

Review

Tumour Hypoxia and Prostate Cancer

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Abstract

Hypoxia is a known hallmark of endocrine tumours including prostate cancer. The extent of this phenomenon varies across different stages of prostate cancer development with more advanced tumours showing higher state of hypoxia. Here we review the current understanding of tumour hypoxia and its implications on treatment outcome including treatment with androgen ablation therapy. In particular, hypoxia selects for cells with geno-phenotypes for survival advantage and malignant progression in both experimental and clinical models of prostate cancer. Evidence showed that tumour hypoxia may serve as a potent therapeutic target in treatment of prostate cancer.

Keywords; Prostate Cancer, hypoxia, angiogenesis, chemotherapeutic resistance

INTRODUCTION

Current data indicates that the prevalence of prostate cancer across the Western World is increasing at an alarming rate. Recent studies have shown that prostate cancer is the most common form of cancer in men in the United State of America, and the second leading cause of death due to cancer (Ahmedin et al., 2008). Approximately 100 men are diagnosed with this disease daily in the United Kingdom (Maitland and Collins 2008). Many published reports show that African American men with prostate cancer have the highest mortality rate reaching 2.4 times as high as white men with the same disease (Odedina et al., 2009). In fact a recent study examined the continuous disparity in mortality rate exiting between prostate cancer patients with African Ancestry in comparison to their counterparts with Western background; that study traced the trail of prostate cancer Trans-Atlantic slave trade (Odedina et al., 2009). This study evaluated the burden of prostate cancer among the Trans – Atlantic Slave Trade population, targeting the black men of West African origin including Ghana, Nigeria, Gambia and other countries with slave trade trails. Tracing the root of high incidence of prostate cancer in both USA and UK, the study found that the

increasing cases of prostate cancer found in the Western countries including UK followed the path of Trans Atlantic Slave Trade; and that West African countries especially Nigeria has similar prostate cancer burden as found in USA (Odedina et al., 2009).

There are many factors that contribute to the development of prostate cancer; the most important of them are age, family history, diet, ethnicity (Nelson *et al* 2003; Cheung et al., 2008). Currently, different phytochemicals are consumed in sub-Saharan Africa in the name of herbs. Most of which contain bioactive substances whose molecular implication are not characterised; while some of these products may have anti cancer effect, others may as well possesses carcinogenic potentials. These need to be established.

Pathogenesis of prostate cancer

Prostate cancer is one of the age related malignancies with high prevalence rate in men. The pathogenesis of this disease is poorly understood, however, on the basis of anatomical locations, prostate cancer is more common in one of the three zones of the prostate. These zones are defined based on their relations to the urethra, and include the transition zone, the central zone, and the peripheral zone (figure 1). The contributions of cells found in these zones in the pathogenesis of prostate cancer

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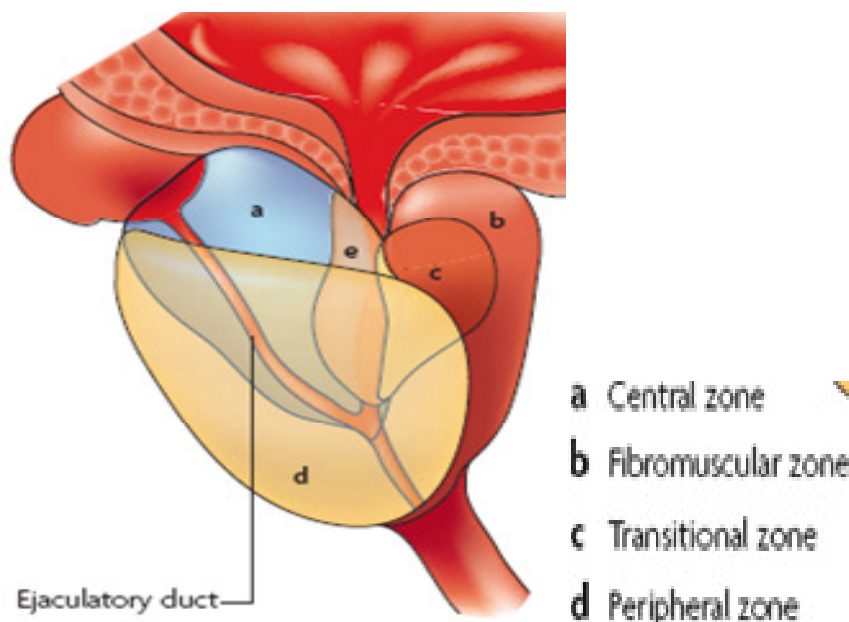


Figure 1. showing the various anatomical zones of the human prostate. Taken from Che and Grignon 2002

vary significantly. For instance, the transition zone which makes up 5 to 10 % of glandular tissue is responsible for 20% of adenocarcinomas of prostate; and the central zone consisting of 25% glandular tissue rarely harbours 1% to 5% of total incidences of prostate cancer (Che and Grignon, 2002). But the peripheral zone consisting mainly of prostatic tissue is responsible for about 70% of all cases of prostate cancer (Nelson et al., 2003). Concerning cell differentiation pathways in the prostate, Hudson et al. (2001) reported that the transition zone retains gradual growth from adulthood through old age with a resultant formation of benign prostatic hyperplasia (BPH). It is not clear whether BPH could lead to prostate cancer, however, it is the second most common indication for men over 65 years undergoing surgery due to obstruction of the urethra (Hudson et al., 2001). It has been suggested that a gain of cell survival or loss of apoptosis by androgen receptor – expressing cells from the luminal compartment of the prostate could result in prostate cancer (Maitland and Collins, 2008).

The Vasculature

It has been established that tumour cannot growth beyond 150 – 200 μm without formation of new blood vessels to deliver sufficient amount of oxygen and nutrient. The process by which the new blood vessels are formed by endothelial progenitors is called vasculogenesis; while angiogenesis involves the action of mural cells with endothelial cells, pericytes, vascular

endothelial growth factor (VEGF) and angiopoietin/Tie-2 system to ensure stabilisation of the vessels (Carmeliet 2003). The new blood vessels are initially small, consisting initially of only the endothelial cells. Micro environmental factor such as vascular endothelial factor (VEGF) has been shown to be positively correlated with aggressive growth in breast cancer, prostate cancer and stomach cancer (Folkman 2002). Previous studies have indentified other factors in the tumour microenvironment that possess angiogenic potential, and include interleukin 8 (IL-8), epidermal growth factor (EGF) and angiogenini (Hobson and Denekamp 1984; Risau 19991; Folkman 2002)

Tumour hypoxia

The term hypoxia is normally used to refer to a state of reduced oxygen partial pressure (see figure 2), below a certain threshold that compromises biological functions in the cell (Hockel and Vauel, 2001; see figure 2). Tumour hypoxia has been classified into two types; chronic and acute hypoxia (Rofstad, 2000). Chronic hypoxia arises as a result of problems orchestrated by the tumour architecture; including blind endings, arteriovenous shunts, developments of vessels from post capillary venues etc, causing severe diffusion gradient of oxygen (Brown and Giaccia, 1998; Rofstad, 2000; Cairns and Hill, 2004). The consequence of a low pO_2 within the tumour microenvironment is poor treatment outcome after treatment with either radiotherapy or chemotherapy. This

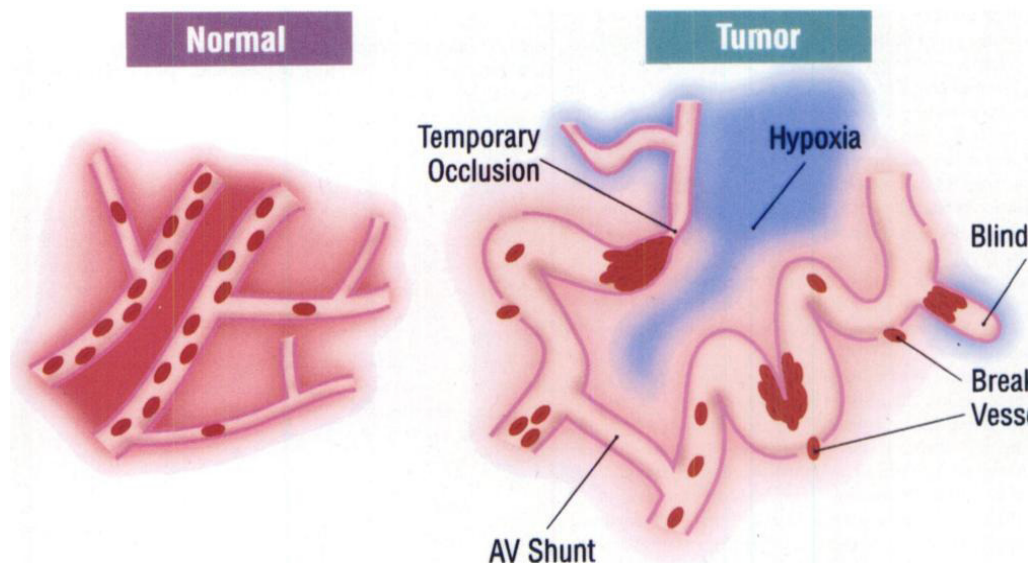


Figure 2. An illustration of the differences between normal oxygen level in the microenvironment in normal tissue and in the solid tumour. The vasculature are irregular, leaking, occluded, chaotic; this may compromise biological functions and alter gene expression profile in hypoxic cancer cells (Brown and Giaccia 1998).

occurs because poorly oxygenated cells are less sensitive to the effects of radiation and since they are in a quiescent state, these cells are also less sensitive to cytotoxic drugs commonly used in treatment of more advanced disease (Hockel and Vaupel, 2001). Evidence has shown that when the oxygen partial pressure (pO_2) in tumours falls below about 7mmHg, this induces genophenotype changes effects in cancer cells which promote resistance to apoptosis inducing agents in the tumour (Vaupel 2008; Butterworth et al., 2008). In addition, some cells adapt to the existing hypoxic stress through the process of genomic modification and change in gene expression profile which results in selection of phenotypes with high metastatic and aggressive potential (Vaupel, 2008; see figure 2).

ANDROGEN ABLATION THERAPY AND TUMOUR HYPOXIA

The first study of hormone deprivation in men with prostate cancer was reported by Huggins and Hodges (1941). Androgen ablation therapy (AAT) is now the mainstay of treatment of locally advanced androgen-dependent prostate cancer (Eisenberger et al., 1998; D'Ancona and Debruyne, 2005; Nishiyama et al., 2005; Tyrrell et al 2006). This method has been shown to improve the quality of life in more than 80% of patients with advanced prostate cancer; however, the median duration of this benefit is only two to three years (D'Ancona and Debruyne, 2005), before the emergence

of androgen resistance state (Eisenberger et al., 1998) and invasive spread to the bones Nadiminty et al 2006. The mechanism underlying the treatment failure is not yet clear, but there are indications that hypoxia may contribute significantly to this process. The pre-clinical and clinical evidence are discussed below

Experimental and clinical evidence

Emerging experimental evidence now demonstrates clearly that androgen ablation therapy may contribute to tumour hypoxia; this compromises biological functions in the cell Hockel and Vaupell 2001 resulting in biochemical changes in the tumour microenvironment which include increased calcium concentration, decreased cytosolic PH, reduced ATP for cellular functions, decreased mitochondrial permeability, decreased degradation of HIF-1, and increased level of nitric oxide (Henrich and Buckler, 2007 Hockel and Vauel, 2001, Semenza, 2003). Rothermund et al 2005 examined gene expression profile of LNCap cells after prolonged androgen ablation treatment *in vitro* using casodex found that membrane metallo endopeptidase, bcl -2, and cyclin G2 which are hypoxia target genes, were over expressed. The study concluded that AAT may induce hypoxia. This report has been confirmed by available data in the author's laboratory. This is in line with the earlier report of Tso et al. (2000) who observed that the androgen deprived LNCaP sub lines (LNCaP-H1) were fast growing, and had the ability to activate the growth of endothelia cells; in

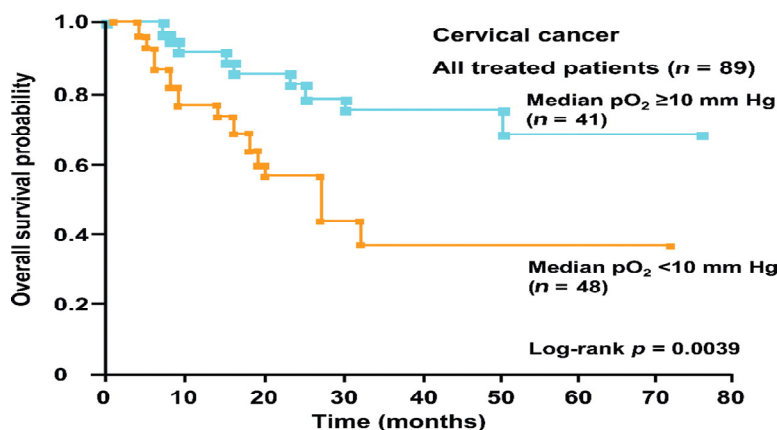


Figure 3. this figure shows that hypoxia is associated with malignant progression and poor overall survival in patients with advanced cancer. As you can see from the figure, patients with lower molecular oxygen level had very poor chance of survival compared to those with higher oxygen level. (Vaupel 2008).

addition there were resistant to both chemotherapy and radiotherapy when exposed to radiation. As mentioned earlier, exposing LNCaP cell line to 0.1% molecular oxygen level allowed to develop to more malignant and metastatic prostate cancer phenotype in vitro (Butterworth *et al.*, 2008). In addition, Zhou *et al.* (2004) also found that the androgen independent prostate cancer cells had decreased apoptosis and acquired higher proliferative rate than the androgen dependent cells; clearly, these features were also identified in hypoxic treated prostate cancer cell in the study of Butterworth *et al.*, 2008. Further studies found exaggerated androgen receptor gene over-expression and emergence of androgen refractory phenotype following androgen ablation (Patel *et al.* 2000; Nessler *et al.*, 2003; Zhou *et al.*, 2004). Available data demonstrates that hypoxia-selected LNCaP sub lines developed androgen independence and were less susceptible to radiation-induced apoptosis and were more tolerant to hypoxia (Butterworth *et al.*, 2008). Put together, AAT which is the mainstay of prostate cancer treatment may induce hypoxia to prostate cancer cells; this may select for cells with more malignant and aggressive potential.

Furthermore, Vaupel 2008 has recently from a clinical studies showed that prostate cancer patients with laboratory evidence of hypoxia had a poor overall survival compared to patients with normoxia (Vaupel, 2008; figure 3). In addition, abundant clinical evidence has accumulated to demonstrate the role of hypoxia in proper tumour control in different forms of solid cancer. It has been shown that tumour hypoxia promotes genomic instability and cause poor response to either chemotherapy or radiotherapy. Unfortunately, the mechanism behind the progression of androgen-

dependent prostate cancer cells to androgen refractory phenotype is still equivocal. Hughes *et al.* (2005) suggested that AAT intervention might induce some structural changes or mutations in the androgen receptors which results in androgen independence. However, the onset of androgen independence did not require any alteration in the status of androgen receptors (Sirotnak *et al.*, 2004).

AAT imposes a positive selection of prostate cancer cells with higher 5 alpha reductase activity which results in the increase in the level of DHT; which causes inhibition of HIF alpha I degradation, allowing it to bind to its beta homologue and activate the oxygen sensing pathways, resulting in over expression of genes responsible for loss of apoptosis, angiogenesis, metastasis and genetic instability (Marignol *et al.* 2008, Semenza, 2003). The works of Butterworth *et al.* 2008 clearly showed that hypoxia induced-LNCaP sublines was less susceptible to apoptosis, resistance to radiotherapy and more tolerant to hypoxia; and that this effect resulted in obvious treatment failure which is a common phenomenon in some clinical scenario of treat resistant prostate cancer. Thus there are sufficient documented data demonstrating that prostate cancer cells with higher metastatic phenotype were driven by hypoxia (Milosevic *et al.*, 2007).

Furthermore, prolonged exposure of prostate cancer cells to androgen ablation resulted in resistance to inhibition of phosphatidyl inositol -3 kinase, pi3K/Akt, indicating that the PTEN had been lost, suggesting that treatment-induced hypoxia may promote prostate cancer progression by triggering the akt/ signalling pathways Marignol *et al.* 2008; this may confer a survival advantage on the cells.

Therefore tumour hypoxia results in activation of

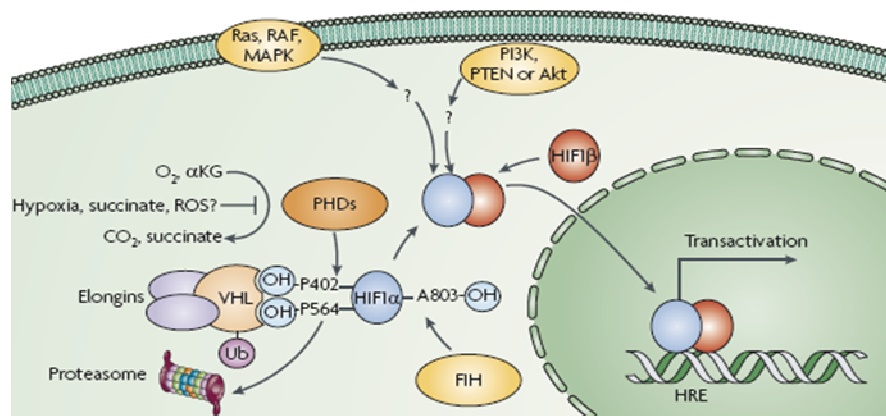


Figure 4. showing the stabilisation of HIF-1 α by hypoxia and other factors like mitochondrial reactive oxygen radicals, and subsequent activation of HIF -1 α induced gene expressions. (Denko 2008).

specific oxygen sensitive pathways including the hypoxia inducible factor I HIF -I, mammalian target of rapamycin mTOR kinase, and the unfolded protein response UPR pathway, Wouters and Koritzinsky 2008. These three pathways are independent but may also act in an integrated manner, with some level of influence on each other leading to common cumulative effects on gene expression, cell survival and tumour genesis, loss of apoptosis and increased angiogenesis and metastasis and resistance to therapy (Wouters and Koritzinsky 2008 Marignol et al., 2008; Dewhirst et al., 2008). For instance, hypoxia induced oxygen sensing signalling through mTOR and HIF I cause inhibition of the rate of mRNA translation initiation and activates the UPR; this has significant effect on gene expression and cellular behaviour that promotes angiogenesis and metastatic spread in the cancer cells (Wouters and Koritzinsky, 2008, Dewhirst et al., 2008).

Hypoxia and prostate cancer treatment

In a recent report from our laboratory; LNCaP cells exposed to hypoxia showed over-expression of genes that indicated a more malignant profile and tendency to metastasize to the bone (Butterworth *et al.*, 2008). Available data from the author's laboratory showed that androgen deprived prostate cancer tumour rapidly experienced dramatic drop in the molecular oxygen level within the tumour microenvironment as early as 24 hours after treatment; this cells developed gene expression profile suggesting that they had acquired the capacity for a single cell migration (Data not provided in this report). These strongly suggest that tumour hypoxia mediates aggressive and metastatic cancer progression. Furthermore, evidence from published data has shown that tumour hypoxic may be associated with over expression of such genes like TWIST, Runx-2, Zeb, Snail etc. These genes have been associated with obvious

increase in tumour metastasis and malignant spread of cancer cells to especially distant organs; this process commonly start with cells expressing evidence of EMT changes and single cell movement. Sufficient data discussed above all suggest that hypoxia targeting might serve as a therapeutic target.

Recently Marignol *et al.* (2008) and Butterworth *et al.* (2008) from our laboratory independently raised concern about the untoward effect of tumour hypoxia on malignant progression of prostate cancer. Till date continued reports have been emerging which suggest that tumour hypoxia could be a molecular target for tumour control in not only prostate cancer but also in other solid cancers (Milosevic et al., 2007). Emerging clinical and experimental evidences indicate that reduction in the degree of tumour hypoxia could increase the overall survival in cancer patients (Vaupel 2008; O'Rourke *et al.*, 2008) see figure 3. A recent phase 1 study of bio-reductive pro-drug Banoxantrone AQ4N, in patients with solid tumour indicates that selective targeting of hypoxic cancer cell which tend to be more malignant may improve survival in cancer patients (Albertella *et al.*, 2008). The bio-reductive drug have been shown to be reduced to a metabolite that has high cytotoxic effect only within the areas of tumour with remarkable hypoxia (Albertella *et al.*, 2008).

Hypoxia signalling through HIF-alpha

Hypoxia is one of the key factors in the tumour microenvironment that controls most of the malignancy – associated processes in the tumour; it is influenced by tumour type, volume, regional micro vascular density (MVD), blood flow, oxygen diffusion and consumption (Isa et al 2006; Serganova et al 2006). An important regulator of hypoxia is hypoxia – inducible factor (HIF) – a heterodimeric protein that contains oxygen regulated subunit (α) and a stable subunit (β) (figure 4). The biological functions of HIF have been extensively

described. HIF-1 is rapidly degraded within 5 minutes of its production in the presence of oxygen; and is ubiquitinated through the action of von Hippel-Lindau (VHL) protein upon recognition of hydroxylated proline residue in the HIF-1 α in presence of oxygen (Serganova et al 2006). However, in hypoxic microenvironment, prolyl hydroxylases are inactivated, and HIF-1 α degradation is blocked and stabilised; this allows HIF-1 α to accumulate and associate with HIF-1 β to form a functional transcription complex that triggers the transcription of a host of hypoxia-inducible genes (figure 4). Evidence has shown that HIF-1 targeted genes are essential for Epithelial to mesenchymal transformation (EMT) during hypoxia mediated fibrogenesis and hypoxia induced metastasis respectively; these mediate transcription of more genes involved in malignant and metastatic cancer progression and angiogenesis (see figure 3 and 4). In conclusion, tumour hypoxia is a recognised predictor of poor treatment outcome in patients treated of advanced localised endocrine cancers including prostate cancer. We have shown that hypoxia by signalling through a master transcription factor HIF-1 controls over many target genes to alter gene expression profile that promote aggressive and malignant tumour growth and metastasis. Tumour hypoxia results in resistance to chemotherapy and radiotherapy and development of survival advantage in cancer cells. Evidence from phase I clinical trial showed that targeting tumour hypoxia using a bioreductive prodrug could improve treatment outcome (Albertella et al., 2008). We therefore suggest that development of a combination therapy that target hypoxia may provide a better treatment protocol for management of prostate cancer.

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