Abstract. Oral squamous cell carcinomas (OSCCs) have the potential for rapid and unlimited growth. Therefore, hypoxic tissue areas are common in these malignant tumours and contribute to cancer progression, resistance to therapy and poor outcome. Out of all proteins induced by hypoxia, hypoxia-inducible factors (HIFs) and their target genes have been most extensively studied. HIF1 is a heterodimeric transcriptional complex that functions as the main regulator of systemic and cellular oxygen homeostasis; it is composed of HIF1α and HIF1β subunits. At physiological concentrations of oxygen, prolyl hydroxylases (PHDs) modify HIF1α and prepare it for proteasomal degradation. In hypoxia, PHDs are inhibited and HIF1α dimerises with HIF1β to form HIF1, which is responsible for the activation of several genes involved in multiple aspects of tumor biology. Among these genes, vascular endothelial growth factor (VEGF) is essential as a regulatory gene of angiogenesis in the adaptation to a hypoxic microenvironment. Previous studies have shown the correlation between HIF1α and VEGF in OSCC and high levels of HIF1α expression appear to predict a poor prognosis. The purpose of the present article is to review the hypoxic condition in OSCC and its correlation with prognosis.

Despite possessing some advantages over other tumour types in terms of diagnosis and therapeutic strategies, oral squamous cell carcinoma (OSCC) is one of the 10 most common malignant human tumours (1, 2), with 9,990 new cases in 2012 in Brazil (3). Every year, around 263,000 cases of oral cavity cancer occur worldwide and 127,000 die from this disease (4).

OSCC is frequently associated with metastasis to draining lymph nodes, present in approximately 50% of patients at the time of diagnosis, and this event is correlated with poor prognosis and reduced survival rates (5). Current therapies are only marginally effective in inhibiting this process (1), as evidenced by a poor 5-year survival rate (approximately 50%) (6). Due to the location of the disease, multimodal tumor therapy, usually prescribed, leads to a reduction in quality of life, making the psychosocial consequences of OSCC greater than those of other malignancies (7).

Solid malignant tumors, such as OSCC, have the potential for rapid and unlimited growth (8). Furthermore, hypoxia is a common feature and contributes to local and systemic cancer progression, resistance to therapy and poor outcome (2, 9).

Hypoxia-inducible factor-1 (HIF1) is a key regulator of the cellular response to hypoxia (2, 8). It is a heterodimer composed of two sub-units: HIF1α and HIF1β (8, 10, 11) (Figure 1). HIF1α functions as a transcriptional activator in hypoxia and binds specifically to the promoters or enhancers of more than 100 genes involved in multiple aspects of tumour biology (8, 11-14) (Figure 2). Among these genes, vascular endothelial growth factor (VEGF) is vital as a regulatory gene of angiogenesis in the adaptation to a hypoxic environment (15).

Experimental evidence has demonstrated the correlation between HIF1α and VEGF in tongue squamous cell carcinomas (SCCs) (15). Additionally, patients with no or weak expression of HIF1α had higher survival rates than those with moderate or high expression of HIF1α (15).

The purpose of the present article is to review the hypoxic condition in OSCC and its correlation with prognosis.
Pathogenesis of Hypoxia and Its Correlation with Cancer Progression

OSCC is a locally aggressive tumour and presents with extensive necrotic areas, in which the acidity and hypoxic levels are very high (16). Data obtained in the past two decades have shown that 50% to 60% of locally advanced solid tumours exhibit hypoxic or anoxic areas (12, 16) that result from an imbalance between the supply and consumption of oxygen (2, 12).

Under normal conditions, oxygen supply matches the metabolic requirements, whereas in malignances, consumption may exceed the supply and result in tissue areas with very low levels of oxygen (12, 17). The development of these regions involves several factors, such as chaotic vasculature, irregular blood flow, structural and functional abnormalities of tumor microvessels, poor oxygen diffusion across an ever-expanding tumor, regions of necrosis, and therapy-induced anaemia leading to reduced O₂ transport capacity of the blood (anaemic hypoxia) (12, 18).

A hypoxic microenvironment plays a critical role in tumour development and progression, as shown in different types of malignances (18). Specifically, in malignant neoplasias of the head and neck region, hypoxia is correlated with a worse prognosis in patients with advanced status who undergo radiation therapy as initial treatment and may be a predictor of the overall survival and disease-free rates (2, 12).

The exposure of cells to hypoxia leads to restricted proliferation and subsequent cell death due to a reduction in overall protein synthesis. Sustained hypoxia can also change
the cell-cycle distribution and the relative number of quiescent cells (12). Hypoxia-induced cell-cycle arrest at the G1/S checkpoint may be triggered by HIF1α-mediated activation of the cyclin-dependent kinase inhibitors p21 and p27 (19).

Hypoxia can induce apoptosis both in normal and neoplastic cells (12). An increased level of p53 under hypoxic conditions may lead to the alternative activation of apoptosis by the downstream effectors apoptotic protease-activating factor-1 (APAF1) and caspase-9 (20) (Figure 3). However, hypoxia also initiates p53-independent apoptosis pathways, including those involving genes of the B-cell lymphoma 2 (BCL2) family (12) (Figure 3). Because hypoxic cells are already below a critical energy state, hypoxia may result in necrotic cell death (12, 21).

The development of tumor aggressiveness is generally associated with the occurrence of mutations in oncogenes or tumour-suppressor genes (21). Hypoxia promotes genomic instability by increasing the number of mutations, and it simultaneously exerts strong selection pressure (22). Tumour cell variants with proteomic and genomic adaptations favourable for survival under hypoxic conditions may have growth advantages over non-adapted cells and expand through clonal selection. The expansion of these clones further intensifies tumor hypoxia, establishing a vicious cycle of increasing hypoxia, malignant progression and treatment resistance (21).

Hypoxia has the potential to regulate cell differentiation by facilitating the maintenance and evolution of cancer stem cell characteristics, thus allowing tumour cells with self-renewal potential to accumulate a multitude of genetic and epigenetic changes over a long period of time and become increasingly malignant (23).

Additionally, some authors have suggested that the hypoxic tumor microenvironment plays an important role in the expression of matrix metalloproteinases (MMPs) (24). Another study reported that silencing of chemokine receptor CXCR4 significantly reduced the mRNA and protein levels of both MMP9 and MMP13, thus modulating
the expression of MMP9 and MMP13 via activation of the extracellular signal-regulated kinases (ERK) signaling pathway (25).

**HIF1 System**

HIF1 was initially identified because of its response to low O2 concentrations, but it is now apparent that HIF1 can be regulated by other factors such as oncogene activation such as Ras (RAS), Src (SRC) and phosphoinositide 3-kinase (PI3K), or loss of tumor suppressors such as von Hippel–Lindau (VHL) or Phosphatase and Tensin Homolog (PTEN), even under normoxic conditions. Furthermore, increased levels of metabolites such as succinate and fumarate, or oxygen by-products such as free radicals, in a tumour can also stabilize HIF1α (17).

Numerous molecular, biological and clinicopathological studies have elucidated the hypoxia-inducible system in tumor biology (26). Out of all proteins induced by hypoxic conditions, HIFs and their downstream target genes have been studied most extensively (12, 17, 18). HIFs include HIF1α, HIF1β, HIF2α and HIF3α. Of these, the most important is HIF1α because it is responsible for activating hypoxia-related transcriptional responses (11, 16, 17).

HIF1 is a basic, helix-loop-helix- period-ARNT-single-minded (PAS) heterodimer composed of HIF1α and HIF1β subunits (11, 14, 17) (Figure 1). HIF1β, also called arylhydrocarbon receptor nuclear translocator, is a constitutively expressed 91-94 kDa protein. HIF1α is an oxygen-regulated subunit of HIF1 and encodes for a gene located on chromosome 14 (14q21-q24) (27).

At physiological concentrations of oxygen, HIF is hydroxylated on proline residues 402 (Pro-402) and 564 (Pro-564) by prolyl hydroxylase domain protein 2 (PHD2) and other prolyl hydroxylases. Such hydroxylated HIF1α is bound by the pVHL and rapidly destroyed by an E3-ubiquitin ligase complex (9, 11, 17).

Under hypoxic conditions, the hydroxylation reactions are inhibited and HIF1α is stabilized, translocated into the nucleus and dimerises with HIF1β to form HIF1, which is responsible for the activation of several target genes involved in physiological and pathological processes (2, 11, 16, 27) (Figure 1). These include genes coded by vascular endothelial growth factor (VEGF), erythropoietin, carbonic anhydrase IX (CAIX) and several enzymes involved in the metabolism of glucose, iron and nucleotides (2, 9, 11, 16, 21, 22) (Figure 2).

Han et al. in order to determine whether 2-[18F]fluoro-2-deoxy-D-glucose positron-emission tomography (FDG-PET) could serve as a useful technique predicting tumour hypoxia and prognosis in tongue cancer, assessed the relationship between FDG uptake and the levels of hypoxia-related markers (28). They observed strong correlations between maximum standardized uptake values (SUVmax) and expression of HIF1α, CAIX and facilitative glucose transporters-1 (GLUT1). In addition, SUVmax, HIF1α expression and tumour grade were significant independent predictors of disease-free survival. The authors suggested that SUVmax may be a good non-invasive biomarker for prediction of hypoxic status and prognosis of patients with T2 tongue OSCC.

A recent study also assessed the relationship between FDG-PET and 18F-fluoromisonidazole (18F-FMISO) PET with the levels of HIF1α in OSCC (29). The 18F-FMISO PET SUVmax was significantly higher in HIF1α-positive cases, however, the authors found no significant correlations between 18F-FDG PET SUVmax and expression of HIF1α. They concluded that the uptake of 18F-FMISO at the primary site of OSCC may indicate a hypoxic environment with expression of HIF1α.

**Expression of HIF1α in OSCC**

The expression of HIF1α is an early event in oral carcinogenesis (9). An interesting study has showed a stepwise and significant increase in the expression of nuclear HIF1α from normal oral mucosa to oral epithelial dysplasia (OED) and from OED to OSCC (30). Additionally, tobacco and alcohol can enhance the expression of HIF1α either directly or indirectly (31, 32). Corroborating these results, Lin et al. found higher mean HIF1α labelling indices in OSCC in drinkers and smokers than in OSCC in non-drinkers and non-smokers (8).

Nuclear HIF1α labelling indices have been shown to have utility as biomarkers for predicting progression and providing an accurate prognosis for patients with OSCC (8, 9). Several studies have shown a significant correlation between HIF1α expression and a poor prognosis (6, 8, 33-36). Zhu et al. reported that HIF1α was significantly associated with T stage, lymph node involvement, histologic differentiation and microvessel density (36). Patients with positive HIF1α nuclear staining had a significantly worse overall survival and disease-free survival.

Kang et al. also found that HIF1α overexpression was significantly associated with poor overall and disease-free survival rates, independent of T stage and lymphatic metastasis (15). The Cox proportional hazards regression model demonstrated that the level of HIF1α expression may be an independent prognostic factor for tongue SCC.

Eckert et al. demonstrated that increased HIF1α expression, alone or in combination with a low CAIX expression, was significantly correlated with a poor prognosis of OSCC (6). Based on these results, the authors suggested a reconsideration of treatment options for patients with poorly differentiated and locally advanced tumours and with low expression of HIF1α and CAIX. Treatment of these patients may require a complete resection of the tumour and the primary lymph node station but would not require adjuvant chemo/radiotherapy.
In contrast, Fillies et al. demonstrated that the expression of HIF1α was correlated with an improved 5-year survival rate and increased disease-free period (26). Similarly, dos Santos et al. showed a correlation between strong HIF1α protein expression and disease-free or local disease-free survival for patients that undertook postoperative radiotherapy (37). Interestingly, surgery-only cases did not show any correlation between HIF1α protein expression and disease-free or local disease-free survival.

**VEGF Inducibility by Hypoxia in OSCC**

VEGF is regarded as the major angiogenesis factor during epithelial carcinogenesis and in tumour metastases (12, 38, 39). It is a dimeric multifunctional glycoprotein with a molecular mass of 34-42 kDa. The human VEGF gene family consists of five members, VEGFA, VEGFB, VEGFC, VEGFD and placental growth factor (PIGF) (9).

Hypoxia has been shown to be an important stimulus for new blood vessel formation (9, 35). This condition triggers the accumulation of HIF1α, which in turn plays a major role in the transcriptional activation of genes encoding pro-angiogenesis factors, including VEGF (40, 41) (Figure 2). Deletion of the HIF1α gene or disruption of HIF1α transcription results in lack of VEGF secretion by tumour cells; suppress angiogenesis and retards solid tumor growth (42).

In accordance with these data, Shang et al. showed that VEGF production was continuously elevated in supernatants from oral cancer cell lines in response to a drop of oxygen level (43). Mohamed et al. also demonstrated that baseline

---

Figure 2. Inhibition of apoptosis. Growth factors interact with receptor tyrosine kinase (RTK) causing their phosphorylation and activation of both phosphoinositide 3-kinase (PI3K) and RAS sarcoma (RAS) proteins. Activated PI3K phosphorylates phosphatidylinositol biphosphate (PIP2), forming phosphatidylinositol trisphosphate (PIP3), which leads to activation of v-akt marine thymoma viral oncogene homolog (AKT) protein kinase. AKT inhibits the BCL2-associated death promoter (BAD) protein, causing an increase in the levels of antiapoptotic B-cell lymphoma 2 (BCL2) and B-cell lymphoma-extra large (BCL-XL), and inhibition of cytochrome c release, resulting in the inactivation of caspases 9 and 3. Beyond that, activated RAS phosphorlylates extracellular signal-regulated kinase (ERK), by activating it. This reduces the levels of pro-apoptotic BCL2-associated X protein (BAX) and Bcl-2 homologous antagonist/killer (BAK), contributing to the inhibition of apoptosis.
levels of VEGF and HIF1α expression were elevated at the transcription and translation levels in oral cancer cell lines as compared to normal keratinocytes (35).

Several studies have analyzed members of the VEGF family (44-48), but each component is related to different characteristics of OSCC, such as survival (49, 50), stage (44-46), severity (45) and tumour metastasis (46-48). In particular, VEGFC plays a pivotal role in tumour growth and metastasis. However, the role of HIF1α as a regulator of VEGFC is still poorly-understood in OSCC (41).

Huang et al. investigated HIF1α-induced VEGF expression and angiogenesis in tongue SCC (41). HIF1α overexpression was correlated with VEGF overexpression, higher lymphatic vessel density, and regional lymph nodal involvement. Based on these results, the authors suggested that HIF1α might play a crucial role in regional lymph node metastasis as a regulator of lymphangiogenesis and angiogenesis with a possibly novel pathway involving VEGFC (51).

**HIF1-targeted Therapeutics**

A growing number of chemical compounds have been shown to block tumor xenograft growth and inhibit HIF activity through a wide variety of molecular mechanisms, including decreased HIF1α mRNA levels, decreased HIF1α protein synthesis, increased HIF1α degradation, decreased HIF subunit heterodimerization, decreased HIF binding to DNA and decreased HIF transcriptional activity (52).

Specifically for OSCC treatment, Kang et al. reported that a histone deacetylase inhibitor, trichostatin A (TSA), inhibited cell proliferation and invasion, blocked the cell cycle, and induced cell apoptosis of the human tongue SCC cell line in vitro (53). Furthermore, TSA reduced both basal levels of and HIF1α protein accumulation, and both protein and mRNA levels of VEGF expression. Based on these results, the authors suggested that TSA could be a promising drug targeting tumor angiogenesis via inhibition of HIF1α and VEGF expression.

Other interesting research was developed by Jung et al., who investigated anti-proliferative, growth-suppressive and pro-apoptotic effects of glucosamine-hydrochloride (GS-HCl) on YD-8 OSCC cells (54). Treatment with this drug strongly inhibited proliferation and induced apoptosis of YD-8 cells. Of further note, as measured by western analyses, GS-HCl treatment led to activation of caspase-3, cytosolic accumulation of cytochrome c, down-regulation of HIF1α and generation of reactive oxygen species (ROS) in YD-8 cells.

**VEGF-targeted Therapeutics**

Some anti-angiogenic agents have been developed and tried for the treatment of oral cancer (55, 56). Harada et al. reported that Cepharanthine, a biscoclaurine alkaloid extracted from *Stephania cepharantha* Hayata, inhibited expression of VEGF and interleukin-8 (IL8), in cultured cells and in cells implanted into the subcutaneous tissue of nude mice (55). In addition, decreased expression of VEGF and IL8 correlated with decreased tumor cell growth and decreased vascularization in vitro and in vivo.

Data from mouse studies suggest that gene therapy via a soluble form of VEGF receptor may be an effective oral cancer therapy that may be applicable in the clinic (57). Li et al. investigated the effects of a molecular blockade of VEGF receptor 2 by inhibiting several critical steps involved in angiogenesis (58). They concluded that molecular inhibition of VEGF receptor-2 alone and in combination with radiation can enhance tumor response through molecular-targeting of tumor vasculature.

**Conclusion**

HIF1α promotes the formation of new blood vessels by up-regulating VEGF. Alteration and overexpression of HIF1α and VEGF have been detected in OSCC and a positive association of both genes with lymphatic vessel density and the presence of lymph node metastases has been described. Even though tumours may be of clinically equivalent stage, head and neck SCC is a heterogeneous disease with distinct patterns of presentation, biological behaviour and responses to treatment. This suggests that additional genetic and molecular markers could be used to supplement TNM staging and the tumour grade.

Although further confirmation in experimental models is still necessary, all these findings suggest that the hypoxia HIF1α VEGF axis may represent an important non-invasive route for predicting the development of lymph node metastasis and prognosis, as well as being a potential target for anti-lymphangiogenic and anti-angiogenic therapies.

**References**

16 Perez-Sayans M, Suarez-Penaranda JM, Pilar GD, Barros- Denko NC: Hypoxia, HIF1 and glucose metabolism in the solid cancer.


