Hypoxia in oral squamous cell carcinoma: A breath of fresh
air in cancer research?

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Abstract

It is an established fact that solid tumors are deprived of oxygen, to varying extents due to aberrant vascular function. The hypoxia caused, influences the properties of tumors from proliferation and angiogenesis to radiation and chemotherapy resistance. This paper is an attempt to review the explosion in research on hypoxia inducible factor in oral squamous cell carcinoma.

Keywords:
Endogenous marker, hypoxia inducible factor, hypoxia, oral squamous cell carcinoma, prognosis

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Received: 13 July 2015; Accepted: 27 August 2015
doi: 10.15713/ins.jmrps.30

Introduction

Oral squamous cell carcinoma (OSCC) by virtue of affecting easily visible and accessible sites should have been diagnosed and treated early with less morbidity and mortality. Unfortunately, despite leaps and bounds of advancements in science, the prognosis remains far from satisfactory. However, the quest to explore the unexplored continues so as to find new prognostic and predictive factors that would allow tailoring of surgical and adjuvant therapy.[1] There are several biomarkers that have been investigated for the prognostication of this unpredictable disease. The umbrella term “biomarker” or “prognostic/predictive marker” summarizes proteins involved in tumor progression and metastasis. The core of such research begins with an understanding of some of the adaptive processes/pathways, which are seen in establishment and progression of tumors. Currently, one of the finest pathways under investigation is tumor hypoxia.[2]

What is Tumor Hypoxia and How is it Caused?

Normally, oxygen supply is on par with the metabolic requirements in tissues. However in malignancies, consumption exceeds the supply and results in tissue areas showing hypoxia.[3] This can be due to a number of factors:

Perfusion-related (acute) hypoxia
Lack of blood supply resulting in ischemic hypoxia is often transient. It is caused due to abnormalities in the tumor microvasculatures such as disorganized vascular network, dilations, elongated and tortuous shape, incomplete endothelial lining, lack of physiological/pharmacological receptors, absence of flow regulation, and intermittent stasis.[4]

Diffusion-related (chronic) hypoxia
Diffusion-related (chronic) hypoxia caused by an increase in diffusion distances with tumor expansion resulting in inadequate oxygen supply for cells distant (>70 μm) from the nutritive blood vessels.[4]

Anemic hypoxia
Tumor-associated or therapy-induced anemia results in hypoxia which is exaggerated when hemoglobin levels are below 10-12 g/dl, especially when it coincides with a low perfusion rate.[4] Contradicting this no association between Hb-levels and upregulation of hypoxia regulated proteins or vascular endothelial growth factor (VEGF) was seen in endometrial adenocarcinomas and head and neck tumors.[5]
Role of Tumor Hypoxia in Tumor Progression

Within the microenvironment of a developing tumor, decreased vascular supply and increased energy demand to maintain proliferation, results in the formation of hypoxic regions. Such a hypoxic stress enables a complex gene expression program. Hypoxia inducible factor-1 (HIF-1) is a master transcriptional regulator of genes regulating oxygen homeostasis. HIF mediates proteomic and genomic adaptations in tumor cells favorable for survival under hypoxic conditions. These cells proliferate through clonal expansion and have a distinct advantage over the non-adapted cells as the clonal expansion intensifies tumor hypoxia, launching a vicious cycle of increasing hypoxia, advancing malignancy and resistance to various treatment modalities. Thus, persistent hypoxia leads to selection of genotypes promoting tumor angiogenesis, epithelial-to-mesenchymal transition (EMT), invasiveness and metastasis, as well as suppressing immune reactivity.

Assessing Tumor Hypoxia

Most widely used method for direct measurement of PO2 in human cancers is direct polarographic (needle-electrode) measurements. Its main drawback is that it cannot distinguish necrosis with low O2 from severe hypoxia in viable tumor areas.

Exogenous and Endogenous Markers

Exogenous markers include drugs, chemicals, or even bacteria that, after intravenous administration specifically accumulate or are bio reducible under hypoxic conditions. Clinically relevant markers are 2-nitroimidazoles, pimonidazole, and EF5.

Less invasive methods with repetitive assessment capacity allowing visualization of whole tumor include radiologic and nuclear medicine imaging techniques such as positron emission tomography (PET) imaging using specific tracers or dynamic contrast-enhanced magnetic resonance imaging. Although glucose utilization is indirectly related to the proliferative activity, and the oxygenation status of the tumor, 18F-fluorodeoxyglucose uptake at best correlates weakly to hypoxia, advancing malignancy and resistance to various treatment modalities. Thus, persistent hypoxia leads to selection of genotypes promoting tumor angiogenesis, epithelial-to-mesenchymal transition (EMT), invasiveness and metastasis, as well as suppressing immune reactivity.

HIF Biology

HIF-1 is an essential mediator of the transcriptional responses of tumors under hypoxia. This heterodimeric transcription factor is composed of α and β subunits. The expression of α subunit is regulated by the oxygen level while β subunit is constitutively expressed. HIF-1α gene is located on chromosome 14 (14q24-q24). In addition to HIF-1α and β, two other proteins have been identified. These are additional α isoforms termed HIF-2α and HIF-3α. The HIF-2α is closely related to HIF-1α, and both are able to interact with hypoxia response elements to upregulate transcriptional activity. By contrast HIF-3α down regulates hypoxic response via an alternatively spliced transcription factor, which inhibits HIF-1α.

At physiological concentrations of oxygen, HIF is hydroxylated on proline residues 402 and 564 by prolyl hydroxylase domain protein 2 (PHD2) and other prolyl hydroxylases. This hydroxylated HIF-1α is bound by the tumor suppressor pVHL (von Hippel–Lindau) and rapidly destroyed by an E3-ubiquitin ligase complex. In hypoxic conditions, hydroxylation of HIF-1α is inhibited and HIF-1α gets stabilized. Following the stabilization, it is translocated into the nucleus and dimerises with HIF-1β to form HIF-1. This activates several target genes involved in various physiological and pathological processes. In addition to the inhibition of PHDs, as the primary mechanism of HIF-1α stabilization, growth factors, and cytokines can bring forth an increase in HIF-1α synthesis. Furthermore, some iron chelator components, seem to stabilize HIF-1α probably through a reaction of free radicals that act directly on the protein or through PHDs.

HIF-1α up-regulates the transcription of more than 100 genes involved in adaptation to tumor microenvironment, e.g. glucose transport, pH stabilization, angiogenesis, and stem cell properties. Some of the approved HIF-α target genes that are potentially correlated with different aspects of tumor biology are VEGF, CA-IX, urokinase-type plasminogen activator receptor and several enzymes involved in the glucose and iron metabolism.

Tumor Hypoxia and OSCC

HIF-1α overexpression has been reported in a number of human cancers, including colon, breast, stomach, pancreas, prostate, kidney, esophagus, and head and neck. The normal mucosa adjacent to tumor in head and neck squamous cell carcinomas (HNSCCs) also showed overexpression of HIFs. The increasing size of the tumor can cause increased interstitial pressure in the adjacent mucosa and increased metabolic demand by the tumor causes shifting and accumulation of metabolic products in tumor tissue causing hypoxia in the adjacent apparent normal mucosa.

The prognostic relevance of HIF-1α in tumors derived from squamous epithelium is controversial. Most cancers over-expressing HIF-1α are associated with increased mortality. Studies using oral cancer cell lines have found that HIF-1 overexpression is sufficient to confer target genes expression essential for tumor proliferation, invasiveness, and survival. Among the hypoxic markers, HIF-1α has been studied commonly either alone or mainly with other markers like HIF-2α, CA-IX, GLUT-1 and
VEGF. Increased HIF-1α expression has been found to be significantly correlated with clinicopathological variables such as clinical stage, histopathological grade, and lymph node involvement in many studies, with correlation with only clinical stage and lymph node involvement in some, and even in some with recurrences. The expression of HIF-1α was localized to the nucleus and was distributed heterogeneously throughout the tumor area especially in the perinecrotic regions, and at the tumor/stroma interface. The heterogeneous expression is due to variations in the flow of blood, increased oxygen consumption and variations in the oxygen gradients within the tumor. In contrast, Fillies et al. found the distribution to be homogeneous in squamous cell carcinoma of the floor of the mouth. Over-expressed HIF-1α was significantly associated with worse prognosis of HNSCCs in Asian countries. In addition, HIF-1α overexpression was significantly associated with worse overall survival in oral carcinoma, nasopharyngeal carcinoma and oropharynx carcinoma, but not in laryngeal carcinoma.

Contradicting the above findings were Beasley et al. who observed increased HIF-1α and improved prognosis in a group of 79 surgically treated patients with HNSCC. Fillies et al. too showed an improved 5 years survival and improved disease-free period in 85 cases of squamous cell carcinoma of floor of the mouth. Similarly, dos Santos et al. showed an association between strong HIF-1α expression and disease-free survival in patients who underwent post-operative radiotherapy.

Some studies showed no correlation between HIF-1α levels and tumor stage or grade, but in some, tumor thickness, nodal involvement, or resection margin status.

The discrepancy may relate to differences in scoring categories and influence of other factors, which may determine whether HIF-1α acts as negative or positive regulator of cell survival. Thus, the function of HIF-1α may depend on the biological context of cancer cell and tumor microenvironment.

**Downstream Targets of HIF**

**Related to angiogenesis and lymphangiogenesis**

Biochemical pathway between tumor hypoxia and neoangiogenesis is known to occur. The levels of VEGF and HIF-1α expression at baseline were increased at the transcriptional and translational levels in the cancer cell lines as compared to normal keratinocytes. Both HIF-1α and HIF-2α correlated with VEGF and microvessel density in OSCC specimens and thus play a role in angiogenesis. HIF-1α might play a role in lymphatic metastasis via the regulation of VEGF-C. HIF-1α expression was significantly correlated with elevated peritumoral and intratumoral lymphatic density, nodal metastases and tumor node metastasis classification. Ephrin, angiogenin, apelin are some of the other angiogenic factors up-regulated by hypoxia and significantly correlated with tumor recurrence, independent prognostic factor for disease-free survival, lymph node metastasis.

**Relation to glucose metabolism**

The largest functional group of genes consistently regulated by HIF-1α is the genes associated with glucose metabolism. HIF-1α increases glucose uptake by transcriptional activation of glucose transporters GLUT-1 and GLUT-3. GLUT-1 upregulation in response to hypoxia has been found in multiple cell and tissue types. In patients where the tumor had increased levels of both HIF-1α and GLUT-1, there was a 5.13 fold increased risk of tumor-related deaths. HIF and GLUT proteins within tongue squamous cell carcinoma (TSCC) appear to be associated with disease stage, grade, and the presence of metastases.

**Cross talk between VEGF and GLUT-1**

Hypoxic stress generates a specific survival program. Under hypoxic conditions HIF-1α regulates expression of downstream genes like GLUT-1 (glycolytic switch) and VEGFs (angiogenic switch). Both pathways may start consecutively. When tumor growth exceeds the angiogenetic growth, the developing tumor mass suffers from inadequate nutrients supply. In such cases, HIF-1α initiates the upregulation of membrane-spanning glucose transport molecules and ensures energy input to the tumor cells. The other hypothesis assumes that tumor cells at an early stage assure their nutrient supply by an upregulation of GLUT-1. It would be more efficient for cancer to organize a sufficient energy supply system under a disclaiming vascular system.

**CA-IX**

CA-IX is regulated by HIF-1α, rather than HIF-2α. The expression of CA-IX was seen to correlate with worse survival rates, with HIF-2α correlating with poor locoregional control in a study done on 198 HNSCCs (33 OSCCs). Though both were independent prognostic factors, their combined expression had an additive effect, thus confirming a relation between both markers. Eckert et al. studied the relationship between HIF-1α and CA-IX taking into consideration clinical and pathological parameters. They found that patients with low expression of both markers survived an average of 54.8 months, those with increased HIF-1α and decreased CA-IX expression survived an average of 37.6 months, and their tumor-related death risk was 4.97-fold. Roh et al. also found a significant relationship between the markers CA-IX and HIF-1α and HIF-2α. Though Winter et al. found CA-IX expression in 56 of 149 HNSCC cases they did not confirm a positive correlation with either HIF-1α or HIF-2α.

**Cadherin**

HIF-1α activation has the potential of modulating the activity of major EMT-triggering pathways by regulating the expression and activity levels of major transcriptional
Hypoxia in oral squamous cell carcinoma

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Disrupts microtubule polymerization

α conditions are transferable to the α Action

Journal of Medicine, Radiology, Pathology & Surgery

Acts via HSP 90

Inhibits proliferation and induces cell death. Similar findings were reported in another study, of singlet oxygen (and therefore normoxia) for maximum radiotherapy since this treatment depends on the generation HIF1-α of tumor hypoxia has been associated with poor prognosis and resistance to radiotherapy and chemotherapy.[9] It was postulated that the tumors expressing HIF1-α were hypoxic and therefore less likely to respond to radiotherapy since this treatment depends on the generation of singlet oxygen (and therefore normoxia) for maximum cell death. Similar findings were reported in another study, which additionally found that HIF-1α was related to increased angiogenesis and resistance to platinum-based chemotherapy.[6] Tumor hypoxia has been found to reduce the efficacy of some cytotoxic drugs including cyclophosphamide, carboplatin (Paraplatin®; Bristol-Myers Squibb; Princeton, NJ), carmustine (BiCNU®; Bristol-Myers Squibb), and melphalan (Alkeran®; Celgene Corporation; Warren, NJ).[4]

Targeted therapy for HIF and its related markers are being investigated or hypothesized (Table 1).[3,6,8,15,19,20] Many of them are still in the clinical trial stage. Whether the stringent association between markers and oxygenation status under standardized in vitro conditions are transferable to the clinical assessment of oxygenation status in patients, needs to be seen.

Conclusion

Even though most of the previous literature points out to the advantages of the use of HIF as a potential prognostic marker in OSCC tumor microenvironment is constantly changing as a result of tumor cell proliferation and migration, cell metabolism, cell loss and temporal and spatial variations in blood supply

Table 1: Therapeutic targets

<table>
<thead>
<tr>
<th>Agents/target</th>
<th>Action</th>
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<tr>
<td>Histone deacetylase inhibitor, TSA</td>
<td>Inhibits cell proliferation and invasion, blocks cell cycle, and induces apoptosis. Targets tumor angiogenesis &quot;via inhibition of HIF-1α and VEGF expression</td>
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<tr>
<td>GS-HCl</td>
<td>Inhibits proliferation and induces apoptosis. Activates caspase-3, cytosolic accumulation of cytochrome C, down-regulation of HIF-1α and generation of ROS</td>
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<tr>
<td>Cepharanthine, a bisococlarine alkaloid extracted from stephania cepharantha hayata</td>
<td>Inhibits expression of VEGF and interleukin-8 and correlates with decreased tumor cell growth and decreased vascularization</td>
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<tr>
<td>Molecular blockade of VEGF receptor 2</td>
<td>Alone and in combination with radiation, it can enhance tumor response through molecular-targeting of tumor vasculature</td>
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<tr>
<td>Drug 2-methoxyestradiol</td>
<td>Acts via HSP 90</td>
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<tr>
<td>Anti-sense or siRNA oligonucleotides</td>
<td>Disrupts microtubule polymerization</td>
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<tr>
<td>Tumor cell apoptosis</td>
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<tr>
<td>Use of synthetic double-stranded ODNs, designated as HIF-1 decoy ODNs</td>
<td>Reduces the binding of activated HIF-1 to its binding site (HRE) in the promoter region of VEGF. Inhibition of HIF-1 transactivation, as misbinding of activated HIF-1 to decoy ODNs containing HREs</td>
</tr>
<tr>
<td>Inhibition of angiogenic apelin, ephrin</td>
<td>Affect angiogenesis</td>
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and hence the hypoxic areas too are not static. So, the veracity of HIF being a prognostic marker is questionable. The variety of factors that control and influence hypoxia and the ever increasing molecules that are influenced by hypoxia complicate matters further making the entire research highly challenging, and further long-term prospective studies involving patients with stage and site adjusted OSCC would answer the role of HIF in tumor progression.

References
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