levels in less-differentiated invasive oral squamous cell carcinoma, while it was expressed at slightly higher levels in well-differentiated and less-invasive intraepithelial carcinoma tissues or highly differentiated oral squamous cell carcinoma than in normal cells [62]. In contrast, Shang and Li found that VEGF expression was significantly higher in patients in patients with stage 1 and 2 tumors as compared to when there were a stage 3 and 4 tumors [63].

4.3 The role of VEGF in prognosis

Interestingly, some studies have focused on the potential prognostic importance of *VEGF* polymorphisms in head and neck cancers [14, 20, 21]. Results showed the association between −1154GG *VEGF* genotype, located in the promoter region of the gene, and higher VEGF production [12]. A study conducted by Ku et al., reported an association of −460C/T polymorphism and VEGF overexpression in oral cancer, showing a higher risk for oral cancer in the patients with a high −460TC ratio [66, 67]. Further investigations showed an association between +960C/T *VEGF* polymorphism and oral cancer [23], indicating that the low production of VEGF by the T allele is correlated with increased risk of oral cancer, and vascular invasion in oral squamous cell carcinoma. In contrast, Vairaktaris et al. [56], did not demonstrate an influence of gene polymorphism on the oral cancer in the logistic regression models. However, the genotype *VEGF* −460CT was associated with early stage tumors. Nasr et al. analyzed the polymorphism *VEGF* −2578C/A and suggested that carriers of *VEGF* −2578C allele may play a role in susceptibility to nasopharyngeal carcinoma [64, 65]. The risk for laryngeal squamous cell carcinoma seems to be linked with the increase of the −1154G/G genotype of the *VEGF* gene, in the −1154G/A polymorphism of the *VEGF* [27]. Moreover, the polymorphism −1154G/A *VEGF* has been shown to be associated with differential expression of VEGF *in vitro*. However, some of the data are contradictory.

4.4 Vascular endothelial growth factor and advances in developing novel therapeutic strategies for oral squamous cell carcinoma

There is a great need for therapies to prevent and/or slow the progression of OSCC. Recent studies focused on potential of drugs that target VEGF or its receptors-signaling system because their angiogenesis-promoting activity at the level of endothelial cells. Therefore, modulation of these factors to inhibit tumor angiogenesis, currently, is a major focus in developing OSCC therapies. The development of angiogenesis inhibitors as anticancer agents have been developed with data showing promising efficacy at reducing OSCC in in vitro models [13, 14]. Recent studies found that administration of angiogenesis inhibitors agents in association with chemotherapy and radiotherapy can improve the efficacy of these treatments. For example, Teicher et al. observed that coadministration of the TNR-470, angiogenesis inhibitor agent, induces a substantial cyclophosphamide-induced tumor cell-killing. Jain's study confirmed these findings suggesting that it was a consequent normalization of the tumor vasculature, as a result of endothelial cell death. Different therapeutic strategies to induce the inhibition of VEGF or its receptor signaling system are being emerging for treatment of OSCC, as VEGFR-1 ribozymes, VEGF toxin conjugates, and soluble VEGF receptors.

5. Conclusions

Angiogenesis is *essential* for the growth and metastasis of solid *tumors*, including oral squamous cell *carcinoma*. The main factor responsible for angiogenesis is *VEGF* and its receptors. Specifically, *VEGF*-A is known to be a *key* angiogenic factor, and its overexpression has been reported in most types of *cancer*, including *oral squamous cell carcinoma*, and it is thought to be a prognostic factor for survival. It was pointed out that VEGF as an attractive candidate for therapeutic intervention, but more studies are needed to clarify the real potential of angiogenesis inhibitors agents in OSCC, to determine optimal timing for VEGF, and to search for drug candidates.

Conflict of interest

The authors declare no conflict of interest.

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