REVIEW

VEGF and VEGFRs expression in oral squamous cell carcinoma

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Abstract

Oral cancer is an important cause of worldwide morbidity and mortality, with substantial economic, physiological, and psychosocial impacts due to its treatment modality and a great risk for recurrences and second primary oral squamous cell carcinomas (OSCC) development. Therefore, it is very important to understand the underlying cell biology of such tumors. It is now a well-accepted fact that angiogenesis is essential for the growth and metastasis of solid tumors, including head and neck squamous cell carcinoma. The main factor responsible for angiogenesis is VEGF and its receptors. It has been demonstrated that VEGFRs are also present on tumor cells themselves and other cells from the tumor microenvironment, in addition to tumoral endothelial cells (ECs). Therefore between these cells take place numerous and different interactions mediated via paracrine/autocrine pathways that promote angiogenesis, uncontrolled tumor proliferation and metastasation. In consequence, estimation of VEGF expression and its receptors became a reliable prognostic tool in OSCCS, predicting the poor disease-free survival, poor overall survival, and metastatic disease. Understanding the distribution and role of VEGF and its receptors in the progression of OSCC will be essential to the development and design of new therapeutic strategies.

Keywords: angiogenesis, oral, squamous carcinoma, VEGF, VEGFR-1, VEGFR-2, VEGFR-3.

⊟ Introduction

Oral squamous cell carcinoma (OSCC) represents an important pathology of the upper digestive tract, being the sixth common cancer diagnosed around the world [1]. Although improvements have been achieved in surgical techniques, radiation therapy protocols, and chemotherapeutic regimes, the overall five-year survival rate for this disease remains at about 50% and has not significantly improved for the past 30 years [2]. One important mechanism involved in the pathobiology of these tumors is angiogenesis.

Angiogenesis plays an important role in tumor growth and metastasations [3], and vascular endothelial growth factor (VEGF) is an angiogenic cytokine expressed by tumors [4]. VEGF is also known as a vascular permeability factor, being considered a key regulator in tumor-induced neoangiogenesis [5]. In humans, VEGF is expressed in practically all solid tumors studied, as well as in some hematological malignancies [6]. In addition, in some human malignancies, VEGF-expression correlates with disease progression and survival [7], while other studies have shown that the expression of VEGF is an independent prognostic factor in patients with breast cancer [8, 9], colon cancer [10] and esophageal squamous cell carcinoma [11].

⊟ The biology of VEGF and its receptors

VEGF gene regulation and expression control

VEGF gene and its isoforms (Figure 1)

VEGF-A, also known as a vascular permeability factor (VPF) is a heparin-binding, dimeric polypeptide, belonging to the platelet-derived growth factor family [12], which currently includes beside VPF, placenta growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D, and VEGF-E [13–19].

From those six members, **VEGF-A** plays essential roles in vasculogenesis and angiogenesis [20]. At least six VEGF isoforms with different amino acid numbers are produced through alternative splicing: $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$ and $VEGF_{206}$ [21]. The majority of cells secrete $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$. While VEGF121 is present diffuse and relatively freely in tissues, approximately half of the secreted $VEGF_{165}$ binds to cell surface heparan sulfate proteoglycans and the remaining $VEGF_{189}$ is almost completely sequestered in the extracellular matrix making this a reservoir of VEGF that can be mobilized via proteolysis [22]. The secreted forms of VEGF induce proliferation of endothelial cells (ECs) and *in vivo* angiogenesis, while heparin-binding forms of

VEGF can bind to cell surface and extracellular matrix proteoglycans and facilitate the release of other

angiogenic factors stored on heparin sulphates from the extracellular matrix [23].
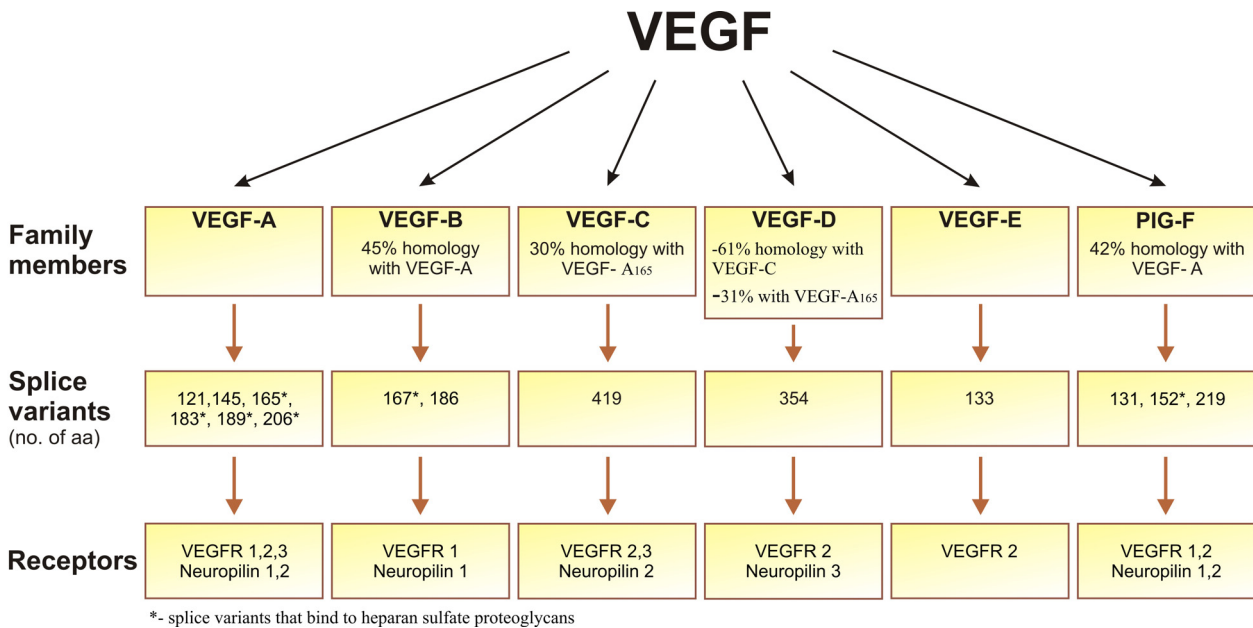

*- splice variants that bind to heparan sulfate proteoglycans

Figure 1 – *Biology of VEGF and its receptors.*

Placenta growth factor (PlGF) is mainly expressed in placenta, heart and lungs [24]. As homodimers, PlGF binds VEGFR-1 and neuropilin-1, while PlGF hetero-dimers bind VEGFR-2 being implicated in angiogenesis regulation [25, 26]. There were reported three human isoforms of PlGF (PlGF-1, -2 and-3), from which only PlGF-2 binds to cell surface heparan sulfate proteo-glycans [27].

VEGF-B is a ligand for VEGFR-1 and neuropilin-1, expressed in humans as two different isoforms: VEGF-B_{167}, mostly sequestered in the extracellular matrix and abundantly expressed in brown fat, in the myocardium and skeletal muscle; and VEGF-B_{186} freely diffusible [28, 29]. The VEGF-B function is most probable linked to high cellular energy metabolism [30, 31].

VEGF-C is produced as a precursor protein, which is activated by intracellular secretory proprotein convertases furin, PC5 and PC7 [32, 33]. The mature form of VEGF-C induces mitogenesis, migration and survival of ECs [34]. The major function of VEGF-C consist in regulation of lymphatic vessel growth by bin-ding to VEGF-receptor-3 (VEGFR-3, Flt-4), a tyrosine kinase receptor which is predominantly expressed in the endothelium of lymphatic vessels [14, 23].

VEGF-D was isolated as a *fos*-inducible factor from mouse skin fibroblasts [35] and the mature form binds *in vivo* and activates VEGFR-2 and VEGFR-3, being mitogenic for EC and angiogenic as well as lymphan-giogenic [34]. In humans, VEGF-D is expressed mostly in the lung and skin during embryogenesis [36] and has been shown to be of prognostic value for lymphatic vessel invasion and also survival in certain human cancers [37].

VEGF-E expression is regulated by the parapox-virus strain NZ7, stimulating angiogenesis in the dermis underlying the site of the viral infection [17, 38]. It was demonstrated that VEGF-E binds with high affinity to

VEGFR-2 [17, 39], stimulating the release of tissue factors, proliferation, chemotaxis and sprouting of cultured vascular ECs *in vitro* and angiogenesis *in vivo*.

VEGF receptors and co-receptors

VEGF-A and all its isoforms bind to VEGFR-1 and VEGFR-2 as well as interact with a family of co-receptors, the neutropilins (neuropilin-1 and neuro-pilin-2) [22, 40].

VEGFR-1 binds VEGF, VEGF-B and PlGF with high affinity and induces weak mitogenic signals in ECs [22, 41]. Activation of VEGFR-1 promotes endothelial cell migration but does not induce cell proliferation [42]. Besides endothelial cell expression, the VEGFR-1 was also observed in osteoblasts, monocytes/macro-phages, pericytes, placental trophoblasts, renal mesangial cells and in some hematopoietic stem cells [22, 43]. By regulating VEGF concentration that may act on VEGFR-2, it may help reduce endothelial cell prolife-ration and prevent overcrowding and vascular disorgani-zation [44]. In addition, an alternatively spliced iso-forms was described, a soluble form of VEGFR-1 that is able to inhibit VEGF-action [44]. VEGFR-1 expression is up-regulated by hypoxia via (transcription hypoxia inducible factor) HIF-dependent mechanism [45]. In the lung VEGFR-1 induce secretion of Matrix Metallo-proteinase 9 (MMP9) at the vascular bed, thus facilita-ting metastasis [46]. Another function of VEGFR-1 at the level of vascular endothelium is to release tissue specific factors in a perivascular specific pattern [47].

VEGFR-2 binds VEGF, VEGF-C, VEGF-D, VEGF-E and PlGF, factors that regulate the expression of this receptor [48, 49]. VEGFR-2 is the major mediator of endothelial cell mitogenesis, proliferation and survival [17, 50, 51]. VEGFR-2 expression is down-regulated in the adult blood vascular ECs, and is again up-regulated in the endothelium of angiogenic

blood vessels [52]. It is also essential for the differentiation of ECs and the induction of microvessel permeability [22, 40, 53]. In addition to the ECs, VEGFR-2 is also expressed on neuronal cells, osteoblasts, pancreatic duct cells, retinal progenitor cells, megakaryocytes and hematopoietic stem cells [22, 54].

VEGFR-3 species bind VEGF, VEGF-C and VEGF-D, and are composed of two isoforms (VEGFR-3short and VEGFR-3long), and regulate lymphangiogenesis, the growth of new lymphatic vessels [55–58]. VEGFR-3 (or Flt-4) is mostly expressed in lymphatic vessels [59] and in hematopoietic cells of monocytic lineage [23, 60]. It was demonstrated on animal models that VEGF-C/VEGFR-3 axis plays a critical role in cancer metastasis by inducing lymphangiogenesis [61–63]; but this axis may have many undefined functions and mechanisms in tumor progression, and further investigations would be necessary. In addition, mutations in VEGFR-3 have been linked with hereditary lymphedema, an autosomal dominant disorder of the lymphatic system that leads to disabilitating swelling of the extremities and, in rare cases, to lymphangiosarcomas [64]. It has been suggested that disturbed VEGFR-3 signaling may play a key role in the development of this disease. In adults, VEGFR-3 is also expressed in a subset of capillary endothelia, although it is absent in endothelia of all large blood vessels [65].

Neuropilins-1 and -2 are more important in immunology and neuronal development, but they are also involved in angiogenesis [66, 67]. 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 [67]. When is co-expressed in cells together with VEGFR-2, Neuropilin-1 enhances the binding of $VEGF_{165}$ to VEGFR-2 and augments tumor angiogenesis *in vivo* [68, 69]. 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 [70].

Regulation of VEGF gene expression

The VEGF gene transcription is upregulated by hypoxia [71]. In this regulation, the major role is played by HIF, which operates in concert with the product of the von Hippel–Lindau (VHL) tumor suppressor gene, that under normoxic conditions target HIF-processing for ubiquitination and degradation [71]. Beside this, a very broad and diverse spectrum of oncogenes, including mutant ras, erbB-2/Her2, activated EGFR and bcr-ab, are implicated in tumoral VEGF-A upregulation [71, 72]. Currently, a variety of growth factors, cytokines as well as nitric oxide have been found to potentiate or inhibit VEGF expression [22, 41].

The biological activities of VEGF and therapeutic implications

The role of VEGF and its receptors in vasculogenesis and angiogenesis

VEGF is involved in every stage of vascular development [42] due to its ability to induce various responses on ECs, including cell proliferation, migration, specialization and survival [22, 42].

Vascular system development is one of the earliest events to occur in organogenesis. The early blood vessels develop by aggregation of *de novo*-forming angioblasts into a primitive vascular plexus (vasculogenesis), which then undergoes a complex remodelling process, in which growth, migration, sprouting and pruning that lead to the development of a functional circulatory system [73]. Many of these events are promoted, guided and aided by VEGF [42], and a decrease of the amounts of VEGF produced during the development of the embryo may lead to decreased angiogenesis with fatal consequences. Thus, in homozygous animals, disruption of the genes encoding the VEGF tyrosine-kinase receptors VEGFR-2 [74] and VEGFR-1 [75] result in severe abnormalities of blood vessel formation.

After birth, angiogenesis still contributes to organ growth but, during adulthood, most blood vessels remain quiescent with only 0.01% of ECs undergoing division, and thus angiogenesis occurs only in the cycling ovary and in placenta during pregnancy. Nevertheless, ECs retain their remarkable ability of dividing rapidly in response to a physiological stimulus, such as hypoxia, so angiogenesis is reactivated during wound healing and repair. When the stimulus becomes excessive, neoangiogenesis may became responsible for many non-neoplastic diseases such as: ocular and inflammatory disorders, obesity, asthma, diabetes, cirrhosis, multiple sclerosis, endometriosis, AIDS, bacterial infections and autoimmune disease, and the list is rapidly growing [76].

The best-known conditions in which angiogenesis is switched on are malignant disorders. Most notably, many tumors promote their own growth and dispersion to form metastases by recruiting host blood vessels to grow into the vicinity of the tumor (so-called tumor angiogenesis) [77]. Many studies revealed the role of VEGF-A in tumor angiogenesis [22, 78]. This growth factor act as angiogenic by binding to two receptor tyrosine kinases (RTK), VEGFR-1 (Flt-1) and VEGFR-2 (KDR, Flk-1). It is now generally accepted that VEGFR-2 is the major mediator of the mitogenic, angiogenic and permeability-enhancing effects of VEGF-A. The role of VEGFR-1 in the regulation of angiogenesis is controversial. While the main action of this receptor is to sequester VEGF, preventing it from interacting with VEGFR-2 in some tumors may promote angiogenesis by recruitment in tumor vasculature of monocytes and other bone marrow-derived cells [45, 79, 80]. Moreover, VEGFR-1 is involved in the induction of matrix metalloproteinases [46] and in a paracrine release of growth factors from ECs [47].

Other roles of VEGF and its receptors

VEGF have also lymphangiogenic effects that may be linked to the recruitment of inflammatory cells, such as macrophages, which express VEGFR-1 and secrete lymphangiogenic factors [81–83]. However, the most potent factor implicated in lymphangiogenesis is VEGFC, which by binding on VEGFR-3 induces migration of ECs committed to the lymphatic endothelial lineage [84].

In addition, VEGF acts as a potent survival factor for ECs during physiological and tumor angiogenesis and it has been shown to induce the expression of anti-apoptotic proteins in ECs [85, 86]. Moreover, VEGF increases the permeability of the endothelium by forming intercellular gaps, vesico-vascular organelles, vacuoles and fenestrations [87], and also causes vasodilatation through the induction of endothelial nitric oxide synthase and the subsequent increase in nitric oxide production [88, 89]. Furthermore, VEGF stimulates inflammatory cells recruitment and promotes the expression of proteases implicated in pericellular matrix degradation in angiogenesis [90–92].

Besides its vascular activity, VEGF is also trophic for nerve cells, lung epithelial cells and cardiac muscle fibers, this further explaining why insufficient VEGF levels contribute to neurodegeneration [93], respiratory distress and, possibly, cardiac failure [76]. Thus, VEGF acts not only on ECs, but also on hematopoietic stem cells, monocytes, osteoblasts and neurons, inducing mobilization of stem cells, chemoattraction of monocytes, bone formation and neuronal protection [22, 94].

Understanding the biology of VEGF and its receptors facilitated the development of therapeutic strategies to promote revascularization of ischemic tissues or to inhibit angiogenesis in cancer, ocular, joint or skin disorders. Therefore, the anti-VEGF treatment combined with chemotherapy or radiation therapy in the attempt to cure some solid tumors proved an increased anti-tumoral effect compared to conventional treatment modalities taken alone [22].

⊟ VEGF and their receptors expression in normal and pre-neoplastic lesions of oral mucosa

Studies regarding VEGF-expression in normal and dysplastic oral epithelium and the potential role of VEGF in oral cancer progression are contradictory.

Thus, some authors found that either the normal and mildly dysplastic oral epithelium have no VEGF-expression or the expression was significant lower than neoplastic epithelium [95–97]. Moreover, Johnstone S and Logan RM [98], indicated a significant up-regulation 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. In our experience, VEGF-expression was up-regulated in cancerous tissues compared to normal oral mucosa [authors' unpublished data] (Figure 2). According to these results VEGF could be implicated in tumor progression by increasing the vascularity during the transition from normal oral mucosa, through variate degrees of dysplasia and to invasive carcinoma [97, 99–105].
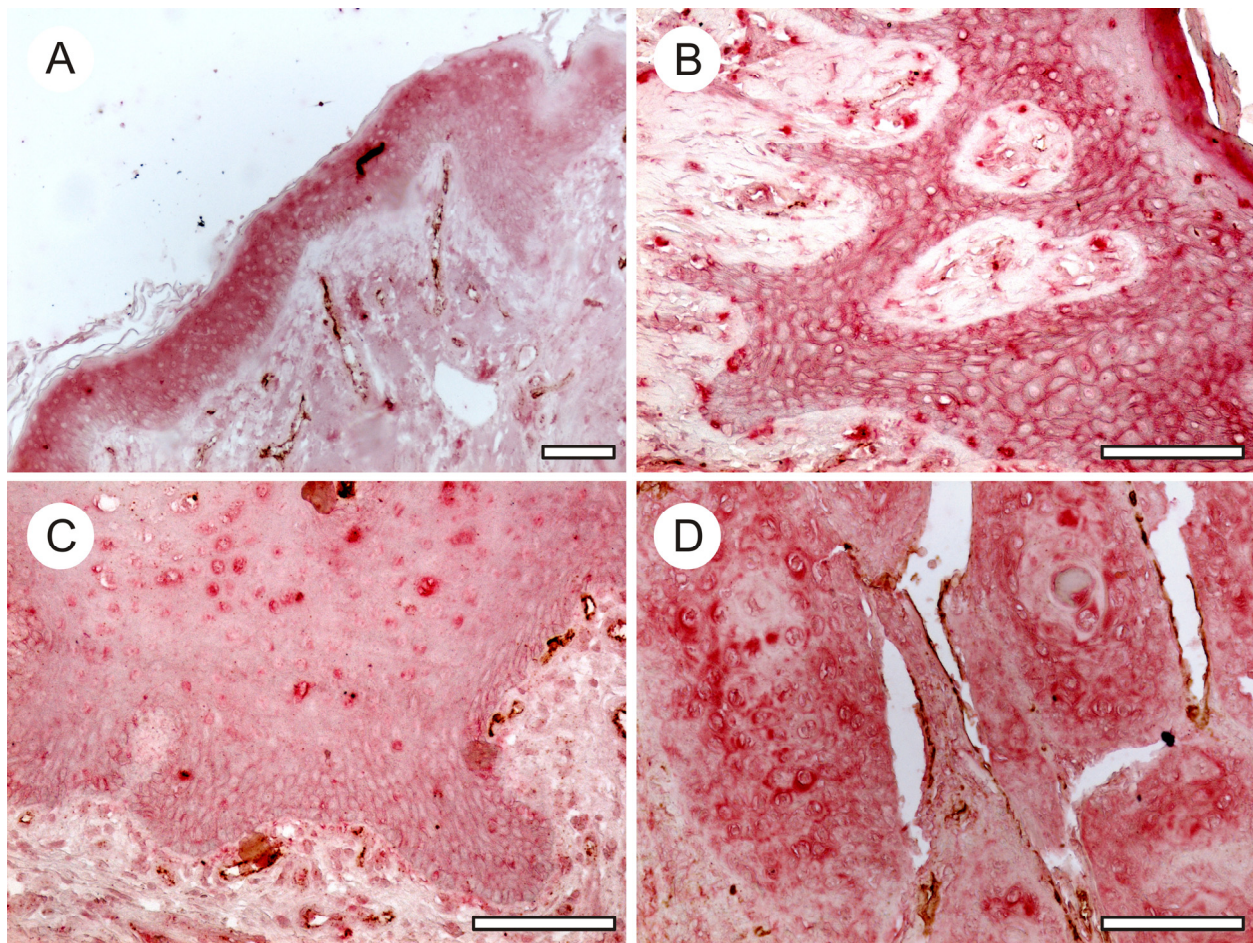

Figure 2 – *Double immunostaining (VEGF – red/CD105 – brown): (a) Normal lower lip; (b) Lower lip mucosa with hyperplasia; (c) Lower lip mucosa with mild to moderate dysplasia; (d) Well differentiated OSCC of the lower lip. Bars: 50 µm.*

On the other hand, other authors do not revealed any differences between VEGF-expressions in normal oral mucosa when compared to epithelial dysplasia [99, 106] or even to cancerous lesions [106]. Moreover, Tae K *et al.* [107] investigating VEGF-expression in head and neck squamous cell carcinomas observed that the level of expression were highest in normal control oral epithelium and decreased as lesions progressed from adjacent normal epithelium to hyperplasia, to dysplasia, and to invasive cancer. In agreement with this results, studies regarding VEGF-expression at other sites then oral cavity, such as skin, vulva, prostate and salivary gland lesions, prove a higher expression of this growth factor in normal tissue and benign lesions compared to cancer [108–111]. As an explication to these results, it was proposed that VEGF is involved in regulating the functions of these tissues under normal physiological conditions.

There are no data regarding VEGF-receptor expression in normal or preneoplastic oral epithelium. In our experience, VEGFR-1 and VEGFR-2 are expressed in the cytoplasm of acinary cells and luminal cells of excretory ducts of minor salivary glands and in macrophages (Figure 3).
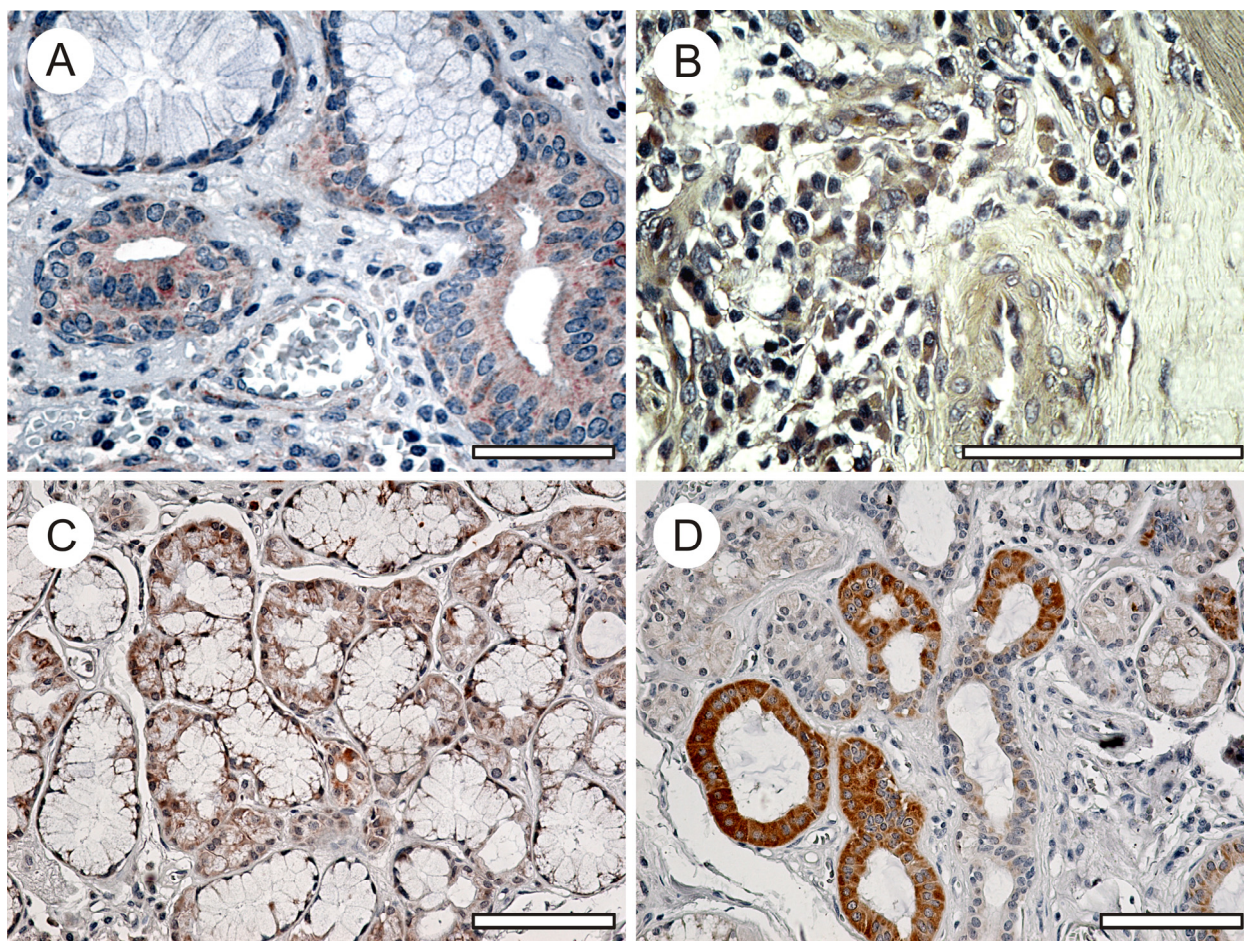

Figure 3 – Single immunostaining (VEGFR-1 – brown): (a) Cytoplasmatic expression in the luminal cells of an excretory duct of minor salivary glands; (b) Cytoplasmic expression in macrophages and stromal fibroblast from the lower lip mucosa; Single immunostaining (VEGFR-2 – brown) (c) Cytoplasmic expression in the acinus cells of oral floor minor salivary glands; (d) Cytoplasmic expression in the luminal cells of an excretory ducts of oral floor minor salivary glands. Bars: 50 μm.

⊡ Expression of VEGF and its receptors in oral cancer tissue

Regarding tumor tissue reactivity to VEGF in oral cancer, data from literature are also disputed. The percentage of VEGF-positive OSCC cases varies from 24% to 100% with a mean positivity of 77% [97, 98, 112–121]. In our experience, this percentage did not exceed 87% in our casuistry.

As we showed, data from literature revealed that the immunostaining pattern of VEGF in the tumoral tissue was heterogeneous, either from case to case but also within the same case but in different regions (Figure 4).

Many studies proved that the level of VEGF-expression in those cases of OSCC-positive to such growth factor increases with tumor invasion depth [115, 122]. Moreover, Shintani S *et al.* [119] proved that VEGF-C and -D expression were frequently up-regulated at the infiltrating tumor front.

Some studies revealed an increased VEGF-expression in tumor cells located near necrotic regions within the tumor [4, 123]. It was suggest that hypoxia was responsible for this result, by regulating both VEGF and HIF-1a expression.

The majority of studies on oral cancer, proved that there was no significant association between VEGF-

expression and the histological degree of tumor differentiation [107, 113, 117, 118, 120, 122]. In contrast with these, other studies, including our work, have found a significant correlation between VEGF-expression and tumor differentiation, the expression of VEGF appearing to be reduced in poorly differentiated OSCCs (Figure 5) [97, 98, 119].

In what it regards the expression of the other VEGF family members in OSCCs, Shintani S *et al.* [119] noticed more numerous positive cases for VEGF-B and VEGF-C than VEGF-A, while VEGF-D was up-regulated in a smaller number of cases.

Neuchrist C *et al.* [124] showed an up-regulation of the lymphangiogenic factor VEGF-C and its receptor VEGFR-3 in head and neck squamous cell carcinomas compared with normal tissue from the head and neck regions. VEGF-C was also detected immunohistochemically in some stromal, mainly perivascular situated, mononuclear cells. The authors suggest that these cells morphologically resemble to macrophages, angioblasts, or dendritic cells, all of which bear VEGF-receptors according to the literature [125–127].
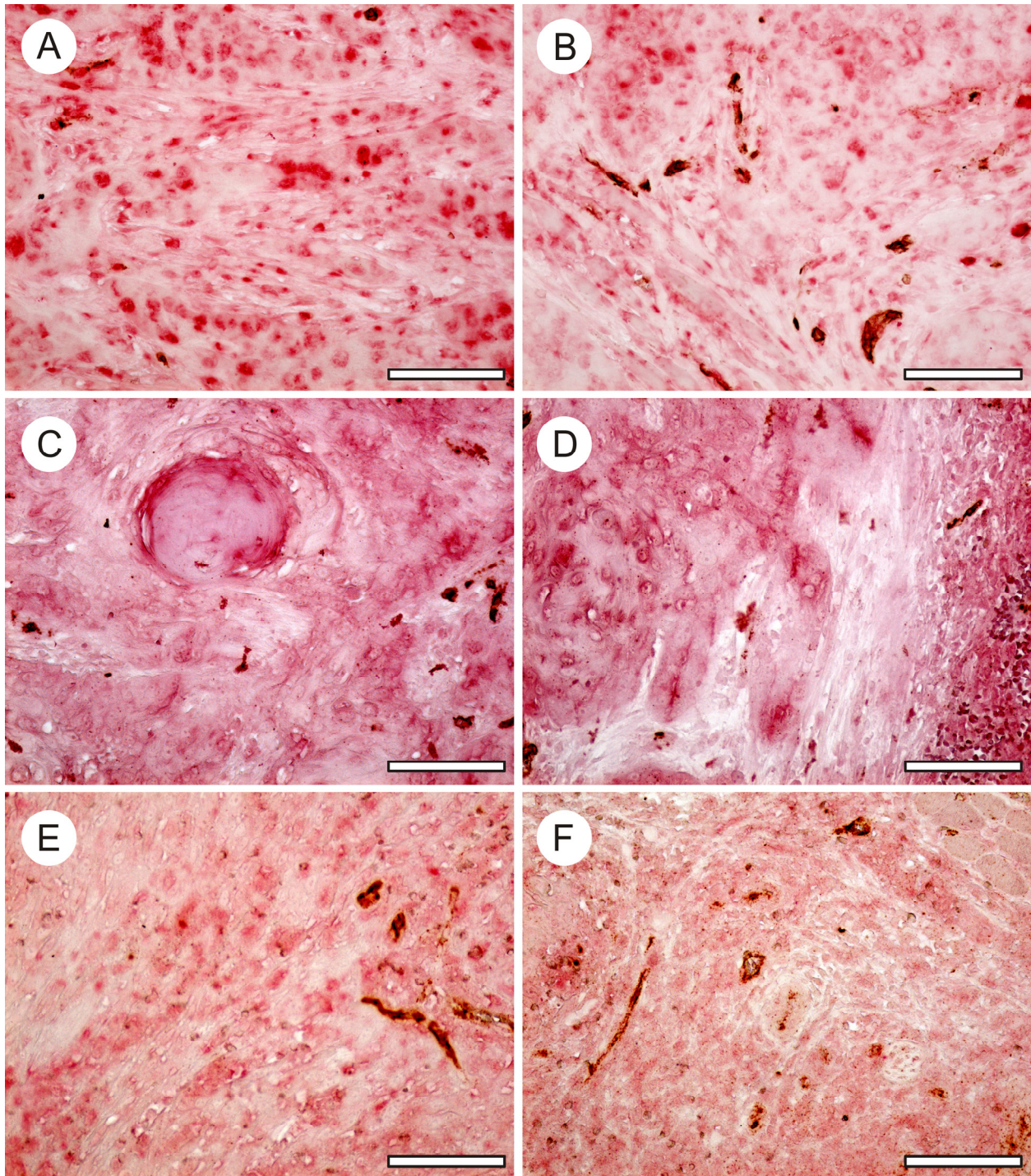

Figure 4 – *Double immunostaining (VEGF – red/CD105 – brown): (a) Inside region of a well differentiated OSCC – lip; (b) Edge region of a well differentiated OSCC – lip; (c) Inside region of a moderate differentiated OSCC – tongue; (d) Edge region of a moderate differentiated OSCC – tongue; (e) Inside region of a oral floor poor differentiated OSCC; (f) Edge region of a oral floor poor differentiated OSCC. Bars: 50 µm.*
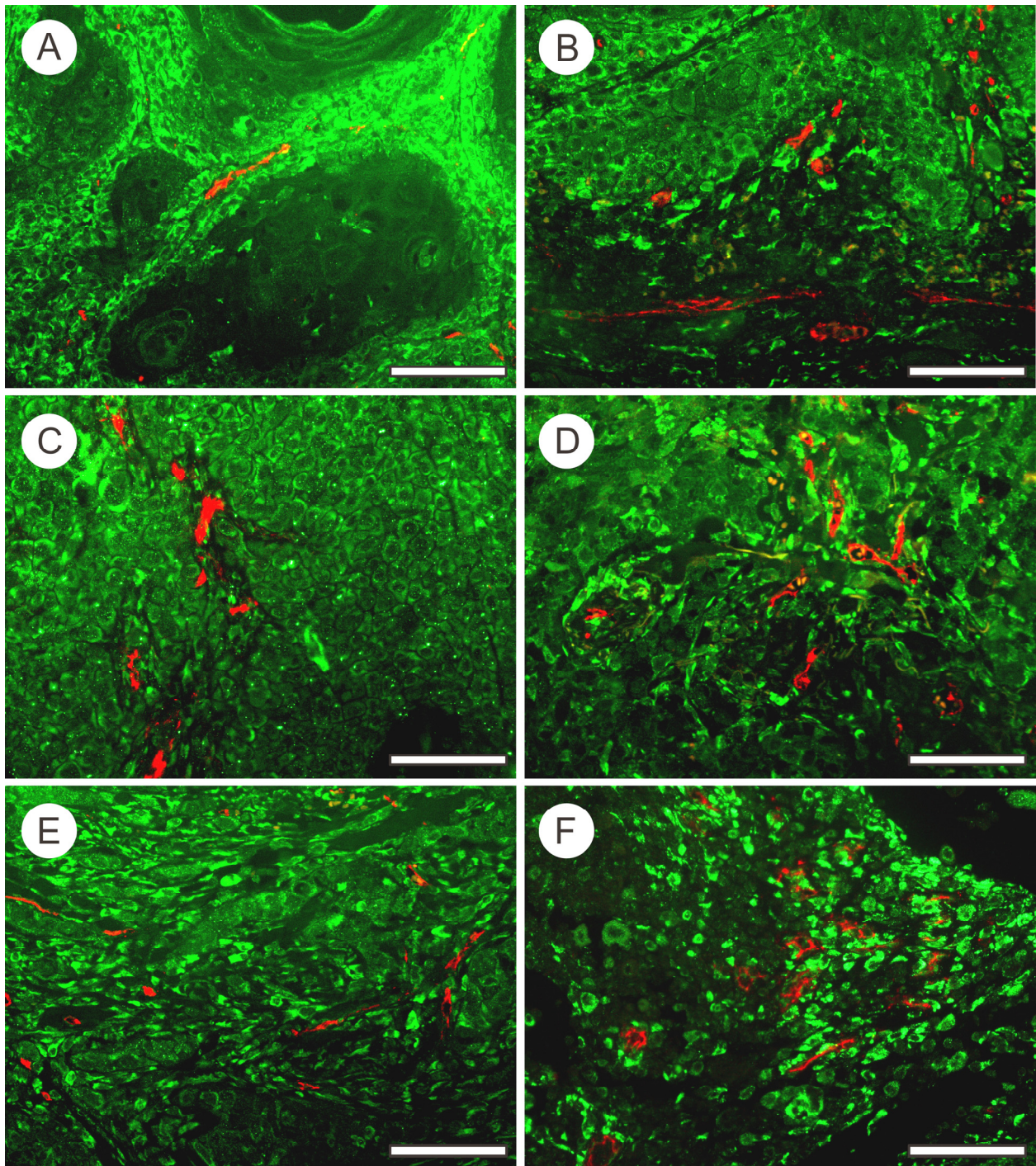
Figure 5 – *Double immunostaining (VEGF – red/CD105 – brown): (a) Inside region of a well differentiated OSCC – lip; (b) Edge region of a well differentiated OSCC – lip; (c) Inside region of a moderate differentiated OSCC – tongue; (d) Edge region of a moderate differentiated OSCC – tongue; (e) Inside region of a poor differentiated OSCC – oral floor; (f) Edge region of a poor differentiated OSCC – oral floor. Bars: 50 μm.*

These results in conjunction with the expression of VEGFR-3, even at a low level on tumor cells, pointed out to the existence of an autocrine growth loop VEGF-C/VEGFR-3 and a paracrine growth loop between tumor cells, macrophages, angioblasts, and stromal vessels.

Many other studies proved that tumor cells of OSCC, in the early stage of development, express VEGF-C [119, 128–138], which in turn may elevate the risk of precocious nodal metastasis. In addition, Ohno F *et al.* [133], observed a decreasing VEGF-C-expression in the tumor cells with the depth of tumor invasion from superficial to deep areas. On the contrary, Nakaya H *et al.* [131] proved that the expression of vascular endothelial growth factor-C increased as tumor invasion progresses.

Most recently, Matsuura M *et al.* [139], revealed that the VEGF-C/FLT-4 autocrine loop in tumor cells was a potential enhancer system to promote cancer progression in oral mucosa.

Studies concerning VEGF-receptors expression in OSCCs revealed that all three receptors (VEGFR-1, VEGFR-2, and VEGFR-3) were consistently expressed

on tumor cells and vascular ECs [140–143], along with a predominant overexpression of VEGFR-2. Regarding their level of expression, Lalla RV et al., [142] found that VEGFR-3 imunostaining was higher than VEGFR-1 and VEGFR-2, the last one having the lowest expression on tumor cells. These results suggest an autocrine regulatory function for VEGF in tumoral grow (Figure 6).

The study conducted by Kyzas PA et al. [141] demonstrated the colocalization of VEGF and VEGFR-2 in cancer cells, and occasionally the co-expression of VEGF/VEGFR-2/Ki-67 in the same cell. Regarding this results, the authors suggest the existence of a VEGF autocrine loop in OSCCs but with the specification that might not be a general property of head and neck squamous cell carcinomas. Thus, it was revealed that tumors located in the lower lip demonstrated VEGF/VEGFR-2 overexpression very rarely, and this might offer a logical explanation for their decreased aggressivity (Figure 7) [141].

On the other hand, the expression of either VEGF or VEGFR-2 in cancer cells located in the same tumor area, suggests the existence of a paracrine (endothelial-independent) loop between cancer cells (Figure 8) [141].

Regarding VEGFR-1 expression in neoplastic cells, Kyzas PA et al. [141] did not observe any correlation neither with VEGF, nor with Ki-67 expression. This fact suggests that autocrine VEGF-pathways have different

manifestation in different neoplasms. However, the authors pointed out that VEGF autocrine action is an enhancer of uncontrolled tumor proliferation and along with tumor angiogenesis regulates tumor metastatic potential, increasing tumor aggressivity (Figure 8).

On VEGFR-3 expression in OSCCs, Neuchrist C et al. (2003) found that this receptor was rather up-regulated on ECs than on tumor cells. However, the authors pointed out about the existence in OSCCs of a VEGF-C–driven autocrine growth loop similar to that of VEGF/VEGFR-2 (Figure 8). These results were confirmed by other studies [139, 144]. In addition Sugiura T et al. [144], observed an intense VEGF-C and VEGF-D staining on the membrane of SCC cells, especially at the invasive edge.

The study of Lalla RV et al., [142] proved the existence of a significant expression of all three VEGFRs on tumor associated macrophages (Figure 9, a, b, e, and f). It suggests that the presence of VEGFRs on macrophages supports the concept that VEGF produced by tumor cells may play a role in the attraction of macrophages into the head and neck squamous cell carcinomas environment. An increasing macrophage infiltration is associated with increased VEGF expression, increased angiogenesis, and probable with a worse prognosis in this cancer similar with breast cancer [145].
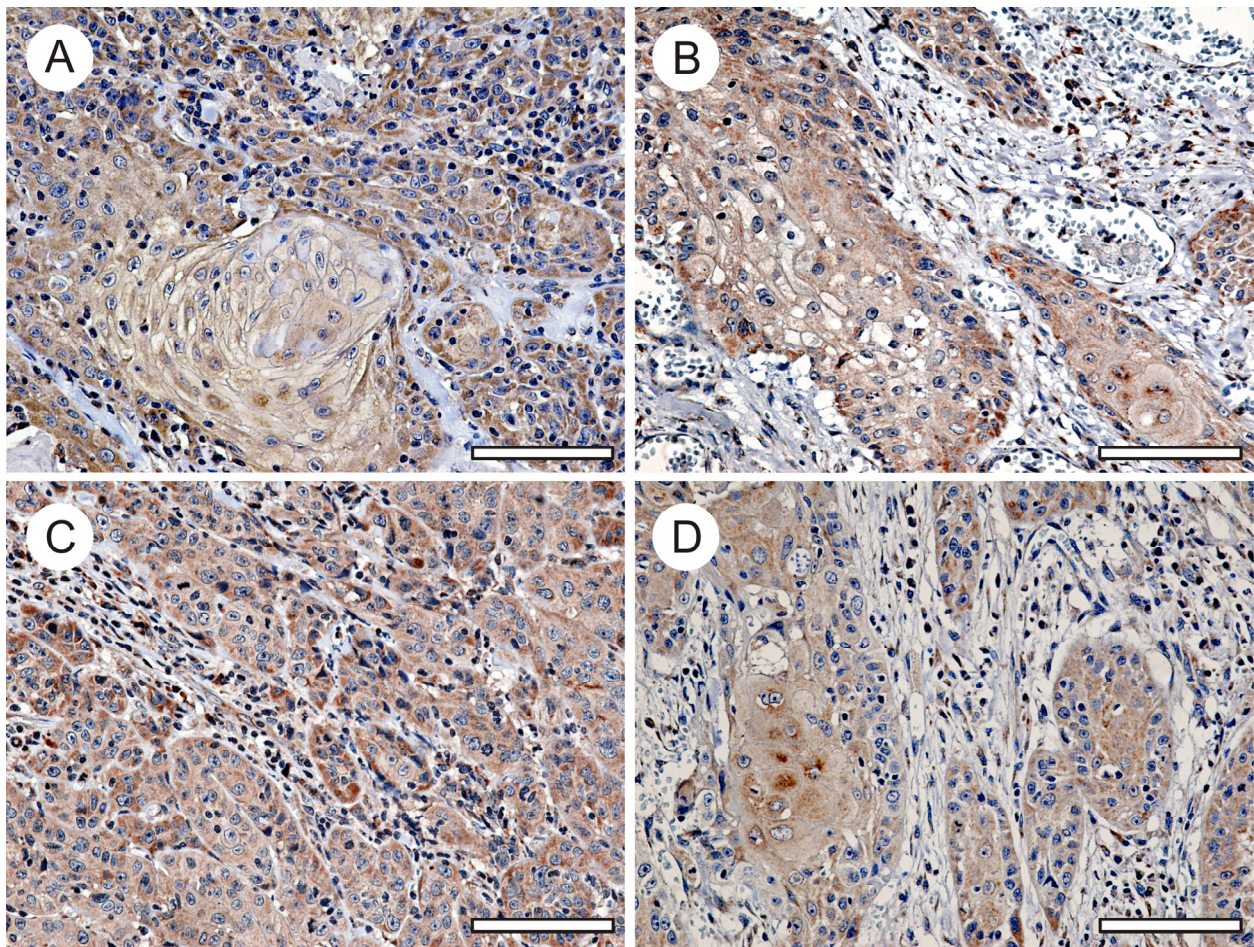

Figure 6 – Single immunostaining (VEGFR-1 – brown): (a) Tumor cytoplasmatic expression inside of a well differentiated OSCC – lip; (b) Tumor cytoplasmatic expression at the edge well differentiated OSCC – lip; (c) Tumor cytoplasmatic expression inside a moderate differentiated OSCC – tongue; (d) Tumor cytoplasmatic expression at the edge of a moderate differentiated OSCC – tongue. Bars: 50 µm.
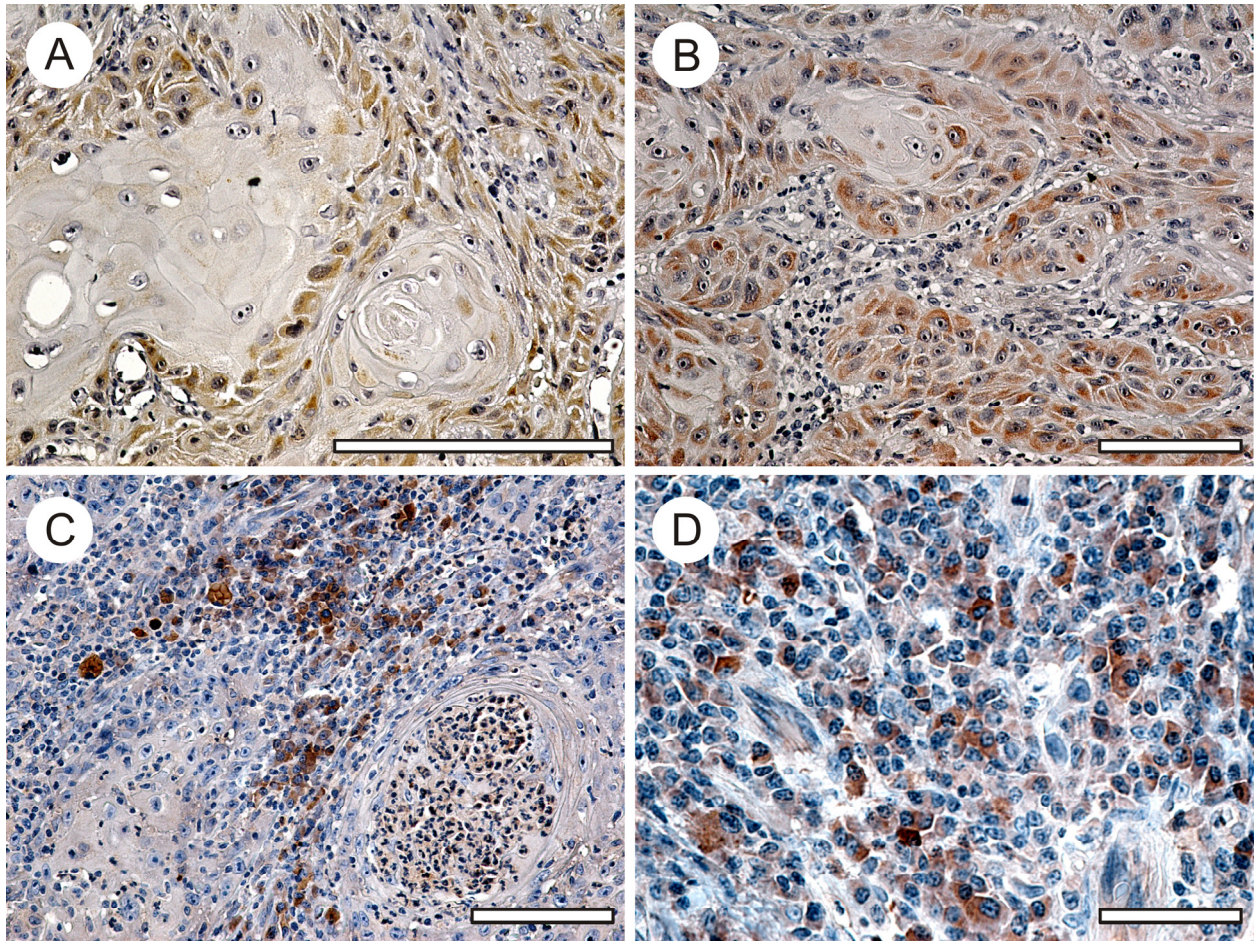
Figure 7 – *Single immunostaining (VEGFR-2 – brown): (a) Tumor cytoplasmatic expression inside of a well differentiated OSCC – lip; (b) Tumor cytoplasmatic expression inside of a moderate differentiated OSCC – lip; (c) Tumor cytoplasmatic expression at the edge of a moderate differentiated OSCC – tongue; (d) Tumor cytoplasmatic expression at the edge of a poor differentiated OSCC – oral floor. Bars: 50 µm.*
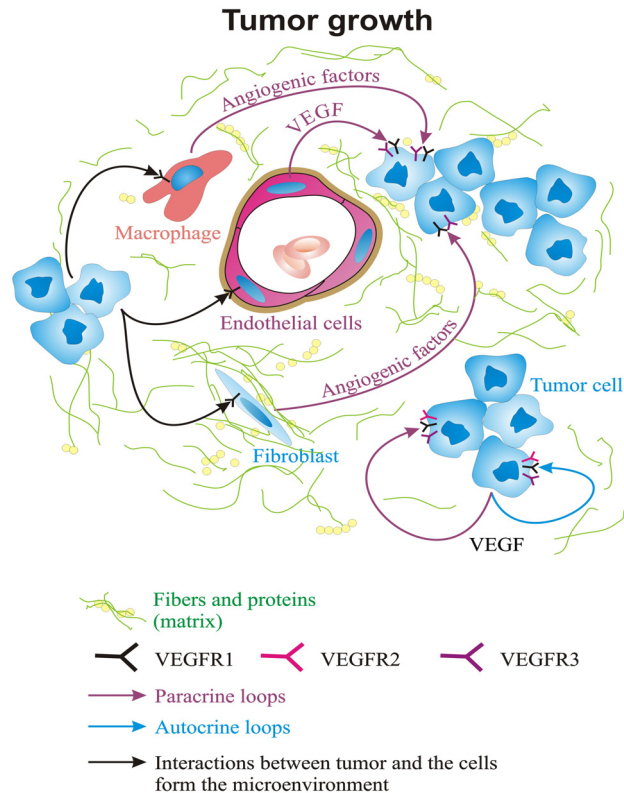

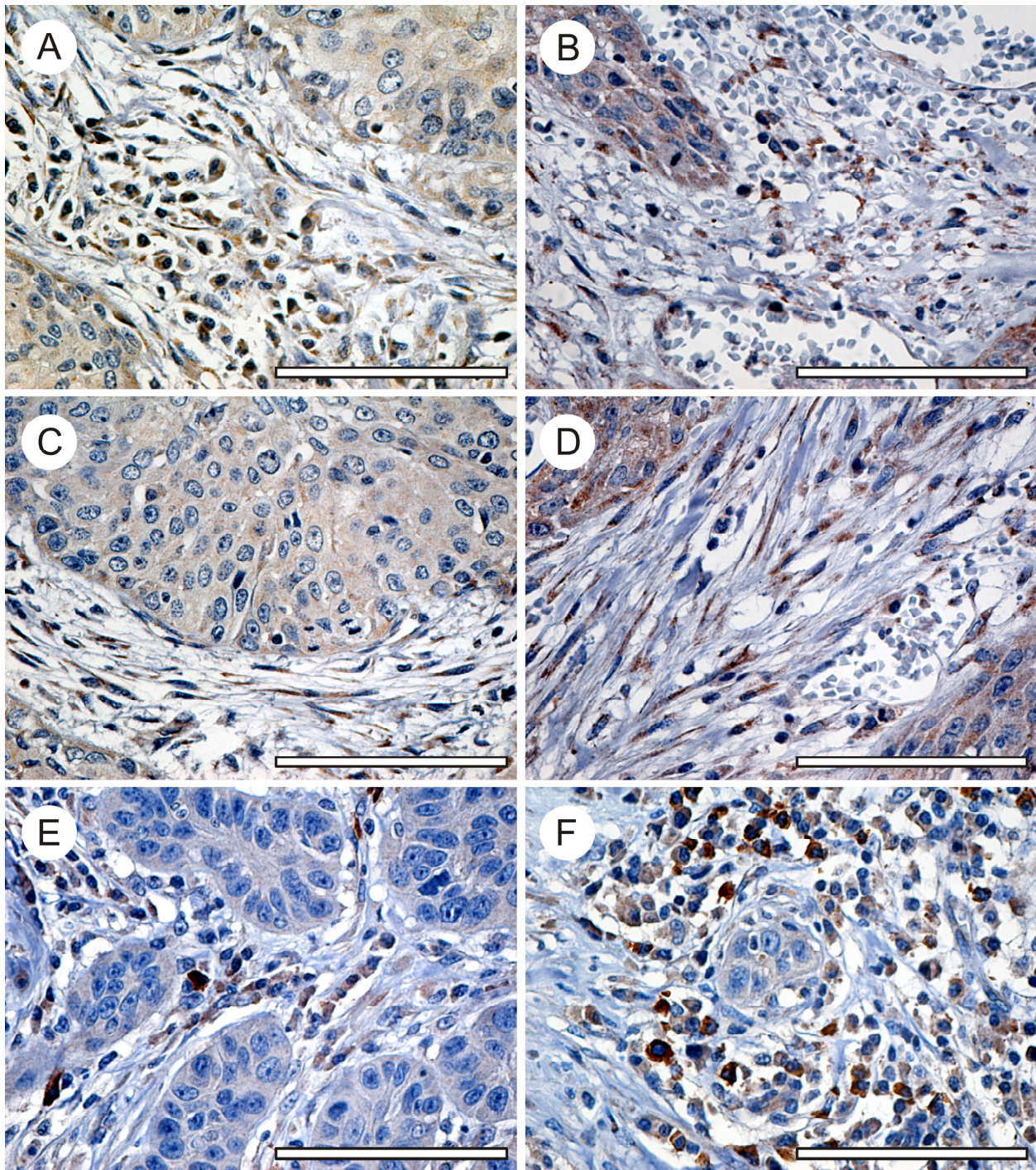**Figure 8** – *Autocrine and paracrine loops of OSCCs growth.*

Figure 9 – *Single immunostaining (VEGR-1 – brown): (a) Macrophage cytoplasmatic expression from inside a moderate differentiated OSCC – lip; (b) Macrophage and fibroblast cytoplasmatic expression from the edge of a moderate differentiated OSCC – lip; (c) Fibroblast cytoplasmatic expression from inside a moderate differentiated OSCC – lip; (d) Fibroblast cytoplasmatic expression from inside a moderate differentiated OSCC – tongue; single immunostaining (VEGR-2 – brown); (e) Macrophage cytoplasmatic expression from inside a moderate differentiated OSCC – lip; (f) Macrophage cytoplasmatic expression from the edge of a poor differentiated OSCC – tongue. Bars: 50 μm.*

Also, Lalla RV *et al.* [142], found that VEGFR-1 and VEGFR-3, but not VEGFR-2, were expressed on stromal fibroblasts in head and neck squamous cell carcinomas (Figure 9, c and d). Therefore, it was suggested that the VEGF family also affects fibroblast function in the tumor environment of these cancers. The authors concluded that recruitment and activation of macrophages and stromal fibroblasts play a role in the

complex regulation of tumor growth and metastasis via the VEGF/VEGFR system [142].

⊟ **VEGF and OSCC angiogenesis**

Traditionally, oral tumor angiogenesis was measured by assessing the highest microvessel density (MVD) within a tissue sample [101, 107, 146] or by estimating the microvascular volume [101].

Our studies revealed a significant difference between angiogenesis in the invasive front when compared to the centre of the tumor [102] and also we found that the co-localization of VEGF and CD105 signals on the tumoral blood microvessels was higher at the invasion front of the OSCC [*in press*] (Figure 10). These results prove that angiogenesis in OSCCs is more active in the invasion front. Investigating the relationship between VEGF-expression and angiogenesis (as relative blood vessel areas) in OSCCs, we established an inversed correlation inside the tumor between these two parameters [*in press*].

Literature data regarding the correlation between VEGF-expression and angiogenesis in oral cancer are also disputed. Some studies have found no correlation between VEGF-expression and angiogenesis in oral dysplasia or carcinoma [99, 107, 143, 147, 148]. Others have showed a trend towards a relation of high VEGF-expression with high MVD in OSCCs, but the observed correlation was not statistically significant [141, 149]. However, several other studies have found that an increased microvascular density is correlated with an increase in VEGF-expression in oral squamous cell carcinomas [97, 104, 112, 114, 119, 150, 151]. Moreover, in the study conducted by Chien CY *et al.* [150], the cumulative five-year disease-free survival rates of patients with low expression of CD105 and VEGF were significantly higher than those patients with high expression of CD105 and VEGF. Regarding VEGF family members, Shintani S *et al.* [119], have noticed a strong correlation between VEGF-A and -B expression and MVD, but no relationship between VEGF-C, -D expressions and MVD.
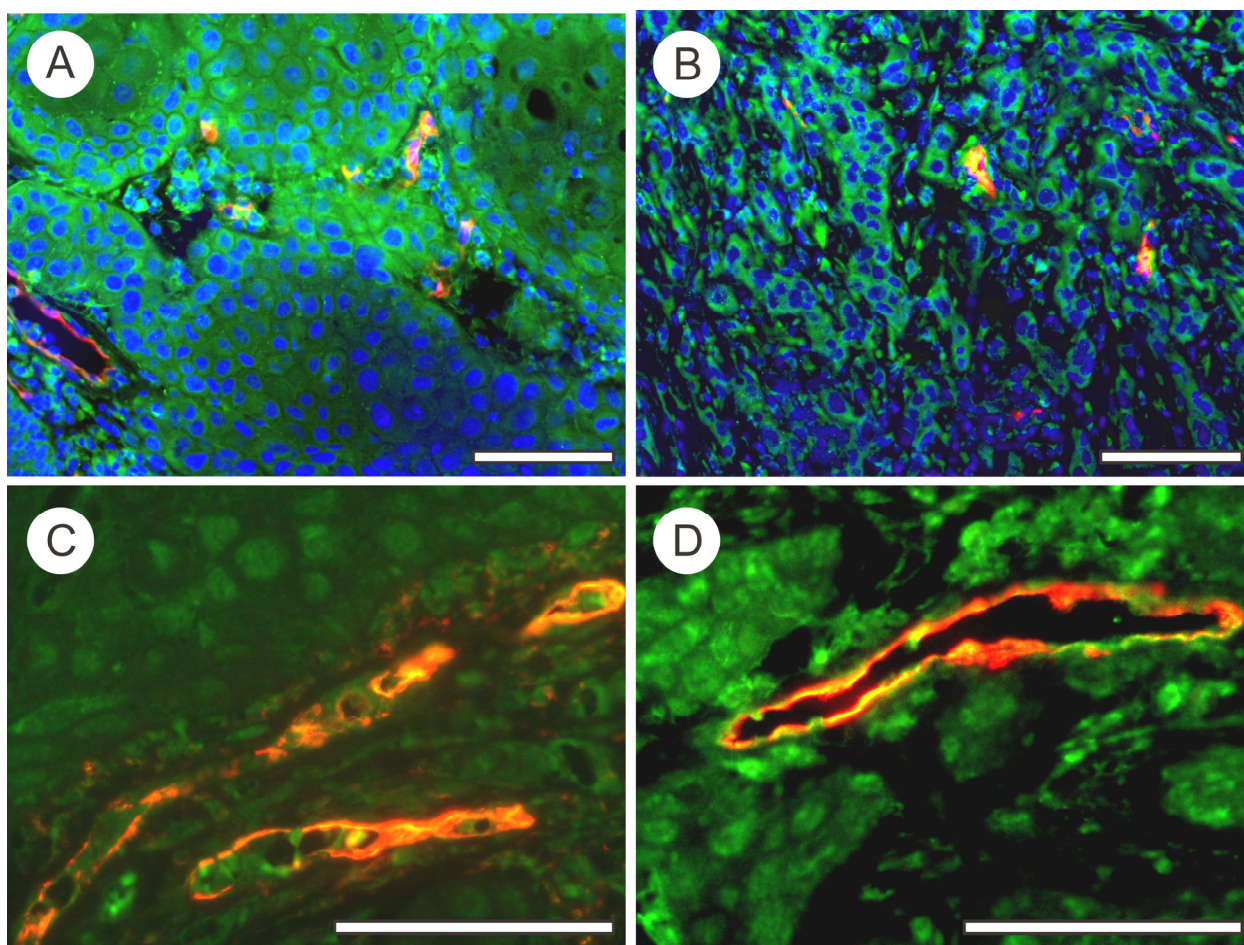

Figure 10 – *Triple fluorescent labeling (VEGF – red, CD105 – brown, DAPI – blue and colocalization of the VEGF and CD105 – yellow): (a) Tumor vessels from inside of a well differentiated OSCC – lip; (b) Tumor vessels from inside of a poor differentiated OSCC – oral floor; double immunostaining (VEGF – red, CD105 – brown, colocalization of the two signal – yellow); (c) Tumor vessels from the edge of a well differentiated OSCC – lip; (d) Tumor vessels from the edge of a moderate differentiated OSCC – tongue. Bars: 50 μm.*

Such differences were owed to potential biases in the qualitative assessment of intensity of VEGF-staining and in the estimation of microvascular density. Also, the majority of the studies highlight the tumor microvessels with pan endothelial markers (CD31, CD34, and vWF), which are far from accurate, as they are neither sensitive nor specific enough to distinguish normal vessels from neoplastic ones. Only Chien CY *et al.* [150] used CD105 for investigating the ongoing angiogenesis, this tumoral endothelial marker being proved to be more precise in reflecting the dynamic status of the angiogenic activity of the tumor [152–154].

One of the explanations for lacking of a significant association between VEGF and MVD is that VEGF acts not only as a vessel-sprouting factor but also as a tumoral growth factor in an autocrine manner. This hypothesis is

sustained by the vascular ECs themselves producing VEGF [155] (Figure 11). Moreover, in tumor angiogenesis are implicated many other angiogenic factors which may act with VEGF in a synergic or an antagonistic manner [156].
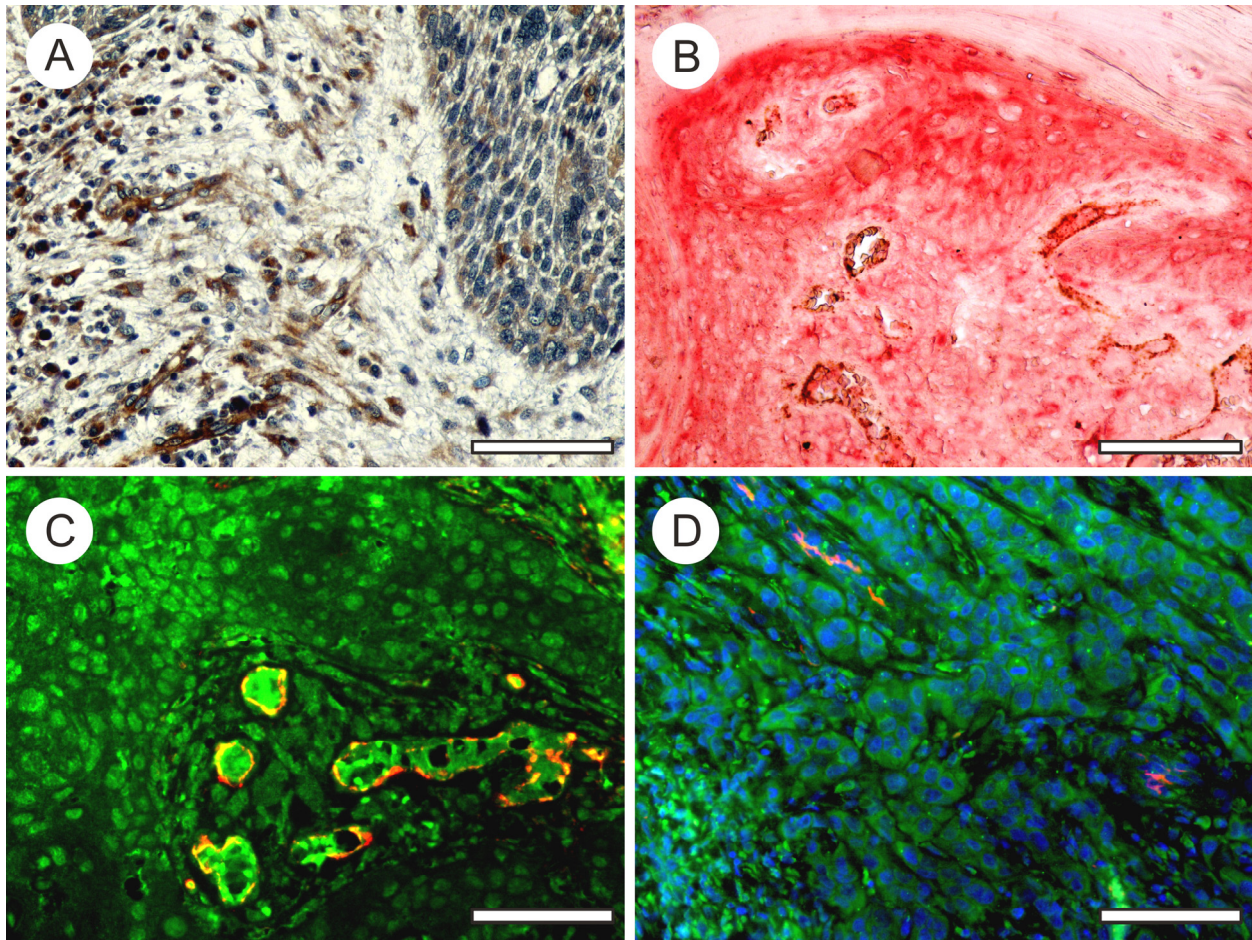

Figure 11 – *Single immunostaining (VEGF – brown): (a) Tumor vessel from the edge of a lip well differentiated OSCC; Double immunostaining (VEGF – red/CD105 – brown); (b) Tumor vessel from inside of a moderate differentiated OSCC – tongue; double immunostaining (VEGF – red, CD105 – brown, colocalization of the two signal – yellow); (c) Tumor vessels from inside of a well differentiated OSCC – tongue; triple fluorescent labeling (VEGF – red, CD105 – brown, DAPI – blue and colocalization of the VEGF and CD105 – yellow); (d) Tumor vessels from the edge of a poor differentiated OSCC – tongue. Bars: 50 μm.*

Sugiura T *et al.* [144] noticed an occasional, VEGF-D-expression in vascular ECs near the carcinoma cell nests. The same authors found a strong correlation between lymphangiogenesis (measured as lymphatic vessel density) and both VEGF-C and VEGF-D in oral SCC patients; the co-expression of both factors being significantly related to lymphangiogenesis.

The same correlation between VEGF-C and lymphatic vessel density in human OSCC was noticed by other authors [118, 124, 133–135, 157, 158]. However, Nakaya H *et al.* [131] could not find any statistically significant correlation between the expression of VEGFC and lymph vessel density.

Moriyama M *et al.* [143], could not find any significant correlation with vessel density, neither for VEGFR-2 nor VEGFR-1. However, the mean vessel density in VEGFR-3-positive patients was significantly higher than that in VEGFR-3-negative patients.

About VEGF-receptors expression on blood ECs, numerous studies indicated the expression of all three VEGFRs at this level (Figure 12) [140, 142, 143, 149, 159].

Lalla RV *et al.*, [142] proved that vascular ECs presented a less consistent and less intense staining for VEGFR-1 and VEGFR-2, while there were no significant differences for VEGFR-3 between ECs and tumor cells, macrophages, or fibroblasts. This pattern of VEGF-receptors expression on blood ECs together with the VEGF production on these cells justifies the existence of an autocrine mechanism for VEGF-induced angiogenesis (Figure 13). The strong expression of VEGFR-3 on blood ECs was clearly proved to be implicated in angiogenesis in head and neck squamous cell carcinomas [142].

Neuchrist C *et al.* [140] proved the existence in the stroma of head and neck squamous cell carcinomas of hemangioblasts, responsible for tumor neoangiogenesis (Figure 13). The authors observed a strong VEGFR-2 positive reaction, but negative to CD34, in many large rounded cells located right onto the endothelium or in the stroma immediately next to inter-cellular gaps, occasionally exhibiting rims of immunoreactivity incompletely enclosing luminae.

Figure 12 – *Single immunostaining (VEGFR-1 – brown): (a) Positive vessels from inside of a moderate differentiated OSCC – lip; (b) Positi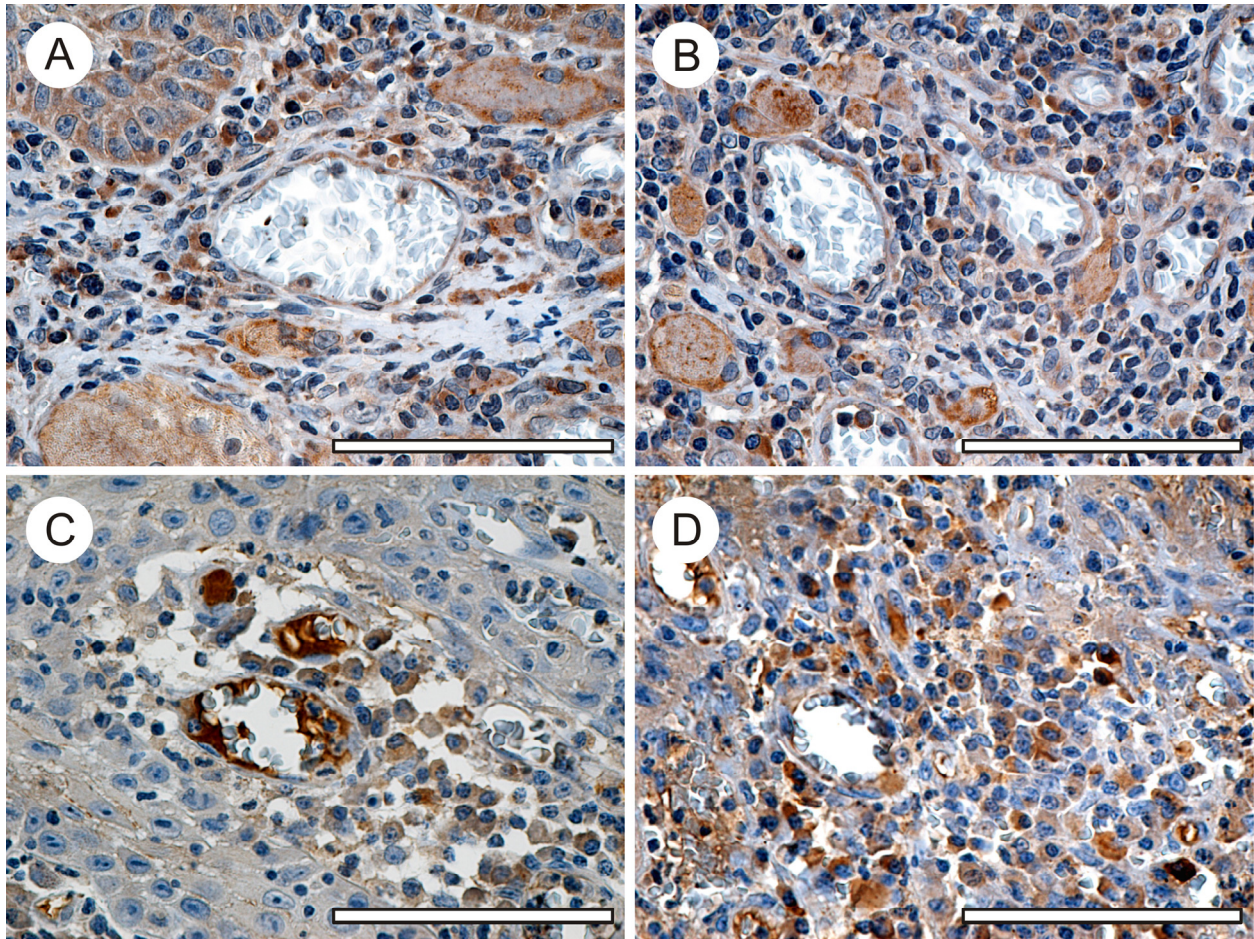ve vessels from the edge of a poor differentiated OSCC – oral floor; single immunostaining (VEGFR-2 – brown); (c) Positive vessels from inside of a moderate differentiated OSCC – tongue; (d) Positive vessels from inside of a poor differentiated OSCC – tongue. Bars: 50 μm.*
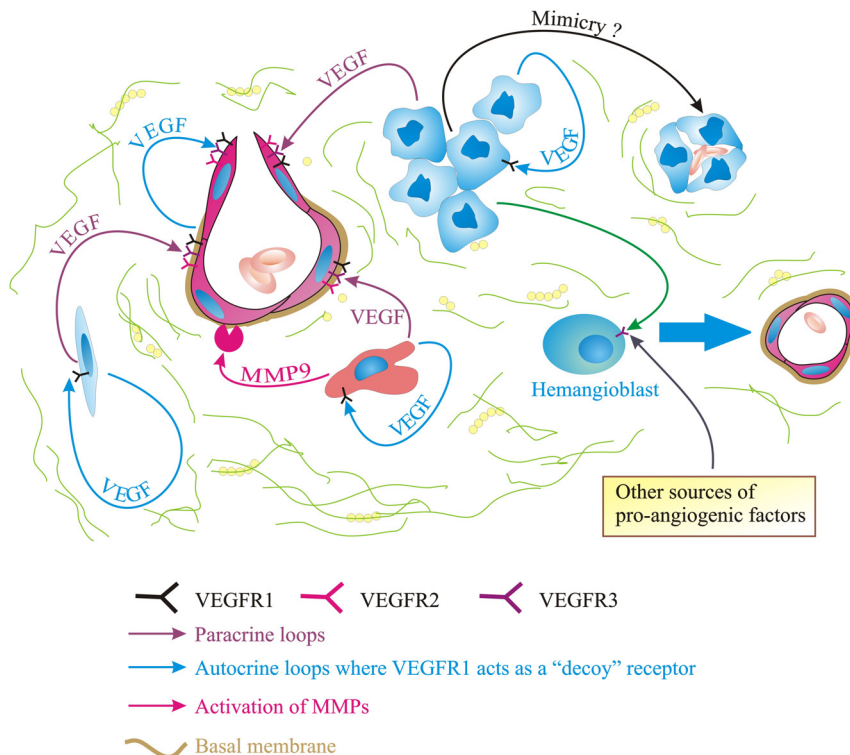

Figure 13 – *Angiogenesis mechanisms in OSCC.*

It was hypothesized that these cells under VEGF-activation might stick to the endothelium, transmigrate, and bud new micro-vessels. However, we must keep in mind that various cell types are able to express VEGFR-2 and further promote the formation of vessel-like structures [126]. Moreover, even tumor cells themselves have been shown to form vessel-like structures with immunoreactivity for VEGFR-2, a process that has been called vascular mimicry (Figure 13) [160].

Some authors revealed that expression of VEGFR-3 in lymphatic vessels of tumor tissue in OSCCs was constantly elevated in contrast to varying degrees of VEGFR-3 immunoreactivity on ECs of tumor microvessels [124, 139, 161].

Neuchrist C *et al.* [124], established that VEGFR-3 was involved as well in tumor lymph angiogenesis as in blood vessel neoangiogenesis (Figure 13). Thus, the authors found typical VEGFR-3-positive lymph vessels that still co-expressed very weakly CD34 and vice versa, CD34 blood vessels that kept a baseline expression of VEGFR-3. Therefore, it was suggested that these are undetermined precursor of the lymphatic and blood vessels which after initial upregulation of VEGFR-3 during the angiogenic process, will be guided toward final lymphatic or vascular specialization.

Matsuura M *et al.* [139], pointed out that VEGF-C/VEGFR-3 autocrine loop of tumor cells is a novel mechanism not only for promoting tumor growth but also for tumor-associated neovascularization, especially lymphangiogenesis, and is likely to contribute to advanced tumor progression in oral squamous cell carcinoma; thus, this pathway may become an effective target in therapy for malignant neoplasms (Figure 14).
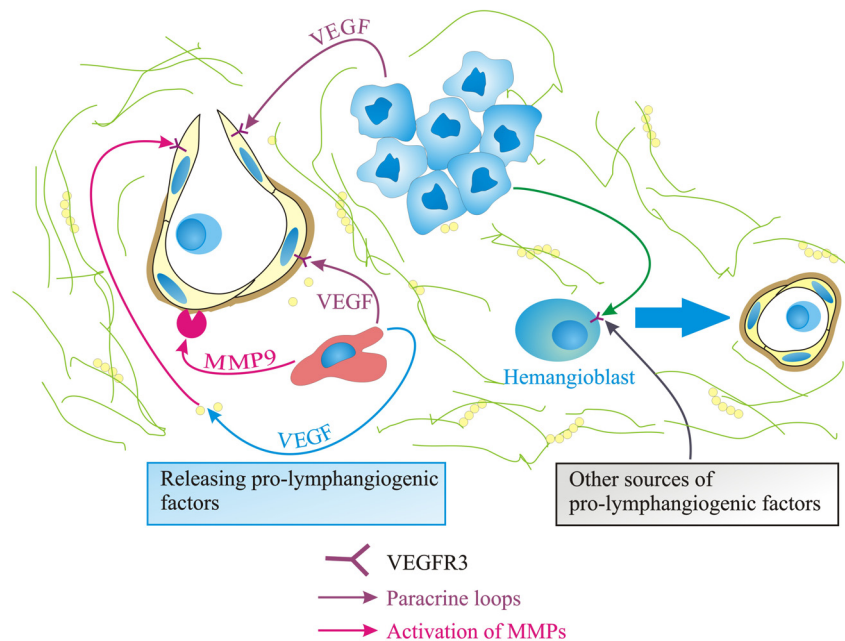
Lymphangiogenesis

Figure 14 – *Lymphangiogenesis mechanisms in OSCC.*

�containing VEGF and OSCC prognosis

In the majority of OSCC studies, authors have trend to establish VEGF correlations with clinico-pathological predictors as: tumor-node-metastasis stage, overall stage, tumor grade, tumor site, pattern of invasion, margin status, tumor necrosis, tumor thick-ness, perineural invasion, vascular invasion, smoking status, sex, race, and age. In this context, we found a significant correlation with tumor size and the presence of lymph node metastasis, but no association with patients' gender, age and tumor topography at oral level [*in press*].

The data regarding VEGF correlation with clinical stages in OSCC are contradictory. Thus, while some authors sustained the existence of a significant correlation with the clinics [121, 149, 151] other groups found no significant correlation between these two parameters [113, 117, 119]. In addition, Zhe S *et al.* [151] proved that VEGF-expression significantly corre-lates with lymph invasion, recurrence, and distant metastasis but does not correlate with patients' gender and age. Kyzas PA *et al.* [149] obtained the same results, but also they established that there were no correlation between VEGF-expression and histological grade, lymph-node status, and smoking habits.

The majority of the studies revealed that there was no correlation between VEGF-C and gender, ethnic group, age, tobacco, alcohol, tumor site, histological grade, clinical stage, local recurrence, and regional recurrence [119, 138, 144, 158]. In addition, Shintani S *et al.* [119] proved that beside VEGF-C also VEGF-A, VEGF-B and VEGF-D had no significant correlation with T-stages. On contrary, Sedivy R *et al.* [134] proved the existence of a correlation between VEGF-C-expression and tumor grade, later occurring regional and distant metastases, while Kyzas PA *et al.* [141] noticed that positive VEGF-C-expression was also strongly correlated with advanced clinical stage. Neuchrist C

et al. [124] found that increased VEGF-C-expression on tumor cells correlated with an increased incidence of recurrences.

Kyzas PA *et al.* [141] proved that both VEGFR-1 and VEGFR-2 did not correlate with age, gender and histological grade in patients with head and neck tumors. In addition, the authors proved the existence of a strong correlation between the two receptors for tumors located in the oral cavity and larynx. Regarding the clinical stage only, VEGFR-2 presented a strong correlation with this parameter.

Neuchrist C *et al.* [124] found no statistically significant correlations between VEGFR-3-expression and the major clinical parameters in head and neck squamous cell carcinomas. They justified these results by the fact that they were counting the entire VEGFR-3-positive vascular network, both mature lymph vessels and not only sprouting microvessels. In addition, we must keep in mind that VEGFR-3 presents two different isoforms which encode a short and a long form of the receptor, from which only the long form of VEGFR-3 was proved to induce cell growth [162]. Therefore, this fact could be another explanation for the lack of correlation between VEGFR-3-expression and the clinical data.

Although many studies have examined the correlation between VEGF-expression and lymph-node metastasis in OSCC, different conclusions have been formulated. Thus, some studies [101, 107, 114, 115, 118, 150, 163, 164]; found a statistically significant association between VEGF-overexpression and the presence of lymph-node metastasis, whereas others [97, 117, 121, 149, 165–168], did not notice any significant association. To support this correlation, Shang ZJ *et al.* [169] revealed a significant association between elevated circulating levels of VEGF and lymph-node status and clinical stages of OSCC. Interestingly, Boonkitticharoen V *et al.* [170] proved that oral and pharyngeal SCC were more aggressive than laryngeal SCC, this aggressivity being owed to the ability of tumors within location to exploit VEGF-A in addition to proliferation in lymphatic progression. The authors concluded that combined expression of Ki-67 and VEGF-A may help in identification of patients at risk for occult metastases in oral and pharyngeal SCC.

Moreover, Shintani S *et al.* [119] showed that VEGF-C and -D might promote development of lymphatic vessels in OSCCs because the tumor invasion and lymph nodes metastatic involvement was closely related to the expression of these VEGF isoforms.

Data from the literature indicates that VEGF-C expression by tumor cells correlates significantly with lymph-node metastasis in different clinical stages of OSCCs [118, 119, 128, 129, 134–136, 144]. In addition, Sugiura T *et al.* [144] proved that both VEGF-C and VEGF-D correlated with lymph-node metastasis. Relative few studies proved that there was no correlation between VEGF-C-expression and lymph node status [138, 149, 158]. Faustino SE *et al.* [138] justified their results by the fact that the majority of the studies did not investigate the occult lymph-node metastasis from OSCC, and most of them analyzed tumors at both early and advanced clinical stages (from I to IV).

Regarding VEGFRs, Moriyama M *et al.* [143] and Kyzas PA *et al.* [141] established that both VEGFR-1 and VEGFR-2 did not correlate with lymph-node status, while VEGFR-3 correlated significantly with lymph-node metastasis [143].

The majority of studies regarding the relationship between VEGF and survival in OSCCs have showed that VEGF correlates with the overall and disease-free survival, indicating that high VEGF-expression correlates with worse survival periods [113–115, 117, 149–151, 164, 168, 171]. Only two studies did not show any correlation between VEGF-expression and survival in OSCC [97, 106]. In addition, VEGF-positive tumors have shown correlations with tumor recurrence [116, 122], although other authors have found only a border-line significance [171].

Kyzas PA *et al.* [149], Miyahara M *et al.* [158] and Sugiura T *et al.* [144] showed that VEGF-C-expression correlated with the overall and disease-free survival in OSCCs, the mean survival times being significantly reduced in patients with up-regulated VEGF-C-expression. On the contrary, Sedivy R *et al.* [134] and Faustino SE *et al.* [138] noticed that VEGF-C-expression by malignant cells was not a significant prognostic factor for patients with OSCC.

Kyzas PA *et al.* [116] noticed that VEGF-expression, VEGFR-2-overexpression and VEGF/VEGFR-2 co-overexpression were correlated with worse survival times in the group of patients with oral and larynx squamous cell carcinomas, but no association was observed between VEGFR-1-expression and the overall survival periods. Moreover, the authors could not establish any correlation between VEGFR-2 and VEGFR-1-expression and recurrence in head and neck tumors. Tanigaki Y *et al.* [136] reported that in OSCCs there was no significant correlation between the survival periods and the expression of VEGF-A, VEGFR-1, or VEGFR-3.

⊟ Therapeutic implication of VEGF and its receptors in OSCCs

The major therapeutical strategies in head and neck squamous cell carcinomas revolve around the radical surgery, despite its economic, physiological, and psychosocial impacts [172, 173]. These difficulties together with high tumor recurrences have guided the research treatment strategies towards the discovery of adjuvant therapies to suppress progression of pre-malignant disease and/or to inhibit head and neck squamous cell carcinoma recurrence or development of second primary tumors [174].

One of the leading targets for such tumor-directed therapies is VEGF and its receptors. Therefore, there were developed monoclonal antibodies that bind VEGF or the VEGFRs and small-molecule inhibitors of VEGR. Bevacizumab was one of the first recombinant humanized monoclonal antibody against VEGF that has been studied extensively in many human cancers, including in head and neck squamous cell carcinomas [175–178].

The literature data showed that combined targeting of the VEGF and EGFR pathways is more effective than targeting individually either pathway alone. EGFR induces angiogenesis *via* VEGF-expression, and EGFR-inhibition can lead to decreased VEGF-secretion and tumor microvessel production *in vivo* [179]. There are clinical trials in different phases which resulted in encouraging responses for this combined therapy [180–184].

A fundamental approach to inhibit angiogenesis during tumorigenesis is the disruption of VEGF/VEGFR-2 pathways [185]. This could suppress tumors' growth by limiting their blood supply; by changing their morphology, the vascular wall structure, and rendering VEGF and VEGFR-2-expression in tumor vasculature to a more normal pattern [186], and thus improving drug penetration in tumors, and also by blocking VEGF autocrine pathway and thus reducing uncontrolled neoplastic cell proliferation.

Currently, many VEGFR-2 inhibitors are undergoing preclinical and clinical evaluation. Yazici YD *et al.* [187] showed for the first time that PTK787 (which inhibits the VEGF-R tyrosine kinases and specifically targets the VEGFR-2-receptor) decreased tumoral growth and tumor vascularization of squamous cell carcinoma of the oral tongue tumors growing orthotopically in nude mice. The most studied agent is SU5416 (semaxanib), which also presents inhibitory activity towards VEGFR-1 [188]. Other tested with successful results were ZD6474 that is a potent VEGFR-2 tyrosine kinase inhibitor, and which also has activity on EGFR tyrosine kinase [189]. Cediranib (Recentin, AstraZeneca), an oral, highly potent, and selective VEGF-signaling inhibitor of all three VEGFRs, has been tested in recurrent and metastatic squamous cell carcinomas of the head and neck setting as a monotherapy in a single-arm phase 2 trial (NCT00458978), with the overall response rate as the primary endpoint [176].

The lymphatic system is more important than the vascular system in metastasis of oral squamous cell carcinoma [190–192]. As we previously discussed, the VEGF-C/VEGFR-3-signaling system is a key regulator for tumor lymphangiogenesis. Therefore, preclinical studies are underway to determine whether inhibition of VEGFR-3-activation might be an effective therapeutic strategy for the suppression of tumor growth and regional metastasis, and several methods of VEGF-C pathway inhibition are currently being evaluated. Fukumoto S *et al.* [193] proved that endostatin inhibited tumor growth by an anti-angiogenic action, and also inhibited lymph node metastasis by down-regulating of VEGF-C-expression in tumor cells of OSCCs. Elser C *et al.* [194] and Choong NW *et al.* [195] tested successfully tyrosine kinase inhibitors such as sorafenib and respective sunitinib on phase II trials in patients with recurrent or metastatic squamous cell carcinoma of the head and neck; they act by blocking the enzymatic activity of both VEGFR-2 and VEGFR-3, and inhibiting both angiogenesis and lymph-angiogenesis in these tumors.

☐ Conclusions

VEGF status has the potential to emerge as an important factor in the diagnosis, prognosis and treatment of OSCCs.

Recent studies ascertained the participation in tumorigenesis, angiogenesis and metastasis in OSCC of VEGF/VEGFR-2 and VEGF-C/VEGFR-3 autocrine loops. In addition, it has been proved that both processes could be influenced by a paracrine growth system in which VEGF is produced not only by tumor cells but also by stromal cells like fibroblasts, macrophages, and even ECs. Therefore, the interactions of the various VEGF/VEGFR systems in the tumor microenvironment are clearly complex, and further investigations are still needed for a complete understanding of their functions.

This will be a most necessary step, as the newly developed anti-angiogenic and anti-tumoral treatments are currently targeting the inhibition of both VEGF and VEGFRs.

References

[1] PARKIN D. M., BRAY F., FERLAY J., PISANI P., *Estimating the world cancer burden: Globocan 2000*, Int J Cancer, 2001, 94(2):153–156.

[2] COOPER J. S., PAJAK T. F., FORASTIERE A. A., JACOBS J., CAMPBELL B. H., SAXMAN S. B., KISH J. A., KIM H. E., CMELAK A. J., ROTMAN M., MACHTAY M., ENSLEY J. F., CHAO K. S., SCHULTZ C. J., LEE N., FU K. K.; RADIAT THER ONCOL GROUP, *Post-operative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck*, N Engl J Med, 2004, 350(19):1937–1944.

[3] FOLKMAN J., *Tumor angiogenesis: therapeutic implications*, N Engl J Med, 1971, 285(21):1182–1186.

[4] SHWEIKI D., ITIN A., SOFFER D., KESHET E., *Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis*, Nature, 1992, 359(6398):843–845.

[5] SIEMEISTER G., MARTINY-BARON G., MARMÉ D., *The pivotal role of VEGF in tumor angiogenesis: molecular facts and therapeutic opportunities*, Cancer Metastasis Rev, 1998, 17(2):241–248.

[6] LEUNG D. W., CACHIANES G., KUANG W. J., GOEDDEL D. V., FERRARA N., *Vascular endothelial growth factor is a secreted angiogenic mitogen*, Science, 1989, 246(4935):1306–1309.

[7] FERRARA N., *Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications*, Semin Oncol, 2002, 29(6 Suppl 16):10–14.

[8] RELF M., LEJEUNE S., SCOTT P. A., FOX S., SMITH K., LEEK R., MOGHADDAM A., WHITEHOUSE R., BICKNELL R., HARRIS A. L., *Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis*, Cancer Res, 1997, 57(5):963–969.

[9] TOI M., HOSHINA S., TAKAYANAGI T., TOMINAGA T., *Association of vascular endothelial growth factor expression with tumor angiogenesis and with early relapse in primary breast cancer*, Jpn J Cancer Res, 1994, 85(10):1045–1049.

[10] TAKAHASHI Y., KITADAI Y., BUCANA C. D., CLEARY K. R., ELLIS L. M., *Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer*, Cancer Res, 1995, 55(18):3964–3968.

[11] UCHIDA S., SHIMADA Y, WATANABE G, TANAKA H, SHIBAGAKI I, MIYAHARA T., ISHIGAMI S., IMAMURA M., *In oesophageal squamous cell carcinoma vascular endothelial growth factor is associated with p53 mutation, advanced stage and poor prognosis*, Br J Cancer, 1998, 77(10):1704–1709.

[12] TISCHER E., GOSPODAROWICZ D., MITCHELL R., SILVA M., SCHILLING J., LAU K., CRISP T., FIDDES J. C., ABRAHAM J. A., *Vascular endothelial growth factor: a new member of the platelet-derived growth factor gene family*, Biochem Biophys Res Commun, 1989, 165(3):1198–1206.

[13] ACHEN M. G., JELTSCH M., KUKK E., MAKINEN T., VITALI A., WILKS A. F., ALITALO K., STACKER S. A., *Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4)*, Proc Natl Acad Sci U S A, 1998, 95(2):548–553.

[14] JOUKOV V., PAJUSOLA K., KAIPAINEN A., CHILOV D., LAHTINEN I., KUKK E., SAKSELA O., KALKKINEN N., ALITALO K., *A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases*, EMBO J, 1996, 15(7):1751.

[15] LEE J., GRAY A., YUAN J., LUOH S. M., AVRAHAM H., WOOD W. I., *Vascular endothelial growth factor-related protein: a ligand and specific activator of the tyrosine kinase receptor Flt4*, Proc Natl Acad Sci U S A, 1996, 93(5):1988–1992.

[16] MAGLIONE D., GUERRIERO V., VIGLIETTO G., DELLI-BOVI P., PERSICO M. G., *Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor*, Proc Natl Acad Sci U S A, 1991, 88(20):9267–9271.

[17] MEYER M., CLAUSS M., LEPPLE-WIENHUES A., WALTENBERGER J., AUGUSTIN H. G., ZICHE M., LANZ C., BÜTTNER M., RZIHA H. J., DEHIO C., *A novel vascular endothelial growth factor encoded by Orf virus, VEGF-E, mediates angiogenesis via signalling through VEGFR-2 (KDR) but not VEGFR-1 (Flt-1) receptor tyrosine kinases*, EMBO J, 1999, 18(2):363–374.

[18] OLOFSSON B., PAJUSOLA K., KAIPAINEN A., VON EULER G., JOUKOV V., SAKSELA O., ORPANA A., PETTERSSON R. F., ALITALO K., ERIKSSON U., *Vascular endothelial growth factor B, a novel growth factor for endothelial cells*, Proc Natl Acad Sci U S A, 1996, 93(6):2576–2581.

[19] SENGER D. R., GALLI S. J., DVORAK A. M., PERRUZZI C. A., HARVEY V. S., DVORAK H. F., *Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid*, Science, 1983, 219(4587):983–985.

[20] BECK L. JR., D'AMORE P. A., *Vascular development: cellular and molecular regulation*, FASEB J, 1997, 11(5):365–373.

[21] ROBINSON C. J., STRINGER S. E., *The splice variants of vascular endothelial growth factor (VEGF) and their receptors*, J Cell Sci, 2001, 114(Pt 5):853–865.

[22] FERRARA N., GERBER H. P., LECOUTER J., *The biology of VEGF and its receptors*, Nat Med, 2003, 9(6):669–676.

[23] KAIPAINEN A., KORHONEN J., MUSTONEN T., VAN HINSBERGH V. W., FANG G. H., DUMONT D., BREITMAN M., ALITALO K., *Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development*, Proc Natl Acad Sci U S A, 1995, 92(8):3566–3570.

[24] PERSICO M. G., VINCENTI V., DIPALMA T., Structure, expression and receptor-binding properties of placenta growth factor (PlGF). In: CLAESSON-WELSH L. (ed), *Vascular growth factors and angiogenesis*, Springer-Verlag, Berlin, 1999, 31–40.

[25] AUTIERO M., WALTENBERGER J., COMMUNI D., KRANZ A., MOONS L., LAMBRECHTS D., KROLL J., PLAISANCE S., DE MOL M., BONO F., KLICHE S., FELLBRICH G., BALLMER-HOFER K., MAGLIONE D., MAYR-BEYRLE U., DEWERCHIN M., DOMBROWSKI S., STANIMIROVIC D., VAN HUMMELEN P., DEHIO C., HICKLIN D. J., PERSICO G., HERBERT J. M., COMMUNI D., SHIBUYA M., COLLEN D., CONWAY E. M., CARMELIET P., *Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1*, Nat Med, 2003, 9(7):936–943.

[26] PARK J. E., CHEN H. H., WINER J., HOUCK K. A., FERRARA N., *Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR*, J Biol Chem, 1994, 269(4):25646–25654.

[27] NAGY J. A., DVORAK A. M., DVORAK H. F., *VEGF-A(164/165) and PlGF: roles in angiogenesis and arteriogenesis*, Trends Cardiovasc Med, 2003, 13(5):169–175.

[28] OLOFSSON B., JELTSCH M., ERIKSSON U., ALITALO K., *Current biology of VEGF-B and VEGF-C*, Curr Opin Biotechnol, 1999, 10(6):528–535.

[29] OLOFSSON B., PAJUSOLA K., VON EULER G., CHILOV D., ALITALO K., ERIKSSON U., *Genomic organization of the mouse and human genes for vascular endothelial growth factor B (VEGF-B) and characterization of a second splice isoform*, J Biol Chem, 1996, 271(32):19310–19317.

[30] ENHOLM B., PAAVONEN K., RISTIMÄKI A., KUMAR V., GUNJI Y., KLEFSTROM J., KIVINEN L., LAIHO M., OLOFSSON B., JOUKOV V., ERIKSSON U., ALITALO K., *Comparison of VEGF, VEGF-B, VEGF-C and Ang-1 mRNA regulation by serum, growth factors, oncoproteins and hypoxia*, Oncogene, 1997, 14(20):2475–2483.

[31] SALVEN P., LYMBOUSSAKI A., HEIKKILÄ P., JÄÄSKELA-SAARI H., ENHOLM B., AASE K., VON EULER G., ERIKSSON U., ALITALO K., JOENSUU H., *Vascular endothelial growth factors VEGF-B and VEGF-C are expressed in human tumors*, Am J Pathol, 1998, 153(1):103–108.

[32] JOUKOV V., SORSA T., KUMAR V., JELTSCH M., CLAESSON-WELSH L., CAO Y., SAKSELA O., KALKKINEN N., ALITALO K., *Proteolytic processing regulates receptor specificity and activity of VEGF-C*, EMBO J, 1997, 16(13):3898–3911.

[33] SIEGFRIED G., BASAK A., CROMLISH J. A., BENJANNET S., MARCINKIEWICZ J., CHRÉTIEN M., SEIDAH N. G., KHATIB A. M., *The secretory proprotein convertases furin, PC5, and PC7 activate VEGF-C to induce tumorigenesis*, J Clin Invest, 2003, 111(11):1723–1732.

[34] SAHARINEN P., TAMMELA T., KARKKAINEN M. J., ALITALO K., *Lymphatic vasculature: development, molecular regulation and role in tumor metastasis and inflammation*, Trends Immunol, 2004, 25(7):387–395.

[35] ORLANDINI M., MARCONCINI L., FERRUZZI R., OLIVIERO S., *Identification of a c-fos-induced gene that is related to the platelet-derived growth factor/vascular endothelial growth factor family*, Proc Natl Acad Sci U S A, 1996, 93(21):11675–11680.

[36] FARNEBO F., PIEHL F., LAGERCRANTZ J., *Restricted expression pattern of VEGF-D in the adult and fetal mouse: high expression in the embryonic lung*, Biochem Biophys Res Commun, 1999, 257(3):891–894.

[37] HE Y., KARPANEN T., ALITALO K., *Role of lymphangiogenic factors in tumor metastasis*, Biochim Biophys Acta, 2004, 1654(1):3–12.

[38] SAVORY L. J., STACKER S. A., FLEMING S. B., NIVEN B. E., MERCER A. A., *Viral vascular endothelial growth factor plays a critical role in orf virus infection*, J Virol, 2000, 74(22):10699–10706.

[39] OGAWA S., OKU A., SAWANO A., YAMAGUCHI S., YAZAKI Y., SHIBUYA M., *A novel type of vascular endothelial growth factor, VEGF-E (NZ-7 VEGF), preferentially utilizes KDR/Flk-1 receptor and carries a potent mitotic activity without heparin-binding domain*, J Biol Chem, 1998, 273(47):31273–31282.

[40] CROSS M. J., DIXELIUS J., MATSUMOTO T., CLAESSON-WELSH L., *VEGF-receptor signal transduction*, Trends Biochem Sci, 2003, 28(9):488–494.

[41] NEUFELD G., COHEN T., GENGRINOVITCH S., POLTORAK Z., *Vascular endothelial growth factor (VEGF) and its receptors*, FASEB J, 1999, 13(1):9–22.

[42] RUHRBERG C., *Growing and shaping the vascular tree: multiple roles for VEGF*, BioEssays, 2003, 25(1):1052–1060.

[43] ZACHARY I., GLIKI G., *Signaling transduction mechanisms mediating biological actions of the vascular endothelial growth factor family*, Cardiovasc Res, 2001, 49(3):568–581.

[44] FONG G. H., ZHANG L., BRYCE D. M., PENG J., *Increased hemangioblast commitment, not vascular disorganization, is the primary defect in flt-1 knock-out mice*, Development, 1999, 126(13):3015–3025.

[45] GERBER H. P., CONDORELLI F., PARK J., FERRARA N., *Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia*, J Biol Chem, 1997, 272(38):23659–23667.

[46] Hiratsuka S., Nakamura K., Iwai S., Murakami M., Itoh T., Kijima H., Shipley J. M., Senior R. M., Shibuya M., *MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis*, Cancer Cell, 2002, 2(4):289–300.

[47] LeCouter J., Moritz D. R., Li B., Phillips G. L., Liang X. H., Gerber H. P., Hillan K. J., Ferrara N., *Angiogenesis-independent endothelial protection of liver: role of VEGFR-1*, Science, 2003, 299(5608):890–893.

[48] Shima D. T., Deutsch U., D'Amore P. A., *Hypoxic induction of vascular endothelial growth factor (VEGF) in human epithelial cells is mediated by increases in mRNA stability*, FEBS Letters, 1995, 370(3):203–208.

[49] Stacker S. A., Caesar C., Baldwin M. E., Thornton G. E., Williams R. A., Prevo R., Jackson D. G., Nishikawa S., Kubo H., Achen M. G., *VEGF-D promotes the metastatic spread of tumor cells via the lymphatics*, Nat Med, 2001, 7(2):186–191.

[50] Gille H., Kowalski J., Li B., LeCouter J., Moffat B., Zioncheck T. F., Pelletier N., Ferrara N., *Analysis of biological effects and signaling properties of Flt-1 (VEGFR-1) and KDR (VEGFR-2). A reassessment using novel receptor-specific vascular endothelial growth factor mutants*, J Biol Chem, 2001, 276(5):3222–3230.

[51] Wise L. M., Veikkola T., Mercer A. A., Savory L. J., Fleming S. B., Caesar C., Vitali A., Makinen T., Alitalo K., Stacker S. A., *Vascular endothelial growth factor (VEGF)-like protein from orf virus NZ2 binds to VEGFR2 and neuropilin-1*, Proc Natl Acad Sci U S A, 1999, 96(6):3071–3076.

[52] Partanen T. A., Alitalo K., Miettinen M., *Lack of lymphatic vascular specificity of vascular endothelial growth factor receptor 3 in 185 vascular tumors*, Cancer, 1999, 86(11):2406–2412.

[53] Cross M. J., Claesson-Welsh L., *FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition*, Trends Pharmacol Sci, 2001, 22(4):201–207.

[54] Matsumoto T., Claesson-Welsh L., *VEGF receptor signal transduction*, Sci STKE, 2001, 2001(112):re21.

[55] Alitalo K., Carmeliet P., *Molecular mechanisms of lymphangiogenesis in health and disease*, Cancer Cell, 2002, 1(3):219–227.

[56] Plate K., *From angiogenesis to lymphangiogenesis*, Nat Med, 2001, 7(2):151–152.

[57] Shibuya M., Claesson-Welsh L., *Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis*, Exp Cell Res, 2006, 312(5):549–560.

[58] Skobe M., Hawighorst T., Jackson D. G., Prevo R., Janes L., Velasco P., Riccardi L., Alitalo K., Claffey K., Detmar M., *Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis*, Nat Med, 2001, 7(2):192–198.

[59] Taipale J., Makinen T., Arighi E., Kukk E., Karkkainen M., Alitalo K., *Vascular endothelial growth factor receptor-3*, Curr Top Microbiol Immunol, 1999, 237:85–96.

[60] Jussila L., Valtola R., Partanen T. A., Salven P., Heikkilä P., Matikainen M. T., Renkonen R., Kaipainen A., Detmar M., Tschachler E., Alitalo R., Alitalo K., *Lymphatic endothelium and Kaposi's sarcoma spindle cells detected by antibodies against the vascular endothelial growth factor receptor-3*, Cancer Res, 1998, 58(8):1599–1604.

[61] Karpanen T., Egeblad M., Karkkainen M. J., Kubo H., Ylä-Herttuala S., Jäättela M., Alitalo K., *Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth*, Cancer Res, 2001, 61(5):1786–1790.

[62] Mandriota S. J., Jussila L., Jeltsch M., Compagni A., Baetens D., Prevo R., Banerji S., Huarte J., Montesano R., Jackson D. G., Orci L., Alitalo K., Christofori G., Pepper M. S., *Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis*, EMBO J, 2001, 20(4):672–682.

[63] Su J. L., Yang P. C., Shih J. Y., Yang C. Y., Wei L. H., Hsieh C. Y., Chou C. H., Jeng Y. M., Wang M. Y., Chang K. J., Hung M. C., Kuo M. L., *The VEGF-C/Flt-4 axis promotes invasion and metastasis of cancer cells*, Cancer Cell, 2006, 9(3):209–223.

[64] Ferrell R. E., Levinson K. L., Esman J. H., Kimak M. A., Lawrence E. C., Barmada M. M., Finegold D. N., *Hereditary lymphedema: evidence for linkage and genetic heterogeneity*, Hum Mol Genet, 1998, 7(13):2073–2078.

[65] Partanen T. A., Arola J., Saaristo A., Jussila L., Ora A., Miettinen M., Stacker S. A., Achen M. G., Alitalo K., *VEGF-C and VEGF-D expression in neuroendocrine cells and their receptor, VEGFR-3, in fenestrated blood vessels in human tissues*, FASEB J, 2000, 14(13):2087–2096.

[66] Bagri A., Tessier-Lavigne M., *Neuropilins as Semaphorin receptors: in vivo functions in neuronal cell migration and axon guidance*, Adv Exp Med Biol, 2002, 515:13–31.

[67] Klagsbrun M., Takashima S., Mamluk R., *The role of neuropilin in vascular and tumor biology*, Adv Exp Med Biol, 2002, 515:33–48.

[68] Klagsbrun M., Folkman J., Angiogenesis. In: Sporn M. B., Roberts A. B. (eds), *Peptide growth factors and their receptors*, Springer-Verlag, New York, 1991, 549–586.

[69] Soker S., Takashima S., Miao H. Q., Neufeld G., Klagsbrun M., *Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor*, Cell, 1998, 92(6):735–745.

[70] Yuan L., Moyon D., Pardanaud L., Bréant C., Karkkainen M. J., Alitalo K., Eichmann A., *Abnormal lymphatic vessel development in neuropilin 2 mutant mice*, Development, 2002, 129(20):4797–4806.

[71] Safran M., Kaelin W. G. Jr., *HIF hydroxylation and the mammalian oxygen-sensing pathway*, J Clin Invest, 2003, 111(6):779–783.

[72] Rak J., Mitsuhashi Y., Bayko L., Filmus J., Shirasawa S., Sasazuki T., Kerbel R. S., *Mutant ras oncogenes upregulate VEGF/VPF expression: implications for induction and inhibition of tumor angiogenesis*, Cancer Res, 1995, 55(20):4575–4580.

[73] Coultas L., Chawengsaksophak K., Rossant J., *Endothelial cells and VEGF in vascular development*, Nature, 2005, 438(7070):937–945.

[74] Shalaby F., Rossant J., Yamaguchi T. P., Gertsenstein M., Wu X. F., Breitman M. L., Schuh A. C., *Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice*, Nature, 1995, 376(6535):62–66.

[75] Fong G. H., Rossant J., Gertsenstein M., Breitman M. L., *Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium*, Nature, 1995, 376(6535):66–70.

[76] Carmeliet P., *Angiogenesis in life, disease and medicine*, Nature, 2005, 438(7070):932–936.

[77] Folkman J., *Cancer medicine*, 5th edition, B. C. Decker, Ontario, 2000, 132–152.

[78] Ferrara N., *Vascular endothelial growth factor: basic science and clinical progress*, Endocr Rev, 2004, 25(4):581–611.

[79] Hattori K., Heissig B., Wu Y., Dias S., Tejada R., Ferris B., Hicklin D. J., Zhu Z., Bohlen P., Witte L., Hendrikx J., Hackett N. R., Crystal R. G., Moore M. A., Werb Z., Lyden D., Rafii S., *Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment*, Nat Med, 2002, 8(8):841–849.

[80] Luttun A., Tjwa M., Moons L., Wu Y., Angelillo-Scherrer A., Liao F., Nagy J. A., Hooper A., Priller J., De Klerck B., Compernolle V., Daci E., Bohlen P., Dewerchin M., Herbert J. M., Fava R., Matthys P., Carmeliet G., Collen D., Dvorak H. F., Hicklin D. J., Carmeliet P., *Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1*, Nat Med, 2002, 8(8):831–840.

[81] CLAUSS M., WEICH H., BREIER G., KNIES U., RÖCKL W., WALTENBERGER J., RISAU W., *The vascular endothelial growth factor receptor Flt-1 mediates biological activities. Implications for a functional role of placenta growth factor in monocyte activation and chemotaxis*, J Biol Chem, 1996, 271(30):17629–17634.

[82] CURSIEFEN C., CHEN L., BORGES L. P., JACKSON D., CAO J., RADZIEJEWSKI C., D'AMORE P. A., DANA M. R., WIEGAND S. J., STREILEIN J. W., *VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment*, J Clin Invest, 2004, 113(7):1040–1050.

[83] RAFII S., AVECILLA S., SHMELKOV S., SHIDO K., TEJADA R., MOORE M. A., HEISSIG B., HATTORI K., *Angiogenic factors reconstitute hematopoiesis by recruiting stem cells from bone marrow microenvironment*, Ann N Y Acad Sci, 2003, 996:49–60.

[84] KARKKAINEN M. J., HAIKO P., SAINIO K., PARTANEN J., TAIPALE J., PETROVA T. V., JELTSCH M., JACKSON D. G., TALIKKA M., RAUVALA H., BETSHOLTZ C., ALITALO K., *Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins*, Nat Immunol, 2004, 5(1):74–80.

[85] BENJAMIN L. E., KESHET E., *Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal*, Proc Natl Acad Sci U S A, 1997, 94(16):8761–8766.

[86] GERBER H. P., DIXIT V., FERRARA N., *Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells*, J Biol Chem, 1998, 273(21):13313–13316.

[87] BATES D. O., HILLMAN N. J., WILLIAMS B., NEAL C. R., POCOCK T. M., *Regulation of microvascular permeability by vascular endothelial growth factors*, J Anat, 2002, 200(6):581–597.

[88] HOOD J. D., MEININGER C. J., ZICHE M., GRANGER H. J., *VEGF upregulates ecNOS message, protein, and NO production in human endothelial cells*, Am J Physiol, 1998, 274(3 Pt 2):H1054–H1058.

[89] KROLL J., WALTENBERGER J., *A novel function of VEGF receptor-2 (KDR): rapid release of nitric oxide in response to VEGF-A stimulation in endothelial cells*, Biochem Biophys Res Commun, 1999, 265(3):636–639.

[90] MANDRIOTA S. J., SEGHEZZI G., VASSALLI J. D., FERRARA N., WASI S., MAZZIERI R., MIGNATTI P., PEPPER M. S., *Vascular endothelial growth factor increases urokinase receptor expression in vascular endothelial cells*, J Biol Chem, 1995, 270(17):9709–9716.

[91] PEPPER M. S., FERRARA N., ORCI L., MONTESANO R., *Vascular endothelial growth factor (VEGF) induces plasminogen activators and plasminogen activator inhibitor-1 in microvascular endothelial cells*, Biochem Biophys Res Commun, 1991, 181(2):902–906.

[92] UNEMORI E. N., FERRARA N., BAUER E. A., AMENTO E. P., *Vascular endothelial growth factor induces interstitial collagenase expression in human endothelial cells*, J Cell Physiol, 1992, 153(3):557–562.

[93] LAMBRECHTS D., STORKEBAUM E., CARMELIET P., *VEGF: necessary to prevent motoneuron degeneration, sufficient to treat ALS?*, Trends Mol Med, 2004, 10(6):275–282.

[94] STORKEBAUM E., LAMBRECHTS D., CARMELIET P., *VEGF: once regarded as a specific angiogenic factor, now implicated in neuroprotection*, Bioessays, 2004, 26(9):943–954.

[95] DENHART B. C., GUIDI A. J., TOGNAZZI K., DVORAK H. F., BROWN L. F., *Vascular permeability factor/vascular endothelial growth factor and its receptors in oral and laryngeal squamous cell carcinoma and dysplasia*, Lab Invest, 1997, 77(6):659–664.

[96] EISMA R. J., SPIRO J. D., KREUTZER D. L., *Vascular endothelial growth factor expression in head and neck squamous cell carcinoma*, Am J Surg, 1997, 174(5):513–517.

[97] LI C., SHINTANI S., TERAKADO N., KLOSEK S. K., ISHIKAWA T., NAKASHIRO K., HAMAKAWA H., *Microvessel density and expression of vascular endothelial growth factor, basic fibroblast growth factor, and platelet-derived endothelial growth factor in oral squamous cell carcinomas*, Int J Oral Maxillofac Surg, 2005, 34(5):559–565.

[98] JOHNSTONE S., LOGAN R. M., *Expression of vascular endothelial growth factor (VEGF) in normal oral mucosa, oral dysplasia and oral squamous cell carcinoma*, Int J Oral Maxillofac Surg, 2007, 36(3):263–266.

[99] CARLILE J., HARADA K., BAILLIE R., MACLUSKEY M., CHISHOLM D. M., OGDEN G. R., SCHOR S. L., SCHOR A. M., *Vascular endothelial growth factor (VEGF) expression in oral tissues: possible relevance to angiogenesis, tumour progression and field cancerisation*, J Oral Pathol Med, 2001, 30(8):449–457.

[100] MACLUSKEY M., CHANDRACHUD L. M., PAZOUKI S., GREEN M., CHISHOLM D. M., OGDEN G. R., SCHOR S. L., SCHOR A. M., *Apoptosis, proliferation, and angiogenesis in oral tissues. Possible relevance to tumour progression*, J Pathol, 2000, 191(4):368–375.

[101] PAZOUKI S., CHISHOLM D. M., ADI M. M., CARMICHAEL G., FARQUHARSON M., OGDEN G. R., SCHOR S. L., SCHOR A. M., *The association between tumour progression and vascularity in the oral mucosa*, J Pathol, 1997, 183(1):39–43.

[102] MĂRGĂRITESCU C., SIMIONESCU C., MOGOANTĂ L., BADEA P., PIRICI D., STEPAN A., CIUREA R., *Endoglin (CD105) and microvessel density in oral squamous cell carcinoma*, Rom J Morphol Embryol, 2008, 49(3):321–326.

[103] PENFOLD C. N., PARTRIDGE M., ROJAS R., LANGDON J. D., *The role of angiogenesis in the spread of oral squamous cell carcinoma*, Br J Oral Maxillofac Surg, 1996, 34(1):37–41.

[104] SHANG Z.-J., LI Z.-B., LI J.-R., *VEGF is up-regulated by hypoxic stimulation and related to tumour angiogenesis and severity of disease in oral squamous cell carcinoma: in vitro and in vivo studies*, Int J Oral Maxillofac Surg, 2006, 35(6):533–538.

[105] TIPOE G. L., JIN Y., WHITE F. H., *The relationship between vascularity and cell proliferation in human normal and pathological lesions of the oral cheek epithelium*, Eur J Cancer B Oral Oncol, 1996, 32B(1):24–31.

[106] SALVEN P., HEIKKILÄ P., ANTTONEN A., KAJANTI M., JOENSUU H., *Vascular endothelial growth factor in squamous cell head and neck carcinoma: expression and prognostic significance*, Mod Pathol, 1997, 10(11):1128–1133.

[107] TAE K., EL-NAGGAR A. K., YOO E., FENG L., LEE J. J., HONG W. K., HITTELMAN W. N., SHIN D. M., *Expression of vascular endothelial growth factor and microvessel density in head and neck tumorigenesis*, Clin Cancer Res, 2000, 6(7):2821–2828.

[108] DOLDI N., ORIGONI M., BASSAN M., FERRARI D., ROSSI M., FERRARI A., *Vascular endothelial growth factor. Expression in human vulvar neoplastic and nonneoplastic tissues*, J Reprod Med, 1996, 41(11):844–848.

[109] PAMMER J., WENINGER W., MILDNER M., BURIAN M., WOJTA J., TSCHACHLER E., *Vascular endothelial growth factor is constitutively expressed in normal human salivary glands and is secreted in the saliva of healthy individuals*, J Pathol, 1998, 186(2):186–191.

[110] WENINGER W., UTHMAN A., PAMMER J., PICHLER A., BALLAUN C., LANG I. M., PLETTENBERG A., BANKL H. C., STÜRZL M., TSCHACHLER E., *Vascular endothelial growth factor production in normal epidermis and in benign and malignant epithelial skin tumors*, Lab Invest, 1996, 75(5):647–657.

[111] WOESSNER R. D., WRIGHT P. S., LOUDY D. E., WALLACE C. D., MONTGOMERY L. R., NESTOK B. R., *Microautoradiographic quantitation of vascular endothelial growth factor mRNA levels in human prostate specimens containing normal and neoplastic epithelium*, Exp Mol Pathol, 1998, 65(1):37–52.

[112] ARORA S., KAUR J., SHARMA C., MATHUR M., BAHADUR S., SHUKLA N. K., DEO S. V., RALHAN R., *Stromelysin 3, Ets-1, and vascular endothelial growth factor expression in oral precancerous and cancerous lesions: correlation with microvessel density, progression, and prognosis*, Clin Cancer Res, 2005, 11(6):2272–2284.

[113] Chuang H. C., Su C. Y., Huang H. Y., Chien C. Y., Chen C. M., Huang C. C., *High expression of CD105 as a prognostic predictor of early tongue cancer*, Laryngoscope, 2006, 116(7):1175–1179.

[114] Joo Y. H., Jung C. K., Kim M. S., Sun D. I., *Relationship between vascular endothelial growth factor and Notch1 expression and lymphatic metastasis in tongue cancer*, Otolaryngol Head Neck Surg, 2009, 140(4):512–518.

[115] Kim S. H., Cho N. H., Kim K., Lee J. S., Koo B. S., Kim J. H., Chang J. H., Choi E. C., *Correlations of oral tongue cancer invasion with matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) expression*, J Surg Oncol, 2006, 93(4):330–337.

[116] Kyzas P. A., Stefanou D., Agnantis N. J., *Immunohisto-chemical expression of vascular endothelial growth factor correlates with positive surgical margins and recurrence in T1 and T2 squamous cell carcinoma (SCC) of the lower lip*, Oral Oncol, 2004, 40(9):941–947.

[117] Maeda T., Matsumura S., Hiranuma H., Jikko A., Furukawa S., Ishida T., Fuchihata H., *Expression of vascular endothelial growth factor in human oral squamous cell carcinoma: its association with tumour progression and p53 gene status*, J Clin Pathol, 1998, 51(10):771–775.

[118] Shang Z. J., Li J. R., *Expression of endothelial nitric oxide synthase and vascular endothelial growth factor in oral squamous cell carcinoma: its correlation with angiogenesis and disease progression*, J Oral Pathol Med, 2005, 34(3):134–139.

[119] Shintani S., Li C., Ishikawa T., Mihara M., Nakashiro K., Hamakawa H., *Expression of vascular endothelial growth factor A, B, C, and D in oral squamous cell carcinoma*, Oral Oncol, 2004, 40(1):13–20.

[120] Smith B. D., Smith G. L., Carter D., Sasaki C. T., Haffty B. G., *Prognostic significance of vascular endothelial growth factor protein levels in oral and oropharyngeal squamous cell carcinoma*, J Clin Oncol, 2000, 18(10):2046–2052.

[121] Uehara M., Sano K., Ikeda H, Sekine J., Irie A, Yokota T., Tobita T., Ohba S., Inokuchi T., *Expression of vascular endothelial growth factor and prognosis of oral squamous cell carcinoma*, Oral Oncol, 2004, 40(3):321–325.

[122] Kyzas P. A., Geleff S., Batistatou A., Agnantis N. J., Stefanou D., *Evidence for lymphangiogenesis and its prognostic implications in head and neck squamous cell carcinoma*, J Pathol, 2005, 206(2):170–177.

[123] Mohamed K. M., Le A., Duong H., Wu Y., Zhang Q., Messadi D. V., *Correlation between VEGF and HIF-1alpha expression in human oral squamous cell carcinoma*, Exp Mol Pathol, 2004, 76(2):143–152.

[124] Neuchrist C., Erovic B. M., Handisurya A., Fischer M. B., Steiner G. E., Hollemann D., Gedlicka C., Saaristo A., Burian M., *Vascular endothelial growth factor C and vascular endothelial growth factor receptor 3 expression in squamous cell carcinomas of the head and neck*, Head Neck, 2003, 25(6):464–474.

[125] Fernandez-Pujol P. B., Lucibello F. C., Zuzarte M., Lütjens P., Müller R., Havemann K., *Dendritic cells derived from peripheral monocytes express endothelial markers and in the presence of angiogenic growth factors differentiate into endothelial-like cells*, Eur J Cell Biol, 2001, 80(1):99–110.

[126] Zangani D., Darcy K. M., Masso-Welch P. A., Bellamy E. S., Desole M. S., Ip M. M., *Multiple different-iation pathways of rat mammary stromal cells in vitro: acquisition of a fibroblast, adipocyte or endothelial phenotype is dependent on hormonal and extracellular matrix stimulation*, Differentiation, 1999, 64(2):91–101.

[127] Ziegler B. L., Valtieri M., Porada G. A., De Maria R., Müller R., Masella B., Gabbianelli M., Casella I., Pelosi E., Bock T., Zanjani E. D., Peschle C., *KDR receptor: a key marker defining hematopoietic stem cells*, Science, 1999, 285(5433):1553–1558.

[128] Kishimoto K., Sasaki A., Yoshihama Y., Mese H., Tsukamoto G., Matsumura T., *Expression of vascular endothelial growth factor-C predicts regional lymph node metastasis in early oral squamous cell carcinoma*, Oral Oncol, 2003, 39(4):391–396.

[129] Li Q. L., Chen F. J., Zeng Z. Y., Yang A. K., Wu Q. L., Zhang H. Z., Hou J. H., [*Expression and clinical significance of VEGF-C and Flt-4 in tongue squamous cell carcinoma*], Ai Zheng, 2006, 25(2):235–240.

[130] López de Cicco R., Watson J. C., Bassi D. E., Litwin S., Klein-Szanto A. J., *Simultaneous expression of furin and vascular endothelial growth factor in human oral tongue squamous cell carcinoma progression*, Clin Cancer Res, 2004, 10(13):4480–4488.

[131] Nakaya H., Kawashiri S., Tanaka A., Noguchi N., Kato K., Hase T., Et Al., *Influences of angiogenesis and lymphangiogenesis on cancerous invasion in experimentally induced tongue carcinoma*, J Oral Pathol Med, 2005, 34(2):87–92.

[132] Nakazato T., Shingaki S., Kitamura N., Saito C., Kuwano R., Tachibana M., *Expression level of vascular endothelial growth factor-C and -A in cultured human oral squamous cell carcinoma correlates respectively with lymphatic metastasis and angiogenesis when transplanted into nude mouse oral cavity*, Oncol Rep, 2006, 15(4):825–830.

[133] Ohno F., Nakanishi H., Abe A., Seki Y., Kinoshita A., Hasegawa Y., Tatematsu M., Kurita K., *Regional difference in intratumoral lymphangiogenesis of oral squa-mous cell carcinomas evaluated by immunohistochemistry using D2-40 and podoplanin antibody: an analysis in comparison with angiogenesis*, J Oral Pathol Med, 2007, 36(5):281–289.

[134] Sedivy R., Beck-Mannagetta J., Haverkampf C., Battistutti W., Honigschnabl S., *Expression of vascular endothelial growth factor-C correlates with the lymphatic microvessel density and the nodal status in oral squamous cell cancer*, J Oral Pathol Med, 2003, 32(8):455–460.

[135] Siriwardena B. S., Kudo Y., Ogawa I., Udagama M. N., Tilakaratne W. M., Takata T., *VEGF-C is associated with lymphatic status and invasion in oral cancer*, J Clin Pathol, 2008, 61(1):103–108.

[136] Tanigaki Y., Nagashima Y., Kitamura Y., Matsuda H., Mikami Y., Tsukuda M., *The expression of vascular endothelial growth factor-A and -C, and receptors 1 and 3: correlation with lymph node metastasis and prognosis in tongue squamous cell carcinoma*, Int J Mol Med, 2004, 14(3):389–395.

[137] Warburton G., Nikitakis N. G., Roberson P., Marinos N. J., Wu T., Sauk J. J. Jr., Ord R. A., Wahl S. M., *Histopathological and lymphangiogenic parameters in relation to lymph node metastasis in early stage oral squamous cell carcinoma*, J Oral Maxillofac Surg, 2007, 65(3):475–484.

[138] Faustino S. E., Oliveira D. T., Nonogaki S., Landman G., Carvalho A. L., Kowalski L. P., *Expression of vascular endothelial growth factor-C does not predict occult lymph-node metastasis in early oral squamous cell carcinoma*, Int J Oral Maxillofac Surg, 2008, 37(4):372–378.

[139] Matsuura M., Onimaru M., Yonemitsu Y., Suzuki H., Nakano T., Ishibashi H., Shirasuna K., Sueishi K., *Autocrine loop between vascular endothelial growth factor (VEGF)-C and VEGF receptor-3 positively regulates tumor-associated lymphangiogenesis in oral squamoid cancer cells*, Am J Pathol, 2009, 175(4):1709–1721.

[140] Neuchrist C., Erovic B. M., Handisurya A., Steiner G. E., Rockwell P., Gedlicka C., Burian M., *Vascular endothelial growth factor receptor 2 (VEGFR2) expression in squamous cell carcinomas of the head and neck*, Laryngoscope, 2001, 111(10):1834–1841.

[141] Kyzas P. A., Stefanou D., Batistatou A., Agnantis N. J., *Potential autocrine function of vascular endothelial growth factor in head and neck cancer via vascular endothelial growth factor receptor-2*, Mod Pathol, 2005, 18(4):485–494.

[142] Lalla R. V., Boisoneau D. S., Spiro J. D., Kreutzer D. L., *Expression of vascular endothelial growth factor receptors on tumor cells in head and neck squamous cell carcinoma*, Arch Otolaryngol Head Neck Surg, 2003, 129(8):882–888.

[143] Moriyama M., Kumagai S., Kawashiri S., Kojima K., Kakihara K., Yamamoto E., *Immunohistochemical study of tumour angiogenesis in oral squamous cell carcinoma*, Oral Oncol, 1997, 33(5):369–374.

[144] Sugiura T., Inoue Y., Matsuki R., Ishii K., Takahashi M., Abe M., Shirasuna K., *VEGF-C and VEGF-D expression is correlated with lymphatic vessel density and lymph node metastasis in oral squamous cell carcinoma: implications for use as a prognostic marker*, Int J Oncol, 2009, 34(3):673–680.

[145] Leek R. D., Hunt N. C., Landers R. J., Lewis C. E., Royds J. A., Harris A. L., *Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer*, J Pathol, 2000, 190(4):430–436.

[146] Homer J. J., Greenman J., Stafford N. D., *Angiogenesis in head and neck squamous cell carcinoma*, Clin Otolaryngol Allied Sci, 2000, 25(3):169–180.

[147] Speight P. M., Morgan P. R., *The natural history and pathology of oral cancer and precancer*, Community Dent Health, 1993, 10 Suppl 1:31–41.

[148] Wong Y. K., Liu C. J., Kwan P. C., Chao S. Y., *Microvascular density and vascular endothelial growth factor immunoreactivity as predictors of regional lymph node metastasis from betel-associated oral squamous cell carcinoma*, J Oral Maxillofac Surg, 2003, 61(11):1257–1262.

[149] Kyzas P. A., Stefanou D., Batistatou A., Agnantis N. J., *Prognostic significance of VEGF immunohistochemical expression and tumor angiogenesis in head and neck squamous cell carcinoma*, J Cancer Res Clin Oncol, 2005, 131(9):624–630.

[150] Chien C. Y., Su C. Y., Hwang C. F., Chuang H. C., Chen C. M., Huang C. C., *High expressions of CD105 and VEGF in early oral cancer predict potential cervical metastasis*, J Surg Oncol, 2006, 94(5):413–417.

[151] Zhe S., Zhang W. F., Chen X. M., Shang Z. J., *Expression of EphA2 and VEGF in squamous cell carcinoma of the tongue: correlation with the angiogenesis and clinical outcome*, Oral Oncol, 2008, 44(12):1110–1117.

[152] Martinez-Lara E., Cañuelo A. R., Siles E., Hernández R., Del Moral M. L., Blanco S., Pedrosa J. A., Rodrigo J., Peinado M. A., *Constitutive nitric oxide synthases are responsible for the nitric oxide production in the ischemic aged cerebral cortex*, Brain Res, 2005, 1054(1):88–94.

[153] Fonsatti E., Sigalotti L., Arslan P., Altomonte M., Maio M., *Emerging role of endoglin (CD105) as a marker of angiogenesis with clinical potential in human malignancies*, Curr Cancer Drug Targets, 2003, 3(6):427–432.

[154] Duff S. E., Li C., Garland J. M., Kumar S., *CD105 is important for angiogenesis: evidence and potential applications*, FASEB J, 2003, 17(9):984–992.

[155] Seghezzi G., Patel S., Ren C. J., Gualandris A., Pintucci G., Robbins E. S., Shapiro R. L., Galloway A. C., Rifkin D. B., Mignatti P., *Fibroblast growth factor-2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: an autocrine mechanism contributing to angiogenesis*, J Cell Biol, 1998, 141(7):1659–1673.

[156] Reinmuth N., Parikh A. A., Ahmad S. A., Liu W., Stoeltzing O., Fan F., Takeda A., Akagi M., Ellis L. M., *Biology of angiogenesis in tumors of the gastrointestinal tract*, Microsc Res Tech, 2003, 60(2):199–207.

[157] Liang X., Yang D., Hu J., Hao X., Gao J., Mao Z., *Hypoxia inducible factor-alpha expression correlates with vascular endothelial growth factor-C expression and lymphangiogenesis/angiogenesis in oral squamous cell carcinoma*, Anticancer Res, 2008, 28(3A):1659–1666.

[158] Miyahara M., Tanuma J., Sugihara K., Semba I., *Tumor lymphangiogenesis correlates with lymph node metastasis and clinicopathologic parameters in oral squamous cell carcinoma*, Cancer, 2007, 110(6):1287–1294.

[159] Michi Y., Morita I., Amagasa T., Murota S., *Human oral squamous cell carcinoma cell lines promote angiogenesis via expression of vascular endothelial growth factor and upregulation of KDR/flk-1 expression in endothelial cells*, Oral Oncol, 2000, 36(1):81–88.

[160] Maniotis A. J., Folberg R., Hess A., Seftor E. A., Gardner L. M., Pe'er J., Trent J. M., Meltzer P. S., Hendrix M. J., *Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry*, Am J Pathol, 1999, 155(3):739–752.

[161] Beasley N. J., Prevo R., Banerji S., Leek R. D., Moore J., van Trappen P., Cox G., Harris A. L., Jackson D. G., *Intratumoral lymphangiogenesis and lymph node metastasis in head and neck cancer*, Cancer Res, 2002, 62(5):1315–1320.

[162] Borg J. P., deLapeyrière O., Noguchi T., Rottapel R., Dubreuil P., Birnbaum D., *Biochemical characterization of two isoforms of FLT4, a VEGF receptor-related tyrosine kinase*, Oncogene, 1995, 10(5):973–984.

[163] Gallo O., Masini E., Bianchi B., Bruschini L., Paglierani M., Franchi A., *Prognostic significance of cyclooxygenase-2 pathway and angiogenesis in head and neck squamous cell carcinoma*, Hum Pathol, 2002, 33(7):708–714.

[164] Mineta H., Miura K., Ogino T., Takebayashi S., Misawa K., Ueda Y., Suzuki I., Dictor M., Borg A., Wennerberg J., *Prognostic value of vascular endothelial growth factor (VEGF) in head and neck squamous cell carcinomas*, Br J Cancer, 2000, 83(6):775–781.

[165] Artese L., Rubini C., Ferrero G., Fioroni M., Santinelli A., Piattelli A., *Microvessel density (MVD) and vascular endothelial growth factor expression (VEGF) in human oral squamous cell carcinoma*, Anticancer Res, 2001, 21(1B):689–695.

[166] Homer J. J., Prentice M. G., Cawkwell L., Birchall M., Greenman J., Stafford N. D., *Angiogenesis and the expression of vascular endothelial growth factors A and C in squamous cell carcinoma of the piriform fossa*, Arch Otolaryngol Head Neck Surg, 2003, 129(10):1110–1114.

[167] Sawatsubashi M., Yamada T., Fukushima N., Mizokami H., Tokunaga O., Shin T., *Association of vascular endothelial growth factor and mast cells with angiogenesis in laryngeal squamous cell carcinoma*, Virchows Arch, 2000, 436(3):243–248.

[168] Tse G. M., Chan A. W., Yu K. H., King A. D., Wong K. T., Chen G. G., Tsang R. K., Chan A. B., *Strong immunohistochemical expression of vascular endothelial growth factor predicts overall survival in head and neck squamous cell carcinoma*, Ann Surg Oncol, 2007, 14(12):3558–3565.

[169] Shang Z. J., Li J. R., Li Z. B., *Circulating levels of vascular endothelial growth factor in patients with oral squamous cell carcinoma*, Int J Oral Maxillofac Surg, 2002, 31(5):495–498.

[170] Boonkitticharoen V., Kulapaditharom B., Leopairut J., Kraiphibul P., Larbcharoensub N., Cheewaruangroj W., Chintrakarn C., Pochanukul L., *Vascular endothelial growth factor a and proliferation marker in prediction of lymph node metastasis in oral and pharyngeal squamous cell carcinoma*, Arch Otolaryngol Head Neck Surg, 2008, 134(12):1305–1311.

[171] Smith B. D., Smith G. L., Carter D., DiGiovanna M. P., Kasowitz K. M., Sasaki C. T., Haffty B. G., *Molecular marker expression in oral and oropharyngeal squamous cell carcinoma*, Arch Otolaryngol Head Neck Surg, 2001, 127(7):780–785.

[172] Gourin C. G., McAfee W. J., Neyman K. M., Howington J. W., Podolsky R. H., Terris D. J., *Effect of comorbidity on quality of life and treatment selection in patients with squamous cell carcinoma of the head and neck*, Laryngoscope, 2005, 115(8):1371–1375.

[173] Chin D., Boyle G. M., Porceddu S., Theile D. R., Parsons P. G., Coman W. B., *Head and neck cancer: past, present and future*, Expert Rev Anticancer Ther, 2006, 6(7):1111–1118.

[174] St John M. A., Abemayor E., Wong D. T., *Recent new approaches to the treatment of head and neck cancer*, Anticancer Drugs, 2006, 17(4):365–375.

[175] Fujita K., Sano D., Kimura M., Yamashita Y., Kawakami M., Ishiguro Y., Nishimura G., Matsuda H., Tsukuda M., *Antitumor effects of bevacizumab in combination with paclitaxel on head and neck squamous cell carcinoma*, Oncol Rep, 2007, 18(1):47–51.

[176] National Institutes of Health, *Clinical trials registry*, available at http://www.clinicaltrials.gov, 2008.

[177] Rapidis A. D., Vermorken J. B., Bourhis J., *Targeted therapies in head and neck cancer: past, present and future*, Rev Recent Clin Trials, 2008, 3(3):156–166.

[178] Seiwert T. Y., Haraf D. J., Cohen E. E., Stenson K., Witt M. E., Dekker A., Kocherginsky M., Weichselbaum R. R., Chen H. X., Vokes E. E., *Phase I study of bevacizumab added to fluorouracil- and hydroxyurea-based concomitant chemoradiotherapy for poor-prognosis head and neck cancer*, J Clin Oncol, 2008, 26(10):1732–1741.

[179] Ciardiello F., Caputo R., Bianco R., Damiano V., Fontanini G., Cuccato S., De Placido S., Bianco A. R., Tortora G., *Inhibition of growth factor production and angiogenesis in human cancer cells by ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor*, Clin Cancer Res, 2001, 7(5):1459–1465.

[180] Bozec A., Sudaka A., Fischel J. L., Brunstein M. C., Etienne-Grimaldi M. C., Milano G., *Combined effects of bevacizumab with erlotinib and irradiation: a preclinical study on a head and neck cancer orthotopic model*, Br J Cancer, 2008, 99(1):93–99.

[181] Bozec A., Sudaka A., Toussan N., Fischel J. L., Etienne-Grimaldi M. C., Milano G., *Combination of sunitinib, cetuximab and irradiation in an orthotopic head and neck cancer model*, Ann Oncol, 2009, 20(10):1703–1707.

[182] Cohen E. E., Davis D. W., Karrison T. G., Seiwert T. Y., Wong S. J., Nattam S., Kozloff M. F., Clark J. I., Yan D. H., Liu W., Pierce C., Dancey J. E., Stenson K., Blair E., Dekker A., Vokes E. E., *Erlotinib and bevacizumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck: a phase I/II study*, Lancet Oncol, 2009, 10(3):247–257.

[183] Gilbert J., Argiris A., *Emerging molecular targeted therapies in squamous cell carcinoma of the head and neck*, Clin Adv Hematol Oncol, 2006, 4(8):611–619.

[184] Vokes E. E., Cohen E. E., Mauer A. M., Karrison T. G., Wong S. J., Skoog-Shuman L. J., *A phase I study of erlotinib and bevacizumab for recurrent or metastastic squamous cell carcinoma of the head and neck*, Proc Am Soc Clin Oncol, 2005, A5504.

[185] Underiner T. L., Ruggeri B., Gingrich D. E., *Development of vascular endothelial growth factor receptor (VEGFR) kinase inhibitors as anti-angiogenic agents in cancer therapy*, Curr Med Chem, 2004, 11(6):731–745.

[186] Tong R. T., Boucher Y., Kozin S. V., Winkler F., Hicklin D. J., Jain R. K., *Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors*, Cancer Res, 2004, 64(11):3731–3736.

[187] Yazici Y. D., Kim S., Jasser S. A., Wang Z., Carter K. B. Jr., Bucana C. D., Myers J. N., *Antivascular therapy of oral tongue squamous cell carcinoma with PTK787*, Laryngoscope, 2005, 115(12):2249–2255.

[188] Fury M. G., Zahalsky A., Wong R., Venkatraman E., Lis E., Hann L., Aliff T., Gerald W., Fleisher M., Pfister D. G., *A Phase II study of SU5416 in patients with advanced or recurrent head and neck cancers*, Invest New Drugs, 2007, 25(2):165–172.

[189] Sano D., Kawakami M., Fujita K., Kimura M., Yamashita Y., Ishiguro Y., Nishimura G., Matsuda H., Tsukuda M., *Antitumor effects of ZD6474 on head and neck squamous cell carcinoma*, Oncol Rep, 2007, 17(2):289–295.

[190] León X., Quer M., Orús C., del Prado Venegas M., López M., *Distant metastases in head and neck cancer patients who achieved loco-regional control*, Head Neck, 2000, 22(7):680–686.

[191] Hong W. K., Bromer R. H., Amato D. A., Shapshay S., Vincent M., Vaughan C., Willett B., Katz A., Welch J., Fofonoff S. *et al.*, *Patterns of relapse in locally advanced head and neck cancer patients who achieved complete remission after combined modality therapy*, Cancer, 1985, 56(6):1242–1245.

[192] Gourin C. G., Johnson J. T., *Surgical treatment of squamous cell carcinoma of the base of tongue*, Head Neck, 2001, 23(8):653–660.

[193] Fukumoto S., Morifuji M., Katakura Y., Ohishi M., Nakamura S., *Endostatin inhibits lymph node metastasis by a down-regulation of the vascular endothelial growth factor C expression in tumor cells*, Clin Exp Metastasis, 2005, 22(1):31–38.

[194] Elser C., Siu L. L., Winquist E., Agulnik M., Pond G. R., Chin S. F., Francis P., Cheiken R., Elting J., McNabola A., Wilkie D., Petrenciuc O., Chen E. X., *Phase II trial of sorafenib in patients with recurrent or metastatic squamous cell carcinoma of the head and neck or nasopharyngeal carcinoma*, J Clin Oncol, 2007, 25(24):3766–3773.

[195] Choong N. W., Kozloff M., Taber D., Hu H. S., Wade J. 3rd, Ivy P., Karrison T. G., Dekker A., Vokes E. E., Cohen E. E., *Phase II study of sunitinib malate in head and neck squamous cell carcinoma*, Invest New Drugs, 2009.

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