Review

Hypoxic Condition and Prognosis in Oral Squamous Cell Carcinoma

PRISCILA OLIVEIRA DE LIMA[1], CAMILA CASSANI JORGE[1],
DENISE TOSTES OLIVEIRA[2] and MICHELE CONCEIÇÃO PEREIRA[1]

[1]Department of Pathology, Federal University of São João Del Rei, Divinópolis, Minas Gerais, Brazil;
[2]Section of Pathology, Department of Stomatology,
Bauru School of Dentistry, University of São Paulo, Bauru, São Paulo, Brazil

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 HF1β 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

Correspondence to: Michele Conceição Pereira, Federal University of São João Del Rei, Campus Centro-Oeste Dona Lindu Avenida Sebastião Gonçalves Coelho, 400, Chanadour, CEP 35501-296, Divinópolis, Minas Gerais, Brazil. Tel: +55 213732150486, Fax: +55 213732211614, e-mail: michelepereira@ufsj.edu.br

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 adaption to a hypoxic microenvironment (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).

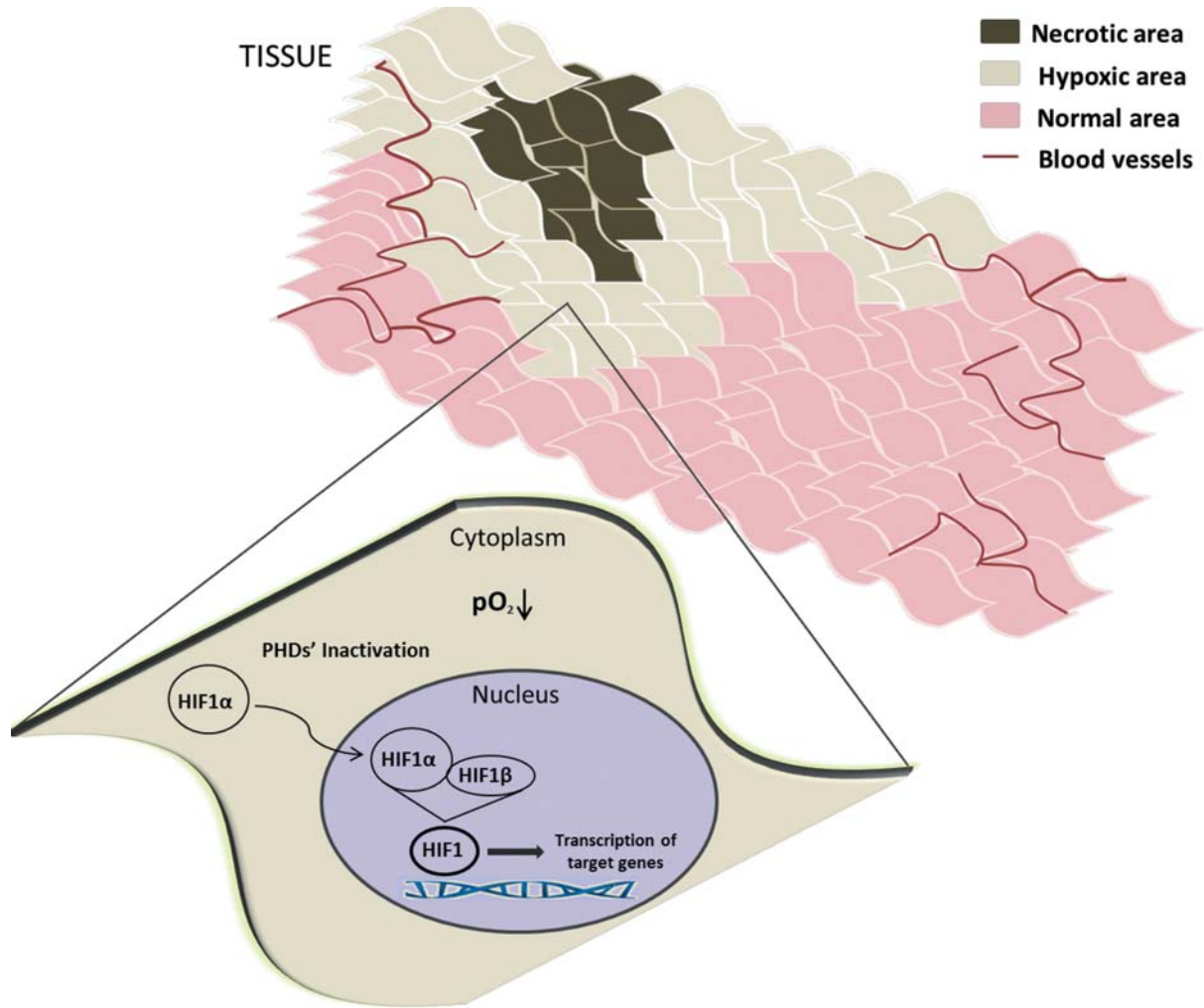The purpose of the present article is to review the hypoxic condition in OSCC and its correlation with prognosis.

Figure 1. *Formation of hypoxia-inducible factor-1 (HIF1) and activation of target genes. In areas of low pO2, the prolyl hydroxylases (PHDs) are inhibited, leading to an accumulation of HIF1α in the cell cytoplasm. HIF1α is translocated to the nucleus, where it dimerises with HIF1β to form HIF1, which is responsible for the activation of several target genes.*

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 O_2 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
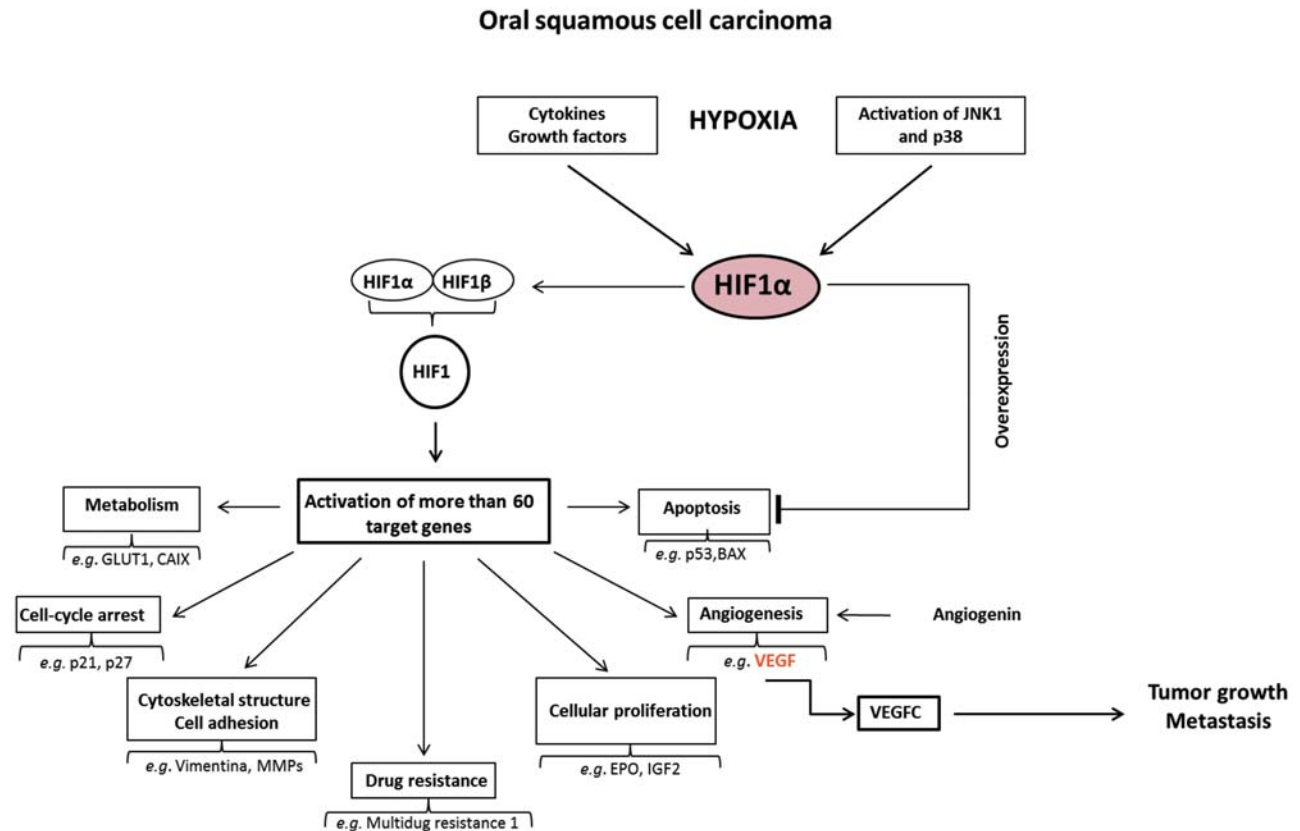
Oral squamous cell carcinoma

Figure 2. *The role of hypoxia-inducible factor 1α (HIF1α) in oral squamous cell carcinoma. Under hypoxia, cytokines and growth factors, as well as activated c-JUN NH2- terminal protein kinase (JNK) and p38 proteins stimulate the accumulation of HIF1α. The formation of HIF1, resulting from the accumulation of HIF1α and its consequent dimerization, leads to activation of more than 60 genes, including vascular endothelial growth factor (VEGF) and BCL2-associated X protein (BAX). VEGF expression and angiogenin stimulate angiogenesis. VEGFC expression leads to tumor growth and metastasis. Interestingly, the overexpression of HIF1α also prevents apoptosis of the cells.*

the cell-cycle distribution and the relative number of quiescent cells (12). Hypoxia-induced cell-cycle arrest at the G_1/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

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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 O_2 concentrations, but it is now apparent that HIF1 can be regulated by other factors such as oncogene activation such as Rat sarcoma (RAS), Sarcoma (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 hydrosylases. 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.
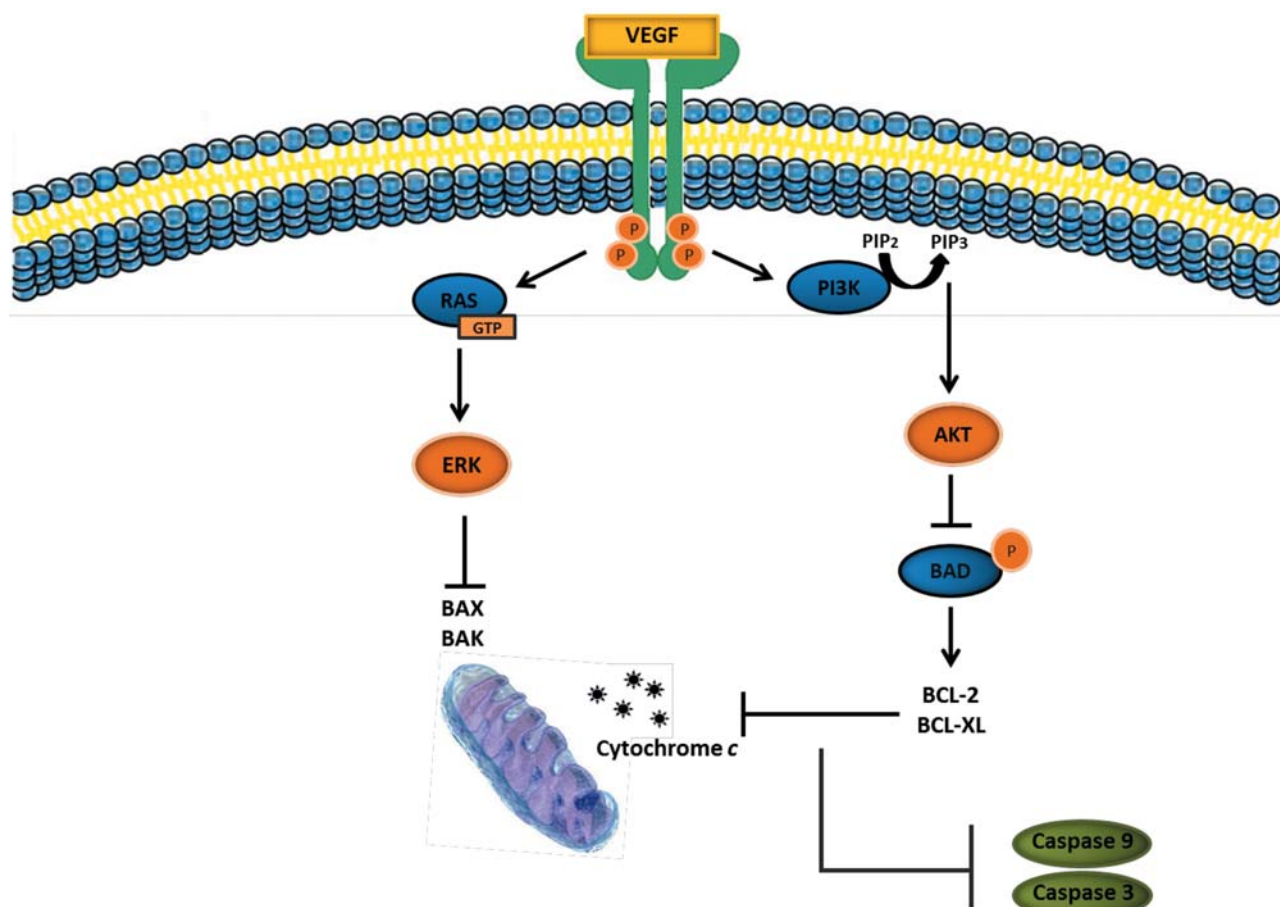
Figure 3. *Inhibition of apoptosis. Growth factors interact with receptor tyrosine kinase (RTK) causing their phosphorylation and activation of both phosphoinositide 3-kinase (PI3K) and Rat sarcoma (RAS) proteins. Activated PI3K phosphorylates phosphatidylinositol biphosphate (PIP2), forming phosphatidylinositol trisphosphate (PIP3), which leads to activation of v-akt murine 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 phosphorylates 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.*

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 responde to a drop of oxygen level (43). Mohamed *et al*. also demonstrated that baseline

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 VEGFC 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

1 Bock JM, Sinclair LL, Bedford NS, Jackson RE, Lee JH and Trask DK: Modulation of cellular invasion by VEGF-C expression in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg *134*: 355-362, 2008.

2 Brennan PA, Mackenzie N and Quintero M: Hypoxia-inducible factor 1α in oral cancer. J Oral Pathol Med *34*: 385-389, 2005.

3 Ministério da Saúde, Instituto Nacional de Câncer: Câncer da cavidade oral. *In*: Estimativa 2012: Incidência de Câncer no Brasil. Rio de Janeiro: Instituto Nacional de Câncer, pp. 40-41, 2011.

4 World Health Organization: International Classification of Diseases. Geneva, 2007.

5 Kim SY, Nam SY, Choi SH, Cho KJ and Roh JL: Prognostic value of lymph node density in node-positive patients with oral squamous cell carcinoma. Ann Surg Oncol *18*: 2310-2317, 2011.

6 Eckert AW, Lautner MH, Schutze A, Bolte K, Bache M, Kappler M, Schubert J, Taubert H and Bilkenroth U: Co-expression of HIF1α and CAIX is associated with poor prognosis in oral squamous cell carcinoma patients. J Oral Pathol Med *39*: 313-317, 2010.

7 Oliveira LR and Ribeiro-Silva A: Prognostic significance of immunohistochemical biomarkers in oral squamous cell carcinoma. Int J Oral Maxillofac Surg *40*: 298-307, 2011.

8 Lin PY, Yu CH, Wang JT, Chen HH, Cheng SJ, Kuo MY and Chiang CP: Expression of hypoxia-inducible factor-1 alpha is significantly associated with the progression and prognosis of oral squamous cell carcinomas in Taiwan. J Oral Pathol Med *37*: 18-25, 2008.

9 Eckert AW, Kappler M, Schubert J and Taubert H: Correlation of expression of hypoxia-related proteins with prognosis in oral squamous cell carcinoma patients. Oral Maxillofac Surg *16*: 189-196, 2012.

10 Eckert AW, Lautner MH, Schutze A, Taubert H, Schubert J and Bilkenroth U: Coexpression of hypoxia-inducible factor-1α and glucose transporter-1 is associated with poor prognosis in oral squamous cell carcinoma patients. Histopathology *58*: 1136-1147, 2011.

11 Semenza GL: Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. Annu Rev Pathol *7*: 7, 2013.

12 Vaupel P and Mayer A: Hypoxia in cancer: Significance and impact on clinical outcome. Cancer Metastasis Rev *26*: 225-239, 2007.

13 Liang X, Zheng M, Jiang J, Zhu G, Yang J and Tang Y: Hypoxia-inducible factor-1 alpha, in association with TWIST2 and SNIP1, is a critical prognostic factor in patients with tongue squamous cell carcinoma. Oral Oncol *47*: 92-97, 2011.

14 Liang X, Yang D, Hu J, Hao X, Gao J and Mao Z: Hypoxia inducible factor-α expression correlates with vascular endothelial growth factor-C expression and lymphangiogenesis/angiogenesis in oral squamous cell carcinoma. Anticancer Res *28*: 1659-1666, 2008.

15 Kang FW, Gao Y, Que L, Sun J and Wang ZL: Hypoxia-inducible factor-1α overexpression indicates poor clinical outcomes in tongue squamous cell carcinoma. Exp Ther Med *5*: 112-118, 2013.

16 Perez-Sayans M, Suarez-Penaranda JM, Pilar GD, Barros-Angueira F, Gandara-Rey JM and Garcia-Garcia A: Hypoxia-inducible factors in OSCC. Cancer Lett *313*: 1-8, 2011.

17 Denko NC: Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat Rev Cancer *8*: 705-713, 2008.

18 Jiang J, Tang YL and Liang XH: EMT: A new vision of hypoxia promoting cancer progression. Cancer Biol Ther *11*: 714-723, 2011.

19 Goda N, Ryan HE, Khadivi B, McNulty W, Rickert RC and Johnson RS: Hypoxia-inducible factor 1α is essential for cell cycle arrest during hypoxia. Mol Cell Biol *23*: 359-369, 2003.

20 Soengas MS, Alarcon RM, Yoshida H, Giaccia AJ, Hakem R, Mak TW and Lowe SW: APAF1 and caspase-9 in p53-dependent apoptosis and tumor inhibition. Science *284*: 156-159, 1999.

21 Vaupel P and Harrison L: Tumor hypoxia: Causative factors, compensatory mechanisms, and cellular response. Oncologist *5*: 4-9, 2004.

22 Hockel M and Vaupel P: Tumor hypoxia: Definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst *93*: 266-276, 2001.

23 Lin Q and Yun Z: Impact of the hypoxic tumor microenvironment on the regulation of cancer stem cell characteristics. Cancer Biol Ther *9*: 949-956, 2010.

24 Miyazaki Y, Hara A, Kato K, Oyama T, Yamada Y, Mori H and Shibata T: The effect of hypoxic microenvironment on matrix metalloproteinase expression in xenografts of human oral squamous cell carcinoma. Int J Oncol *32*: 145-151, 2008.

25 Yu T, Wu Y, Helman JI, Wen Y, Wang C and Li L: CXCR4 promotes oral squamous cell carcinoma migration and invasion through inducing expression of MMP9 and MMP13 *via* the ERK signaling pathway. Mol Cancer Res *9*: 161-172, 2011.

26 Fillies T, Werkmeister R, van Diest PJ, Brandt B, Joos U and Buerger H: HIF1-α overexpression indicates a good prognosis in early stage squamous cell carcinomas of the oral floor. BMC Cancer *5*: 84, 2005.

27 Ryu MH, Park HM, Chung J, Lee CH and Park HR: Hypoxia-inducible factor-1α mediates oral squamous cell carcinoma invasion *via* up-regulation of α5 integrin and fibronectin. Biochem Biophys Res Commun *393*: 11-15, 2010.

28 Han MW, Lee HJ, Cho KJ, Kim JS, Roh JL, Choi SH, Nam SY and Kim SY: Role of FDG-PET as a biological marker for predicting the hypoxic status of tongue cancer. Head Neck *34*: 1395-1402, 2012.

29 Sato J, Kitagawa Y, Yamazaki Y, Hata H, Okamoto S, Shiga T, Shindoh M, Kuge Y and Tamaki N: ^{18}F-fluoromisonidazole PET uptake is correlated with hypoxia-inducible factor-1α expression in oral squamous cell carcinoma. J Nucl Med *54*: 1060-1065, 2013.

30 Lin PY, Yu CH, Wang JT, Chen HH, Cheng SJ, Kuo MYP and Chiang CP: Expression of hypoxia-inducible factor-1 alpha is significantly associated with the progression and prognosis of oral squamous cell carcinomas in Taiwan. J Oral Pathol Med *37*: 18-25, 2008.

31 Li L, Chen SH, Zhang Y, Yu CH, Li SD and Li YM: Is the hypoxia-inducible factor-1α mRNA expression activated by ethanol-induced injury the mechanism underlying alcoholic liver disease? Hepatobiliary Pancreat Dis Int *5*: 560-563, 2006.

32 Michaud SE, Menard C, Guy LG, Gennaro G and Rivard A: Inhibition of hypoxia-induced angiogenesis by cigarette smoke exposure: impairment of the HIF-1α/VEGF pathway. FASEB J *17*: 1150-1152, 2003.

33 Uehara M, Sano K, Ikeda H, Nonaka M and Asahina I: Hypoxia-inducible factor 1 alpha in oral squamous cell carcinoma and its relation to prognosis. Oral Oncol *45*: 241-246, 2009.

34 Roh JL, Cho KJ, Kwon GY, Ryu CH, Chang HW, Choi SH, Nam SY and Kim SY: The prognostic value of hypoxia markers in T2-staged oral tongue cancer. Oral Oncol *45*: 63-68, 2009.

35 Mohamed KM, Le A, Duong H, Wu Y, Zhang Q and Messadi DV: Correlation between VEGF and HIF-1α expression in human oral squamous cell carcinoma. Exp Mol Pathol *76*: 143-152, 2004.

36 Zhu GQ, Tang YL, Li L, Zheng M, Jiang J, Li XY, Chen SX and Liang XH: Hypoxia-inducible factor 1α and hypoxia-inducible factor 2α play distinct and functionally overlapping roles in oral squamous cell carcinoma. Clin Cancer Res *16*: 4732-4741, 2010.

37 dos Santos M, Mercante AM, Louro ID, Goncalves AJ, de Carvalho MB, da Silva EH and da Silva AM: HIF1α expression predicts survival of patients with squamous cell carcinoma of the oral cavity. PLoS One *7*: 18, 2012.

38 Lopez de Cicco R, Watson JC, Bassi DE, Litwin S and Klein-Szanto AJ: Simultaneous expression of furin and vascular endothelial growth factor in human oral tongue squamous cell carcinoma progression. Clin Cancer Res *10*: 4480-4488, 2004.

39 Margaritescu C, Pirici D, Stinga A, Simionescu C, Raica M, Mogoanta L, Stepan A and Ribatti D: VEGF expression and angiogenesis in oral squamous cell carcinoma: An immunohisto-chemical and morphometric study. Clin Exp Med *10*: 209-214, 2010.

40 Lin KT, Lien JC, Chung CH, Kuo SC and Huang TF: Bp5250 inhibits vascular endothelial growth factor-induced angiogenesis and HIF-1α expression on endothelial cells. Naunyn Schmiedebergs Arch Pharmacol *385*: 39-49, 2012.

41 Huang C, Sun Z, Sun Y, Chen X, Zhu X, Fan C, Liu B, Zhao Y and Zhang W: Association of increased ligand cyclophilin A and receptor CD147 with hypoxia, angiogenesis, metastasis and prognosis of tongue squamous cell carcinoma. Histopathology *60*: 793-803, 2012.

42 Semenza GL: Regulation of mammalian O_2 homeostasis by hypoxia-inducible factor 1. Annu Rev Cell Dev Biol *15*: 551-578, 1999.

43 Shang ZJ, Li ZB and Li JR: 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 *35*: 533-538, 2006.

44 Faratzis G, Tsiambas E, Rapidis AD, Machaira A, Xiromeritis K and Patsouris E: VEGF and Ki-67 expression in squamous cell carcinoma of the tongue: An immunohistochemical and computerized image analysis study. Oral Oncol *45*: 584-588, 2009.

45 Shang ZJ, Li JR and Li ZB: Up-regulation of serum and tissue vascular endothelial growth factor correlates with angiogenesis and prognosis of oral squamous cell carcinoma. J Oral Maxillofac Surg *65*: 17-21, 2007.

46 Naruse T, Kawasaki G, Yanamoto S, Mizuno A and Umeda M: Immunohistochemical study of VEGF expression in oral squamous cell carcinomas: Correlation with the mTOR-HIF-1α pathway. Anticancer Res *31*: 4429-4437, 2011.

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

48 Siriwardena BS, Kudo Y, Ogawa I, Udagama MN, Tilakaratne WM and Takata T: VEGF-C is associated with lymphatic status and invasion in oral cancer. J Clin Pathol *61*: 103-108, 2008.

49 Maeda T, Matsumura S, Hiranuma H, Jikko A, Furukawa S, Ishida T and 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 *51*: 771-775, 1998.

50 Smith BD, Smith GL, Carter D, Sasaki CT and Haffty BG: Prognostic significance of vascular endothelial growth factor protein levels in oral and oropharyngeal squamous cell carcinoma. J Clin Oncol *18*: 2046-2052, 2000.

51 Liang XH, Zheng M, Jiang JA, Zhu GQ, Yang J and Tang YL: Hypoxia-inducible factor-1 alpha, in association with TWIST2 and SNIP1, is a critical prognostic factor in patients with tongue squamous cell carcinoma. Oral Oncol *47*: 92-97, 2011.

52 Semenza GL: Hypoxia-inducible factors: Mediators of cancer progression and targets for cancer therapy. Trends Pharmacol Sci *33*: 207-214, 2012.

53 Kang FW, Que L, Wu M, Wang ZL and Sun J: Effects of trichostatin A on HIF-1α and VEGF expression in human tongue squamous cell carcinoma cells *in vitro*. Oncol Rep *28*: 193-199, 2012.

54 Jung CW, Jo JR, Lee SH, Park YK, Jung NK, Song DK, Bae J, Nam KY, Ha JS, Park IS, Park GY, Jang BC and Park JW: Anti-cancer properties of glucosamine-hydrochloride in YD-8 human oral cancer cells: Induction of the caspase-dependent apoptosis and down-regulation of HIF-1α. Toxicol In Vitro *26*: 42-50, 2012.

55 Harada K, Ferdous T, Itashiki Y, Takii M, Mano T, Mori Y and Ueyama Y: Cepharanthine inhibits angiogenesis and tumorigenicity of human oral squamous cell carcinoma cells by suppressing expression of vascular endothelial growth factor and interleukin-8. Int J Oncol *35*: 1025-1035, 2009.

56 Myoung H, Hong SD, Kim YY, Hong SP and Kim MJ: Evaluation of the antitumor and antiangiogenic effect of paclitaxel and thalidomide on the xenotransplanted oral squamous cell carcinoma. Cancer Lett *163*: 191-200, 2001.

57 Okada Y, Ueno H, Katagiri M, Oneyama T, Shimomura K, Sakurai S, Mataga I, Moride M and Hasegawa H: Experimental study of antiangiogenic gene therapy targeting VEGF in oral cancer. Odontology *98*: 52-59, 2010.

58 Li J, Huang S, Armstrong EA, Fowler JF and Harari PM: Angiogenesis and radiation response modulation after vascular endothelial growth factor receptor-2 (VEGFR2) blockade. Int J Radiat Oncol Biol Phys *62*: 1477-1485, 2005.