

Hypoxia in prostate cancer

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Most human solid tumours contain areas which are less oxygenated than normal tissues. Hypoxia increases resistance to radiotherapy, surgery and chemotherapy, and directly alters the function of tumour cells, stimulating them to de-differentiate and to release angiogenic factors with a view to increasing the blood and oxygen supply. Tumour hypoxia promotes malignant progression and metastasis formation. HIF-1 is a heterodimeric transcription factor composed of regulated HIF-1 α and constitutively expressed HIF-1 β . Tumour-associated activation of HIF-1 α seems to be primarily, however the result of adaptation to oxygen shortage.

The presence of the HIF-1 α subunit overexpression has been confirmed in many tumours, in prostate cancer, among others; the role it plays in its progression is yet to be explained. Numerous studies strongly emphasize the importance of evaluating the status of the HIF-1 α transcription factor in predicting the clinical and biochemical recurrence of prostate cancer and its resistance to castration.

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Introduction

Prostate cancer is one of the most frequently diagnosed solid malignant tumours in the male population in the world. Prostate cancer is the second leading cause of neoplasms in men, accounting for over 13% of malignant tumours in Poland. Most tumours affect men over 65 years of age. Over the past three decades, the number of cases has increased five times [1]. Most patients have organ-confined or locally advanced tumours at the time of diagnosis [2].

The majority (90%) of prostate cancers are adenocarcinomas. Rare histopathological forms include sarcoma, squamous cell carcinoma, basal cell carcinoma, urothelial carcinoma, small cell carcinoma and others. The degree of histopathological variation in prostate cancer is evaluated based on the Gleason classification, which provides important prognostic information. A higher Gleason score means a higher risk of progression, recurrence after radical treatment, growth of metastases and death.

Biochemical recurrence will occur in 15–50% of patients despite radical treatment; a part of patients will develop metastasis and die [2–3]. The rate of relapse depends on

prognostic factors, such as the TNM stage, the degree of malignancy according to Gleason as well as the initial PSA (prostate-specific antigen) concentration. However, these factors only partially explain the variation in treatment effects.

Advances in medical genomics and molecular medicine have revolutionized cancer research in recent years. Research into targeted therapies is underway, with the aim of ensuring long-term tumour control while causing fewer side effects than current standard treatments.

Strategic issues in prostate cancer treatment and diagnosis include not only identifying the group of patients with aggressive disease, but also developing personalized treatment methods tailored to individual patients. More efficient prostate cancer markers than those used to date still need to be found. Priority should be given to finding markers for identifying high-risk patients in whom standard treatment modalities are unsatisfactory, and who would be eligible for clinical trials using more aggressive methods. Identifying the markers of prostate cancer would provide insight into the aetiology of the disease and into molecular mechanisms underlying its progression, thus setting the direction for the

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search for new therapeutic targets. Progressive disorders of genes which regulate cell proliferation, differentiation and migration as well as signalling at the intracellular level affect the development of endothelial neoplasia in the first place and the occurrence of invasive prostate cancer in consequence. Hypoxia, a hallmark of many human malignancies, is considered to be a crucial modulator of the clinical features of cancer and the response to treatment, which is affected by genomic and molecular changes that induce local progression and the formation of metastases [4, 5]. Increasingly, the tumour microenvironment and hypoxia are investigated as potential prognostic factors in prostate cancer [6]. The expression of specific tumour hypoxia markers is an important predictor of therapeutic outcomes in many neoplasms, treated not only with ionizing radiation, but with surgery as well [7].

Hypoxia in a tumour

Hypoxia, as a pathophysiological consequence of the mismatch between oxygen supply and demand, is a feature of neoplasm resulting from high metabolic requirements of rapidly dividing neoplastic cells that depend on dynamic, unpredictable and ineffective neovascularization. Most solid tumours grow on their own blood supply, provided in a process called angiogenesis. Increased oxygen demand leads to the growth of a chaotic network of blood vessels. In spite of intense neovascularization, tumours are characterised by low vascular density and a poorly developed network of arterioles, which impairs and reduces the efficiency of blood transport in the tumour.

Thomlinson and Gray were the first to report the presence of hypoxia in a malignant tumour in 1955 [8]. They examined histopathology slides from fresh tissue specimens of human lung cancer. They observed vascularized areas of tumour which supplied oxygen and nutrients to tumour cells at a distance of 150–180 microns. Thomlinson and Gray discovered an area of living tumour cells capable of proliferation, surrounded by a rim of blood vessels, and a necrotic area in which anoxia developed along with the growth of the peripheral area of the tumour. Chronic hypoxia was defined by the presence of hypoxic, viable yet non-oxygenated cells at the border between the oxygenated area and necrotic area.

Today we know that cancer is a heterogeneous population of cells with varying degrees of oxidation. Clinically significant concentrations of hypoxia are detected in 50–60% of all malignancies [9]. Their oxidation is lower than in normal tissue, in which partial oxygen pressure ranges from 20 mm Hg in the liver and brain to 70 mm Hg in the kidneys [10, 11]. When the oxygen level in tissues falls below physiological norms, their capacity to perform normal cellular functions becomes impaired. Oxygen concentration below 10 mm Hg increases the expression of HIF-1 factor and activates many

other molecular pathways which ensure the maintenance of basic cellular functions. This complex and dynamic response has massive implications such as increased angiogenesis, transition from aerobic to anaerobic metabolism, the inhibition of apoptosis and activation of growth factors as well as irreversible changes in cell genome [12–14].

The relative level of oxygen in the tumour during irradiation affects the effectiveness of radiotherapy. At partial oxygen pressure below 10 mmHg, neoplastic cells become hypoxic and 2–3 times more radioactive than well-oxidised cells. Radiation resistance of these cells is related to the lack of oxygen, which is responsible for the fixation of DNA damage caused by irradiation [15]. Radiation resistance of hypoxic cells in tumours is highest at pO₂ below 5 mmHg [16, 17].

Although it is acknowledged that hypoxic cell fractions are present in most neoplasms, their impact on progression, the formation of metastases and response to therapy varies, and it is unlikely to be equally relevant in all patients.

HIF-1 is one of the most important proteins that allow cells to adapt to low levels of oxygen in the environment.

HIF-1 protein

HIF-1 protein (hypoxia-induced factor) is a heterodimeric transcription factor composed of two alpha and beta subunits [18, 19]. The following three genes have been identified in humans: HIF1A, EPAS1 and HIF3A, which encode HIF-1 α , HIF-2 α and HIF-3 α , respectively [20, 21]. HIF-1 α and HIF-2 α have a similar structure, functions and regulatory pathways, whereas HIF-3 α acts as an inhibitor of the transcriptional response to hypoxia. Under aerobic conditions, the alpha subunit of HIF-1 protein is degraded in the cell via ubiquitization. The beta subunit is an oxygen-independent constitutive protein located in the cell nucleus [22]. The process of alpha subunit degradation is initiated by posttranslational hydroxylation of amino acids proline 402 and proline 564 [23–25]. Because of hydroxylation, the alpha subunit of HIF protein is recognized by the von Hippel-Lindau protein (pVHL), a fragment of the ubiquitin ligase complex. A product of the VHL suppressor gene — the Hippel-Lindau protein (pVHL) — is attached to thus modified alpha subunit, followed by the ubiquitin molecule. The degradation of the alpha subunit takes place in the proteasome.

Under hypoxic conditions no hydroxyl groups are attached to the alpha subunit and no hydroxylation and interaction with pVHL takes place. Subsequently, the HIF-1 α protein preferentially associates with transcriptional coactivators p300 and CBP. HIF-1 protein breakdown by ubiquitization becomes inhibited in consequence. At this point, the alpha subunit enters the cell nucleus and binds to the beta subunit, which stimulates gene expression in response to hypoxia. Hypoxia-regulated genes include those involved in angiogenesis (encoding VEGF — vascular endothelial

growth factor), glycosylation (encoding GLUT — glucose transporters), and pH control (encoding carbonate anhydrase IX) [22, 26].

Under hypoxic conditions, HIF-1 protein can induce the expression of several dozen genes that encode proteins responsible for energy metabolism, neovascularization, intracellular pH and the migration of tumour cells.

Even though the presence of HIF-1 α transcription factor overexpression has been confirmed in many cancers including breast, rectum, prostate and cervix cancers [27–32], its role in progression remains to be explained. High concentrations of HIF-1 α in renal and breast cancer cell lines increased tumour cell survival; in ovarian cancer, they were associated with enhanced apoptosis. The correlation between the increased expression of HIF-1 α and resistance to apoptosis as well as a worse prognosis is not the same in every neoplasm. In a non-small cell lung cancer study, Volm et al. have shown a relationship between the HIF-1 α expression and tumour cell apoptosis and increased median survival [32]. Another study in a similar group of patients did not confirm these findings [33].

In many tumours HIF-1 α overexpression is observed at an early stage of tumour development and it correlates with an increased density of vascularization in the lesion. Bos et al. have found that ductal *in-situ* carcinoma, which is an early pre-invasive stage of breast cancer, is characterized by an increased expression of HIF-1 α protein, which is associated with higher vascular tumour density [34]. The same phenomenon has been observed in ovarian cancer and in brain tumours, suggesting that HIF-1 activity contributes to amplified production of proangiogenic factors such as VEGF [35, 36]. The relationship between HIF-1 α expression and tumour progression as well as the level of vascularization has also been proven in brain tumours [36].

Examining the expression of HIF-1 α in nasopharyngeal carcinoma, Aebersold et al. have shown that patients with the HIF-1 α expression in > 10% of neoplastic cells had a 3times lower chance of complete remission after radiotherapy compared to those with increased expression of HIF-1 α protein in < 10% of tumour cells [37].

Hypoxia in prostate cancer

Four key methods are used in clinical practice to identify hypoxic cells in tumours: immunohistochemical evaluation of protein expression — intracellular and extracellular hypoxia markers, microelectrodes, the imaging of hypoxic area and the imaging of haemoglobin oxidation.

The existence of hypoxic areas in prostate cancer has been proved to date by many researchers [38, 40–42]. With immunohistochemistry tests, which use hypoxic cell markers and direct oxygen electrode measurements, clinically relevant levels of hypoxia are detected in 30–90% of prostate cancer cases. Unfortunately, little is known about the

influence of hypoxia on progression-free time and on long-term effects of radiotherapeutic and surgical treatment in prostate cancer. Vergis et al. examined tissue microarrays obtained from a group of 201 patients with locally advanced prostate cancer who underwent neoadjuvant hormone therapy and radiotherapy. Their research showed that increased expression of HIF-1 protein and VEGF gene were independent factors associated with biochemical recurrence. Vergis et al. have demonstrated that increased expression of transcriptional factor HIF-1 α is an independent predictor of biochemical progression in patients with prostate cancer treated with radiotherapy or prostatectomy [43]. Their findings are consistent with the results presented by Movsas et al. [42] who examined a group of 57 patients with low- and intermediate-risk prostate cancer who received brachytherapy. Movsas et al. have found a significant relationship between tumour oxidation and time to biochemical recurrence. This fact may indicate that hypoxia is a clinically important determinant of disease progression. However, it should be borne in mind that increased expression of HIF-1 may also be induced by hypoxia-independent factors such as altered expression of oncogens and tumour suppressors, oxygen free radicals, androgens and other growth factors [44–46].

Researchers from the Princess Margaret Cancer Centre in Toronto conducted a prospective study in a group of 247 prostate cancer patients in which Eppendorf microelectrodes were used for measuring oxygen pressure in prostate prior to radiotherapy. Median pO₂ was 6.8 mm Hg and the median percentage of hypoxia was less than 10 mm Hg [47]. Their findings show that hypoxia is associated with early biochemical recurrence and local recurrence in prostate after radiotherapy or radiotherapy associated with hormone therapy.

Researchers from the F. Łukaszczyk Centre of Oncology in Bydgoszcz analysed 43 paraffin blocks of prostate adenocarcinoma from patients after primary prostatectomy. They evaluated the severity of hypoxia in prostate tissues on the basis of HIF-1 α protein expression analysis. The presence of hypoxia in primary tumour was observed in 90.7% of prostate cancer cases. The researchers have also reported a statistically significant correlation between the HIF-1 α binding index and the tumours' histological grade (degree of malignancy) on the Gleason scale [48]. In many other studies, no significant correlation was found between the HIF-1 α expression and standard prognostic factors in prostate cancer [27, 28, 49–51].

Zapatero et al. conducted a retrospective study evaluating the expression of HIF-1 alpha in a group of 86 men with intermediate and high-risk prostate cancer patients subjected to hypofractionated radiation therapy combined with hormonal therapy. The median follow-up time was 10 years. Surprisingly, cytoplasmic overexpression of HIF-1 α was associated with the improved overall survival [52].

Prostate cancer is a hormone-dependent tumour whose systemic treatment consists chiefly in eliminating androgens from the body. The combination of radiotherapy with hormone therapy in the treatment of locally advanced prostate cancer reduces clonogenic tumour cells and, thus, offers a greater chance of eliminating the remaining cells during radiotherapy. Moreover, the use of hormone therapy — by reducing the number of cells within the tumour — improves the oxygenation of the remaining cancer cells. Recent research has shown that hormonal therapy enhances oxygenation in prostate cancer cells [53]. However, although hormone therapy is highly efficient, some patients have progression after a period of time, which occurs because prostate cancer cells are no longer dependent on endogenous androgens. In the 1990's Visakorpi et al. demonstrated that under such circumstances, signal transduction pathways related to androgen receptors still functioned in prostate carcinoma cells and that the receptors themselves are activated regardless of the presence of endogenous androgens [54]. With testosterone at castration levels, castration resistant prostate cancer (CRPC) is developed. CRPC is a deadly form of cancer, with a high metastatic potential. As many as 84% of patients have developed metastasis when resistance to castration has been diagnosed. CRPC is linked with a significant deterioration of the quality of life [55]. Searching for factors that will help identify the patients who will develop resistance to castration is of prime importance. Currently there are only a few biomarkers for identifying CRPC. Therapeutic possibilities are scarce for patients with this diagnosis [56, 57].

Ranasinghe et al. evaluated the role of HIF-1 α in the formation of CRPC under *in vitro* conditions [58]. They examined three human prostate cancer cell lines (PC3, DU 145 and LNCaP) and have shown that HIF-1 α is an independent risk factor for CRPC development. HIF-1 α expression was independent of the Gleason scale, tumour stage and treatment that had been administered. Multivariate analysis has demonstrated that the risk of developing castration resistant prostate cancer in patients with HIF-1 α overexpression subjected to hormone therapy was 10 times higher. This observation clearly highlights the importance of assessing the status of transcriptional factor HIF-1 α in predicting the development of prostate cancer castration resistance and in identifying patients eligible for novel second-line hormone therapy.

Summary

Hypoxia is a negative predictor of treatment outcome not only with via radiotherapy, but also via surgery and chemotherapy [59, 60]. It represents a strong rationale for research into the association of standard oncological treatment with hypoxia-guided factors. Finding a clinically

relevant hypoxia test that will be inexpensive, non-invasive and reproducible remains to be the greatest challenge.

List of abbreviations:

VHL — von Hippel-Lindau

pVHL — von Hippel-Lindau protein

VEGF — vascular endothelial growth factor

HIF-1 — hypoxia-inducible factor

HIF-1 α — hypoxia-inducible factor 1 alpha

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