J Oral Pathol Med (2005) 34: 385–9

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REVIEW ARTICLE

Hypoxia-inducible factor 1α in oral cancer

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BACKGROUND: Hypoxia is a common feature of many cancers. It contributes to local and systemic tumour progression as well as potentially compromising radiotherapy and chemotherapy. Hypoxia-inducible factor 1α (HIF-1α) is an essential component in changing the transcriptional response of tumours under hypoxia. It targets the transcription of over 60 genes involved in many aspects of cancer biology including cell survival, glucose metabolism, cell invasion and angiogenesis.

METHODS: In this review, we discuss the relevant literature on HIF-1α with specific emphasis on oral cancer. We also present some of our preliminary data on HIF-1α in oral cancer.

RESULTS: Although there are a few conflicting reports of its prognostic significance, over expression of HIF-1α seems to play an adverse role in the malignant progression of head and neck cancer by facilitating the adaptation of cells to hypoxia as well as contributing to the invasive properties and angiogenesis in these tumours.

CONCLUSIONS: HIF-1α has an important role to play in pathophysiology of oral cancer, both under normal and hypoxic conditions. The pharmacological manipulation of HIF-1α has marked effects on tumour growth, and it could prove to be an important target for drug therapy, both in oral cancer and in other hypoxia-dependent disease states.

J Oral Pathol Med (2005) **34**: 385–9

Keywords: Hypoxia-inducible factor; oral cancer; review

Introduction

A thorough understanding of the aetiology and pathogenesis of oral cancer is vital in the search for novel treatment modalities. Recently much work has been carried out at a molecular level and recent discoveries have helped us to understand more fully the complex nature of this aggressive disease. Tumour hypoxia is a common feature of many cancers and essentially occurs when the growth of the tumour outstrips the accompanying angiogenesis. All cells must be within 1–2 mm^3 of a blood supply for survival (1). It is therefore not surprising that many parts of a developing tumour are hypoxic. Clinically, hypoxia may be deleterious by compromising the effects of radiotherapy (2) and/or chemotherapy (3). The transcriptional factor hypoxia-inducible factor 1α (HIF-1α) plays an essential role in the adaptive response of cells to reduced oxygen tension (4). It functions as a master regulator of oxygen and undergoes conformational changes in response to varying oxygen concentrations (5).

Basic biology

The dimeric transcription factor hypoxia inducible factor (HIF-1) is a key regulator of the cellular response to hypoxia (6). Its nuclear translocation follows cytosolic accumulation of its α-subunit (HIF-1α), which results from inhibition of the O_2-sensitive prolyl hydroxylases (PHDs) (7). These enzymes, at physiological O_2 concentrations, constantly modify HIF-1α and target it for proteasomal degradation (8, 9). Following inhibition of PHD, HIF-1α dimerizes with the constitutive HIF-1β to form HIF-1, which is responsible for activation of a variety of genes. More than 60 target genes that are activated by HIF-1 have been identified (for review see Ref. 10). These include genes encoding for vascular endothelial growth factor (VEGF), erythropoietin, and many enzymes involved in glucose, iron, and nucleotide metabolism (11). VEGF is the most potent endothelial-specific mitogen known and several studies have demonstrated it to have a central role in angiogenesis (12). HIF-1α can be viewed as a messenger that is sent from the cytoplasm to the nucleus to activate transcriptional responses to hypoxia.

Although inhibition of PHDs due to hypoxia is recognized as the main mechanism responsible for the stabilization of HIF-1α, it has become evident that this can also occur by mechanisms that are not completely clear. Some growth factors and cytokines bring about an increase in the synthesis of HIF-1α (13, 14). Furthermore, some compounds that chelate iron seem to stabilize it through a still-undefined mechanism,

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Accepted for publication March 14, 2005

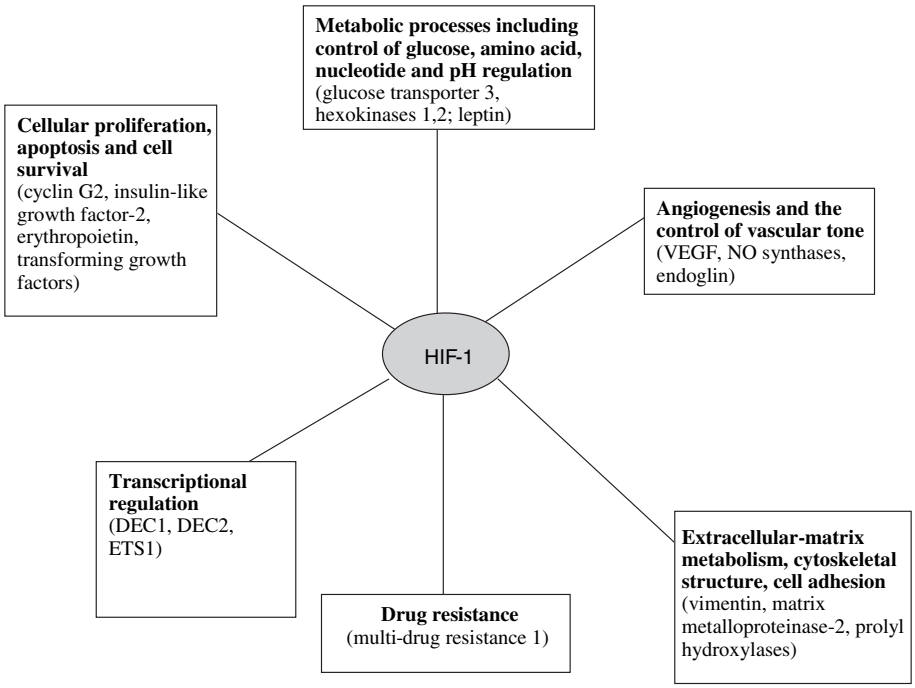

Figure 1 Processes (and gene groups) that are transcriptionally activated by HIF-1.

probably involving a free radical reaction, acting on the protein itself or affecting the action of the PHDs (15).

The presence of HIF-1α has been observed in different types of cancer and there is some indication that this is associated with malignancy and lack of response to treatment (16). A simplified representation of the processes influenced by HIF-1α is shown in Fig. 1. Originally, the presence of HIF-1α in cancer was attributed to tumour hypoxia (17) but recent evidence suggests that other mechanisms may also be involved (18, 19). In our recent studies, we found widespread immunohistochemical expression of HIF-1α in many oral cancers with the pattern of both at the invading tumour margin as well as in the potentially hypoxic, more central areas of tumours (Fig. 2). This finding confirms that hypoxic-independent mechanisms are likely to be responsible for HIF-1α expression.

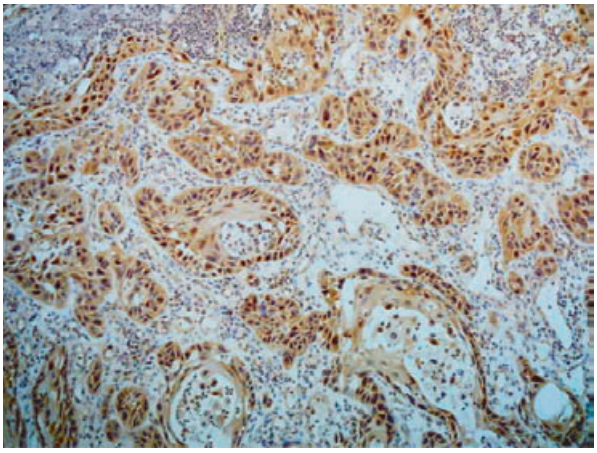

Figure 2 Widespread nuclear and cytoplasmic expression of HIF1α in oral cancer.

Other HIF proteins

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 up-regulate transcriptional activity (13). By contrast HIF-3α is involved in down-regulation of the hypoxic response via an alternatively spliced transcription factor, which may function as an inhibitor of HIF-1α (20).

Effects of HIF-1α in oral cancers

When compared with other cancers, there are only a few studies to date that have investigated the role of HIF-1α in oral and head and neck cancers.

Most cancers over-expressing HIF-1α are associated with increased mortality. In experimental models, HIF-1α depletion severely impedes tumour growth and angiogenesis (21). Adverse effects on patient survival are found in cervical (22), breast (23), ovarian (24), endometrial (25) and stomach cancers (26). However, in head and neck cancers there are conflicting results relating to the over-expression of HIF-1α. Studies using oral cancer cell lines have found that HIF-1 α over-expression is sufficient to confer target genes expression essential for tumour proliferation and survival. Furthermore, the use of anti-sense oligonucleotides targeted to HIF1 induced tumour cell apoptosis in human tongue cancer cell lines, implicating the important role of HIF-1α in maintaining cancer cell viability (27).

Similar findings have been reported recently using the drug 2-methoxyestradiol (2ME2) (28). This is a natural compound with HIF-1α inhibitory activity that is currently being evaluated in phases 1 and 2 clinical trials for advanced solid tumours and multiple myeloma. Using cell lines and a xenograft model, Ricker et al. found that 2ME2 exhibited anti-tumour and anti-angiogenic activity, as measured by CD31 immunostaining (28). It was suggested that 2ME2 in combination with paclitaxel may have a future role to play in the treatment of recurrent or advanced head and neck squamous cell carcinoma.

Furthermore, using an *in vitro* model and oral cancer cells lines, over-expression of HIF-1 α has been shown to be associated with increased and tumour cell invasiveness (29). In clinical studies, Aebersold et al. found that HIF1-α expression correlated with poor prognosis and response to radiotherapy in head and neck cancer patients (18). It was postulated that the tumours 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 (30).

In contrast to the above studies, Beasley et al. found that the immunohistochemical over-expression of HIF-1α was associated with improved prognosis in head and neck cancer (31). It was suggested that the expression (or absence) of HIF-1α varied between different tumour subtypes and the overall phenotype was important in determining outcome.

Cancer development is known to be the result of a multi-step progression during which mutation and clonal selection lead to the development of the cancer phenotype. Selection must operate at the cellular level and the malignant clone represents the summation of properties selected through an autonomous survival advantage. When a genetic mutation involves an extensive physiological pathway then the clonal selection will involve many functions that are linked through that pathway. These functions may then be acquired by the malignant clone irrespective of whether or not they make a positive contribution towards selective tumour advantage. In respect of hypoxia and HIF-1α pathways, the huge scale of interconnection suggests that these pathways may be particularly prevalent and important. Pathways that preserve oxygen homeostasis link angiogenesis and HIF-1α activation and it is possible that these pathways are co-selected during the acquisition of traits, which confer a cell-autonomous survival advantage. This could explain the paradox that tumours often develop an angiogenic system, which is inefficient in the delivery of oxygen (10).

Hypoxia regulates both VEGF and HIF-1α expression in head and neck squamous cell carcinoma, thus establishing a biochemical pathway between tumour hypoxia and neoangiogenesis in these tumours (32). The mechanism of activation of HIF-1α in head and neck squamous cell carcinoma has recently been studied. Under hypoxic conditions, two protein kinases, Jun N terminal kinase (JNK 1) and p38 kinase were found to induce HIF-1α and VEGF expression. These enzymes belong to a family of stress activated protein kinases that are activated by a variety of stressful stimuli including hypoxia (33).

Role of nitric oxide (NO) in HIF-1α expression

Increases in nitric oxide (NO) generation have been observed in certain forms of cancer and there is also evidence for an association between concentrations of NO, malignancy and resistance to treatment (34). At physiological concentrations NO decreases the stabilization of HIF-1α in hypoxia due to its ability to inhibit mitochondrial respiration and redirect O_2 to reactivate PHD (35). However, at the high concentrations produced in certain forms of cancer, NO also increases the presence of HIF-1α by an action probably involving a free radical mechanism (36). It is already known that NO seems to have an adverse role in oral cancer, and expression of the enzyme inducible NO synthase (iNOS) also correlate with neck node metastasis (37). However, the mechanism of how NO produces these effects has not been fully elucidated in oral cancer. We have recently found that NO production by NOS enzymes influences HIF-1α expression under normoxic and mildly hypoxic conditions (6% oxygen). Using three oral cancer cell lines and Western blot analysis, we found that HIF-1α protein expression could be reduced or prevented by the NOS antagonist drug, L-NMMA, implying that NO plays a role in HIF-1α expression. However, this action did not occur under more severe hypoxia (3% oxygen) or anoxic (0.5% oxygen) conditions (Fig. 3). The use of the anti-oxidant agents N-acetyl cysteine and ascorbic acid inhibited HIF-1α expression under both normoxia and hypoxia (3% oxygen) suggesting that HIF-1α expression is influenced by a free radical mechanism (Quintero et al. unpublished data). Therefore, both NO and other free radicals (such as reactive oxygen species, ROS) appear to aid HIFα

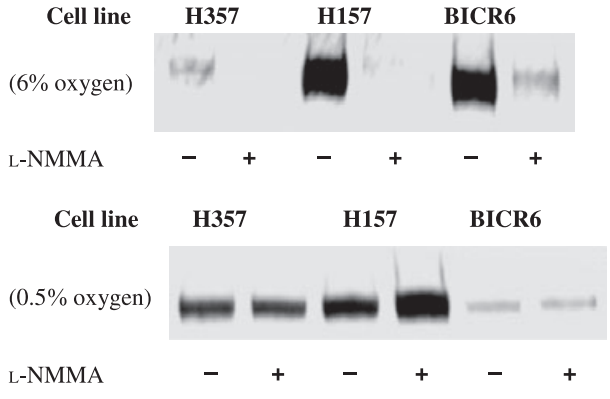

Figure 3 Western blot showing reduction or inhibition of HIF-1α protein expression by the NOS antogonist drug L-NMMA (shown as +) at 6% oxygen, but not at 0.5% oxygen concentration.

Table 1 Some drugs with HIF-1α inhibitory activity

Agent	*Molecular targets*	*Tumours*	*Status*
17-AAG	HSP-90 (see text)	Melanoma, myeloma, prostate	Clinical trials
2-ME2	Microtubule polymerization	Advanced tumours breast, myeloma lung	Clinical trials
Trastuzumab	Tyrosine kinase	Breast, lung, ovarian	Clinical trials
Celebrex	Cyclo-oxygenase-2	Lung, brain	Clinical trials
Imatinib	Platelet-derived growth factor receptor	Leukaemias, GI tumours	Approved agent

expression in oral cancer. This may partly explain how NO and ROS appear to have an adverse role in oral cancer, by working upstream to HIF-1α.

Therapeutic manipulation of HIF-1α

In most human cancers, HIF-1α expression seems to be associated with tumour progression and development. Hypoxic cancer cells are also more likely to be resistant to radiotherapy and chemotherapy, and interestingly, HIF-1α itself mediates resistance to both radiotherapy (18) and chemotherapy (16). Therefore, inhibition of HIF-1α activity might enhance existing radiotherapy and chemotherapy regimes. By reducing the angiogenic affect, it may reduce tumour growth and dissemination. Over-expression of HIF-1α is associated with radiation resistance and increased mortality regardless of tumour grade, stage or other biomarkers in head and neck cancer (18). Constitutive or hypoxia induced expression of HIF-1α in tongue squamous cell carcinoma confers target gene expression essential for tumour proliferation and survival. Interfering with HIF-1α pathways by antisense or siRNA strategy may provide a therapeutic target for oral squamous cell carcinoma (27).

The National Cancer Institute (NCI) Developmental Therapeutic Program is developing a number of novel therapeutic agents. These include the agents 17AAG, which induces HIF-1α degradation acting via heat shock protein (HSP 90) (38). Another agent, 2ME2 (39) has also been shown to reduce HIF-1α levels by disrupting microtubule polymerization. As previously discussed, it is possible that 2ME2 may have a role to play in head and neck cancer (28) or that other agents may be useful. There are a number of other inhibitor drugs that are also undergoing clinical trials (listed in Table 1), although none of these seem to specifically target HIF-1α. Although no clinical trials have been published to date on the use of drugs that inhibit HIF-1α, it seems likely that trials will commence in the near future.

Conversely, drug-associated HIF-1α induction may be useful in pathologies associated with hypoxia such as myocardial infarction and other diseases associated with vascular occlusion (40).

Conclusions

By acting on a large number of target genes, HIF-1 seems to facilitate both cancer growth and spread in the majority of human cancers, including oral squamous cell carcinoma. It may also reduce the efficacy of existing radiotherapy and chemotherapy regimes. The therapeutic targeting of HIF-1α is an exciting area for possible therapy in oral cancer, and the results of clinical trials are awaited with interest.

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