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# Tumor Hypoxia and the Progression of Prostate Cancer

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Tumor cell hypoxia is an innate environmental factor encountered during the development of many types of human tumors, including malignant prostate tumors. For prostate cancer, however, tumor cell hypoxia may be an even more critical element in tumor development and progression. Recent evidence suggests that androgenic steroids are important regulators of blood flow to prostate tumors and suppressors of tumor cell hypoxia. In addition, because prostate tumor cells are similar to other eukaryotic cells, they have the ability to respond to hypoxic conditions with drastic changes in gene expression mediated by the upregulation of a unique transcription factor, hypoxia-inducible factor-1. This response increases cancer cells' metabolic resistance to hypoxia, and also enhances the ability of prostate cancer cells to attract a more vigorous blood supply by upregulating the expression of pro-angiogenic factors. Because such changes would, in essence, increase the potential aggressiveness of affected prostate cancer cells, it is clear that tumor hypoxia has the potential for being a very important factor in prostate cancer cell biology. This review focuses on recent studies regarding the occurrence and potential role of hypoxia in prostate cancer, including hypoxia-inducible factor-1 and its related signaling pathways.

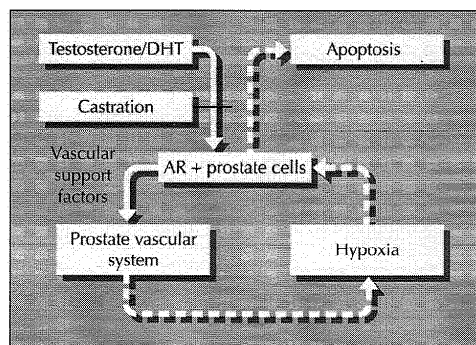
## Introduction

The prostate gland is the leading site for cancer incidence among men in the United States [1]. Our ability to treat this disease is improving, as indicated by recent overall decrease in prostate cancer death rates (dropping by an average 1.6% annually between 1990 and 1996). Nevertheless, carcinoma of the prostate remains the second most common cause of cancer death in men in the United States [1]. One well understood and striking biologic aspect of prostate cancer is the stimulating effect of male (andro-

genic) steroids on this disease. For this reason, androgen ablation therapy is a mainstay of treatment for patients with advanced prostate cancer, and has been established to temporarily stabilize the patient and even cause regression in metastatic lesions. However, this therapy is not definitively curative; treated patients inevitably develop an androgen-resistant form of the disease that is also unresponsive to other therapies. The transient effectiveness of androgen ablation therapy is believed to be a result of its apoptotic effect on some fraction of prostate cancer cells. Normal prostate epithelial cells are known to undergo apoptosis in response to androgen ablation (such as castration) [2], and recent studies suggest that this is likely the result of drastic reduction of blood flow to the tissue. The ultimate result of this process is the onset of a hypoxic environment conducive to the loss of prostate epithelial cells via apoptosis (Fig. 1) [3,4]. Because therapeutic resistance of prostate cancer is believed to arise from a subset of cancer cells that have the ability to resist apoptosis following castration, it is therefore possible that an endogenous hypoxia-response survival mechanism may participate in the pathways through which prostate cancer cells inevitably acquire their therapeutic resistance. Additionally, based on our increasing knowledge of hypoxia-response pathways in eukaryotic cells, it is clear that hypoxia can be a stimulus for a cellular response that can contribute to the potential aggressiveness of any tumor cell, including prostate cancer cells. In this review we present an update on the potential role of hypoxia in prostate cancer development and progression, including the role of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) and its downstream signaling pathway.

## Hypoxia in Human Tumors

Because the accelerated growth of cancerous cells within a human tumor is rarely accompanied by vascular development sufficient to support the growing tumor mass, most human tumors are likely to encounter hypoxia at some point in their development. On one hand, this phenomenon is potentially beneficial to the patient because tumor cells starved for oxygen are much less likely to divide and are much more susceptible to the onset of apoptosis. Indeed, as originally proposed by Folkman *et al.* [5], the



**Figure 1.** Prostate cell hypoxia subsequent to castration: the loss of prostatic epithelial cells after castration results from the hypoxic environment of the tissue in the absence of androgens. AR—androgen receptor; DHT—dihydrotestosterone.

inability of tumor cells to attract a developing blood supply may be one of the more important factors in maintaining some tumors in an indolent state. On the other hand, we already know that tumor hypoxia has the potential to be problematic for patients with cancer, because hypoxic tumor cells are generally resistant to therapies (such as radiation) whose efficacy depends on the generation of free radical oxygen byproducts. An even more important consideration, however, is the recent concept that hypoxia might actually be a stimulus for the generation of more aggressive tumor cells. Activation of a tumor cell's hypoxia-response system alters its metabolism, improving survivability under low-oxygen conditions and increasing its angiogenic potential by upregulating the production of critical angiogenic factors [6•]. The cellular hypoxia-response system relies on the presence of hypoxic conditions to drastically alter the gene expression profile in the hypoxic cell. The expression of certain enzymes, particularly those that enable efficient cell glycolysis (to generate energy) and glucose transport proteins (to increase expression of protein substances) is upregulated by hypoxia. Additionally, hypoxia can act as a stimulus to increase the expression of protein substances, thereby improving a cell's potential for attracting increased oxygenation. These protein substances can stimulate the development and growth of a tumor's local vascular system through the action of peptide factors. These peptide factors can, for example, induce systemic erythropoiesis or more specifically, actively promote angiogenesis through the production of vascular endothelial growth factor (VEGF). Indeed, with the use of oxygen-sensitive electrodes, it is possible to directly measure in situ tumor oxygen levels. It has already been demonstrated that tumor hypoxia can be an independent prognostic indicator of poor patient outcome for patients with head and neck cancers, cervical cancers, and soft tissue sarcomas [7–9].

In 1999, hypoxic regions were first described in the tumors of 12 different prostate cancer patients using custom-made Eppendorf electrodes [10•]. These electrodes directly measured partial pressure of oxygen ( $PO_2$ ) in tumor tissue and compared this with the  $PO_2$  in adjacent muscle. In this preliminary report, increasing levels of hypoxia (less than 5 and 10 mm Hg  $O_2$ ) were observed with increasing clinical stage. An update of this work with 55 patients was reported in 2000 [11]. In this later report, a multivariate, linear analysis among patients undergoing brachytherapy demonstrated that the significant predictors of  $PO_2$  were tissue type, disease stage, and patient age. In order to test the hypothesis that increased levels of prostate tumor hypoxia were associated with activation of the cancer cell's hypoxia response, the same group correlated median tumor oxygenation levels with immunohistochemically determined levels of VEGF expression in malignant cells of 13 prostate tumors: a significant correlation was reported between increasing hypoxia and percentage of VEGF positive cells in the tumor [12]. The authors have pointed out, however, that these micro-electrode measurements may not reflect global oxygenation status of the entire tumor, and therefore, should be combined with noninvasive techniques that indicate global tumor oxygenation [13]. Such a noninvasive technique was recently reported with the use of dynamic oxygen mapping with MRI to record changes based on sequential maps of regional tumor  $PO_2$  [14]. Preliminary investigations performed on rodents revealed considerable heterogeneity within any given tumor. However, large tumors (greater than 3.5  $cm^3$  in mass) were significantly less well-oxygenated than smaller tumors (less than 2  $cm^3$ ). Faster growing, less-differentiated tumors were also found to be less well-oxygenated than size-matched tumors of slower growing sublines [14]. Future studies with larger patient bases and longer follow-up periods will be required to determine the true clinical significance of tumor hypoxia in prostate cancer.

#### Androgen Action in Hypoxia Suppression

Development and progression of both normal and malignant prostate tissue is stimulated by the presence of androgenic steroids. The basis for this stimulatory action is still poorly understood, but the results of a series of recent studies on androgen action in the normal prostate gland of laboratory rats suggests that this effect is, at least partially, due to the regulation of blood flow to the tissue. When androgens were withdrawn from rats (by surgical castration) blood flow to the prostate rapidly decreased [15]. Among the factors responsible for this rapid loss of prostatic blood flow were an equally rapid loss of capillary elements (vasodegeneration via apoptosis of capillary endothelial cells) that supplied the prostate glands, and a concomitant severe vasoconstriction of the larger prostatic vascular elements surviving castration. These vasodegen-

erative events were subsequently followed by loss of extensively hypoxic prostatic epithelium [4]. A similar finding was also demonstrated in an androgen-dependent animal tumor model system [16]. Based on these results, we have proposed that the onset of hypoxia is the likely cause of the apoptotic response to castration by normal and malignant prostate epithelial cells [4]. Prostatic endothelial cells are not known to express androgen receptor protein (AR): a prerequisite for primary androgen effects in any given cell. Therefore, we have proposed that the previously described complex response of prostatic vasculature to castration is based on a change in production of vascular support factors that are linked to AR-producing prostate cells (Fig. 1). If this model is correct, then the concept that prostate cancer cells can alter their gene expression patterns in response to hypoxia to enhance their survival in an oxygen-poor environment has significant implications, not only for the treatment of this disease, but also for our concepts as to why prostate tumors ultimately become unresponsive to androgen-deprivation therapy.

#### Hypoxic Regulation of Gene Expression

**Structure and function of hypoxia-inducible factor-1 $\alpha$**   
The ability to maintain oxygen homeostasis is essential for the survival of eukaryotic cells. Therefore, under conditions of hypoxia, eukaryotic cells can initiate a hypoxia-response program. It is this response that is thought to play an important role in the pathogenesis of many serious disorders such as cerebral and myocardial ischemia, chronic heart and pulmonary diseases, and cancer [17]. One of the better characterized responses of an eukaryotic cell to acute or chronic hypoxia involves changes in gene expression mediated by the transcriptional regulatory protein complex referred to as HIF-1. In actuality, the functional HIF-1 complex is a heterodimer composed of an  $\alpha$  and a  $\beta$  subunit [18]. Whereas the  $\beta$  subunit (also known as the aryl hydrocarbon nuclear translocator) is constitutively expressed in most cells, the  $\alpha$  subunit is generally not expressed except under conditions of hypoxia. Exposure of a mammalian cell to hypoxia (or to cobalt ions) induces the expression of the 120-kD protein referred to as HIF-1 $\alpha$ . Upon dimerization with the  $\beta$  subunit, the HIF protein-complex can enter the nucleus wherein it stimulates transcription of gene products that increase the metabolic resistance of cells to hypoxia and apoptosis (eg, glycolytic enzymes, glucose transporters, adenylate kinase-3, insulin-like growth factor 2, pyruvate kinase M, and carbonic anhydrase-9). Other gene products (such as VEGF) are produced to increase the propensity of the cell to attract a better blood supply (Fig. 2) [6,18–20]. It is critical to recognize that the biologic activity of the hypoxia-response mechanism is determined by the expression of the HIF-1 $\alpha$  protein subunit of this transcription complex. More interesting, the expression of

the HIF-1 $\alpha$  protein is basically regulated at the post-translational level rather than at the transcriptional level. Most cells express HIF-1 $\alpha$  mRNA even when they express little (or no) HIF-1 $\alpha$  protein. Under nonhypoxic conditions, newly translated HIF-1 $\alpha$  protein is rapidly ubiquitinated and then subject to almost immediate proteasomal degradation [17]. Indeed, the half-life of newly translated HIF-1 $\alpha$  protein in a nonhypoxic cell is thought to be very brief, only 1 minute or less in duration. Induction of the HIF-1 $\alpha$  protein in cells exposed to hypoxia is a result of the transient suppression of the normal HIF-1 $\alpha$  degradation process and a dramatic prolongation in the half-life of the HIF-1 $\alpha$  protein. Several studies have already identified a specific region within the HIF-1 $\alpha$  protein that is responsible for this rapid degradation process: this region has been named the oxygen-dependent degradation domain (ODD) [17]. This Pro-Ser-Thr-rich protein stabilization domain is located between amino acids 429 and 608 of the HIF-1 $\alpha$  polypeptide [21].

It is also of interest that the von Hippel-Lindau protein (pVHL), thought to be important for the development of renal cancer, functions as a regulator of HIF-1 $\alpha$  degradation. The pVHL binds to the ODD domain of HIF-1 $\alpha$  and plays a critical role in its potential for ubiquitination [22]. In the presence of oxygen, HIF-1 $\alpha$  is targeted for destruction by an E3 ubiquitin ligase complex containing the pVHL. It was recently discovered that human pVHL binds to a short HIF-derived peptide when a conserved proline residue (HIF-1 $\alpha$  P564) at the core of this peptide is hydroxylated by the enzyme HIF-1 $\alpha$  prolyl-hydroxylase. Because proline hydroxylation requires molecular oxygen and iron, this protein modification is thought to play an important role in oxygen sensing [23,24]. In tumor cells, loss of pVHL or p53 activity results in increased HIF-1 $\alpha$  expression and the subsequent increased transcription of downstream target genes such as VEGF [22]. The expression of HIF-1 $\alpha$  protein and VEGF mRNA under nonhypoxic conditions is also induced by activation of the signal transduction pathway involving phosphoinositol 3-kinase (PI-3K), serine/threonine kinases protein kinase B (AKT), and FKBP-rapamycin-associated protein (FRAP) (Fig. 3) [25]. Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), a tumor suppressor with phosphoinositol phosphatase activity, negatively regulates the PI-3K pathway and, therefore, loss of PTEN activity can also lead to increased HIF-1 $\alpha$  protein expression (Fig. 3) [26].

#### Hypoxia-inducible factor-1 $\alpha$ and prostate cancer

Whereas HIF-1 $\alpha$  was not found to be expressed in the normal rat prostate, Zhong *et al.* [27] evaluated HIF-1 $\alpha$  expression and HIF-1 binding activity in several standard rat prostate cancer cell lines. Expression of HIF-1 $\alpha$  mRNA in rat prostate cancer cell lines was found to be associated with increased cell growth rates and metastatic potential in these cells [27]. This study was the first one to indicate

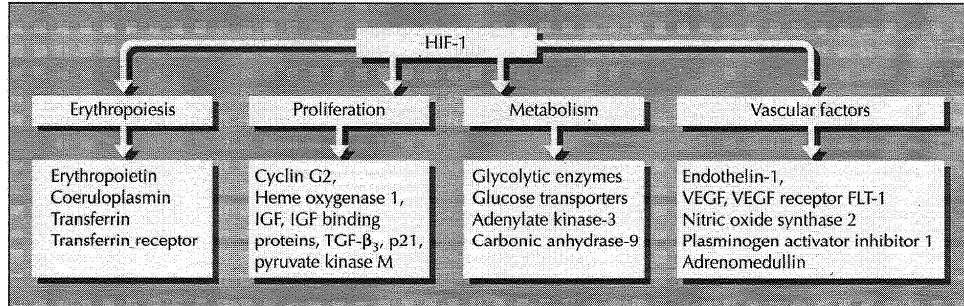


Figure 2. Target genes of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ).

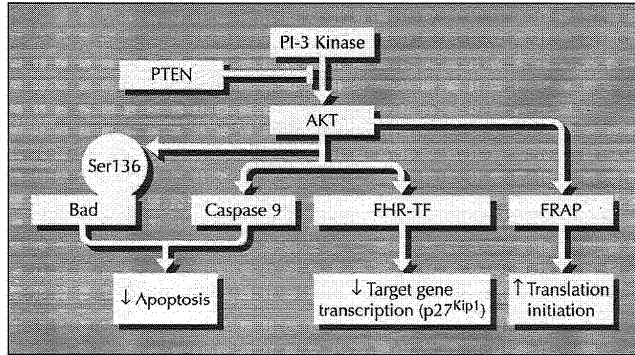
that HIF-1 $\alpha$  may become dysregulated in prostate cancer and promote tumor progression via transcription of hypoxia-adaptive genes. Regarding the standard human prostate cancer cell lines, only the PC-3 line was found to be expressing HIF-1 $\alpha$  protein under normal conditions, but all other cell lines examined had the potential to express HIF-1 $\alpha$  when exposed to hypoxia. The abnormal constitutive expression of HIF-1 $\alpha$  by PC-3 cells (without hypoxia) may be explained in a recent study in which comparative genomic hybridization showed that the region 14q21-q24 containing the HIF-1 $\alpha$  gene is amplified in the PC-3 cell line [28]. The gene was later mapped to locus 14q23 by fluorescence in situ hybridization and shown to be highly amplified in the PC-3 cell line as compared with five other prostate cancer cell lines (LNCaP, DU-145, NCI-H660, Tsu-Pr, and JCA-1) [29•].

Immunohistochemical evaluation of primary prostate tumors showed an HIF-1 $\alpha$  protein overexpression in 13 of 19 different solid tumor types, including prostate cancer [30••]. With specific regard to prostate cancer, a HIF-1 $\alpha$  positivity could be demonstrated in the majority of prostate cancer specimens examined (nine of 11), but in none of the benign prostatic tissue specimens. In this study, HIF-1 $\alpha$  expression was correlated with both aberrant p53 accumulation and increased cell proliferation activity in the tumors. These results provided the first clinical data indicating that HIF-1 $\alpha$  may play an important role in human cancer progression. These data were also confirmed by another study conducted in the United Kingdom that investigated the distribution of HIF-1 $\alpha$  and HIF-2 $\alpha$  in various normal human tissues and solid tumors, including prostate cancer [31]. Salnikow *et al.* [32] tested the functional activity of HIF-1 in human prostate cell lines ranging from normal epithelial cells (PrEC), hormone-dependent LNCaP, hormone-independent DU-145, and PC-3, to highly metastatic PC-3M cancer cell lines. They demonstrated that HIF-1-stimulated transcription was the lowest in the PrEC and LNCaP cells and the highest in PC-3M cells (with amplified HIF-1 $\alpha$  gene and constitu-

tively activated HIF-1 $\alpha$  protein expression). Also, the expression of HIF-1-dependent gene products Cap43 and GAPDH was found to be the highest in PC-3M cells. Since p53 function is also lost in advanced prostate cancer, the combination of high inducibility of HIF-1-dependent genes, loss of p53 function, and loss of sensitivity to p21 inhibition suggests a hypoxic phenotype associated with aggressive cancer behavior [32].

Focusing on the molecular events of angiogenesis and the initiation and progression of prostate cancer, Huss *et al.* [33] used immunohistochemistry and in situ analyses of tissue specimens using the transgenic mouse model to study the expression patterns of several factors, including HIF-1 $\alpha$  and VEGF. The authors propose two angiogenic events consistent with the progression of the mouse model and clinical prostate cancer. In one event, an early angiogenic "initiation switch" correlates expression of HIF-1 $\alpha$  and VEGF receptor, in addition to recruitment and elaboration of intraductal vasculature in prostatic intraepithelial neoplastic lesions. A later progression switch is consistent with high-level expression of VEGF protein in the prostatic tissues and sera of mice harboring advanced, poorly differentiated, and androgen-independent tumors.

Our contemporary understanding of the biology of HIF-1 $\alpha$  suggests that progression of prostate cancer could be the result of a hypoxic environment in the tumor itself. Alternatively, prostate cancer could result with dysregulation of HIF-1 $\alpha$  expression, either through an amplification of the HIF-1 $\alpha$  gene (as is found in PC-3 cells), through suppression of the normal HIF-1 $\alpha$  degradation process in cancer cells, or even, perhaps, through mutations in the HIF-1 $\alpha$  degradation-sensitive region that renders this protein unregulatable through the normal degradation process. In any case, the abnormally high expression of HIF-1 $\alpha$  protein by prostate tumor cells clearly has the potential to make these cells more aggressive by the induction of metabolic resistance and angiogenesis-promoting gene products in these cells.



**Figure 3.** The phosphatidylinositol 3-kinase/PTEN/AKT signaling pathway. AKT—serine/threonine kinase (protein kinase B); FHR TF—forkhead related transcription factor; FRAP—FKBP-12 rapamycin-associated protein; PI-3 kinase—phosphatidylinositol 3-kinase; PTEN—phosphatase and tensin homologue deleted on chromosome 10.

### HIF-1 and the phosphatidylinositol 3-kinase signaling pathway

Oncogenic transformation is characterized by activation of growth-promoting oncogenes and loss of function of tumor suppressor genes. Both types of dysregulation occur in the signaling pathway mediated by PI-3K [34] (Fig. 3). PI-3K phosphorylates inositol lipids at the D-3 position, producing phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate [35]. A downstream target of PI-3K is the serine-threonine kinase AKT (also known as protein kinase B) that is activated by phosphatidylinositol-dependent kinase 1 [36]. Downstream targets of AKT action include BAD, an apoptosis inhibitor, and FRAP, an activator of p70<sup>S6k</sup>, which is required for ribosomal biogenesis and cell cycle progression. The tumor suppressor PTEN negatively regulates the PI-3K/AKT pathway, which promotes cell proliferation and inhibits cell death. PTEN acts through dephosphorylation of phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate [37]. In prostate cancer, loss of PTEN function correlates with increased angiogenesis and appears to be critical for progression to hormone refractory disease [38].

In 2000, Zhong *et al.* [25••] demonstrated that activation of the PI-3K/PTEN/AKT/FRAP pathway resulted in increased expression of HIF-1 $\alpha$  protein, HIF-1 transcriptional activity, and VEGF protein expression in human prostate cancer cell lines. They were able to demonstrate that in human prostate cancer cells (DU-145, PC-3, PPC-1, and TSU), basal-, growth factor-, and mitogen-induced expression of HIF-1 $\alpha$  could be blocked by LY294002 (wortmannin) and rapamycin, inhibitors of PI-3K and FRAP, respectively. HIF-1-dependent gene transcription was blocked by dominant-negative AKT or PI-3K and by wild-type PTEN, whereas transcription was stimulated by constitutively active AKT or dominant-negative PTEN [25].

These results were confirmed by independent groups studying rat pheochromocytoma (PC-12) and human fibrosarcoma (HT1080) cells. Hypoxia effects on the cells

that resulted in AKT phosphorylation and increase of one of its downstream substrates, glycogen synthase kinase-3 (GSK-3), were blocked by pretreatment with the PI-3K inhibitor wortmannin [39,40]. These data indicate that pharmacologic agents that target PI-3K, AKT, or FRAP in tumor cells inhibit HIF-1 $\alpha$  expression, and therefore, may contribute to increased therapeutic efficacy in the future.

### Conclusions

Hypoxia is a common phenomenon found in many types of malignant tumors including prostate cancer. Although this condition has the potential to suppress tumor growth or progression (by inhibiting oxidative energy production and increasing potential for apoptosis), there is also the critical potential of increasing the aggressiveness of prostate tumor cells via its ability to stimulate the cancer cell's hypoxic response. Such a response would induce the expression of cancer cell gene products that could protect the prostate cancer cell against therapies used against this disease, specifically androgen withdrawal and radiation. In addition, these factors can increase the propensity of the prostate cancer cell to attract a viable blood supply by stimulating increased angiogenesis.

The prostate cancer cell's hypoxic response is primarily mediated by HIF-1, a transcription factor complex that is functionally expressed in cells mainly in response to inadequate oxygen supply. The activity of this transcription complex is based on stabilization of its  $\alpha$  subunit protein under hypoxic conditions. Downstream gene products induced by the HIF-1 transcription complex include a number of different products that increase the potential for oxygen delivery to the cancer cell or provide metabolic adaptation to the cancer cell, enabling it to survive under reduced oxygen availability. Recent studies indicate that overexpression of HIF-1 $\alpha$  is a common occurrence in human prostate cancers and that this abnormality may play a major role in the biology of this disease. Pharmacologic inhibitors of HIF-1 and

its related pathways, therefore, might be a promising therapeutic perspective for the future.

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Short communication paper describing the amplification of the HIF-1 $\alpha$  gene in the PC-3 cell line, which expresses the gene even under normoxic conditions.

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