Hypoxia - an Explanation of Prostate Cancer Progression Mechanisms

Paweł Szymański¹, Krzysztof Roszkowski²*

¹ Department of Radiotherapy the F. Łukaszczyk Oncology Centre, Bydgoszcz 85-796, Romanowskiej 2, Poland
   Tel. +48523743746; E-mail: szeptycp@wp.pl

² Department of Oncology, Radiotherapy and Gynecologic Oncology, Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz 85-796, Romanowskiej 2, Poland

* Correspondence: Tel. +48523743472; E-mail: roszkowskik@cm.umk.pl

ABSTRACT: Prostate cancer (PC) is a common malignancy in males in most industrialized countries, where it is the most commonly diagnosed cancer affecting men after middle age (>50 years). Although the screening and surgical procedures for prostate cancer have improved, successful treatment is still a major challenge. In the tumor microenvironment, hypoxia is one of the crucial factors which promote an aggressive phenotype of tumor cells and decrease the effectiveness of standard treatment. It implies that tumor cells surviving hypoxic stress are likely to be a significant source of viable clonogens that can repopulate tumors with more malignant/metastatic cells. Unfortunately, most treatment protocols are less effective against hypoxic cells which are resistant not only to radiotherapy, but also to standard cytotoxic chemotherapy. There is now a considerable amount of clinical evidence that tumors with a higher proportion of hypoxic cells have a poor diagnosis. Tolerance of hypoxic conditions varies in different tumor types. However, prostate cancer cells seem to be highly tolerant of hypoxia. The main problem concerning the effectiveness of prostate tumor therapies are changes in the biology of hypoxic tumor cells after standard hormone- and radiotherapy. Despite the many studies of tumor hypoxia, very little attention has been given to the oxygen concentration in the conditions of in vitro cancer cells studies. To date, there has been no comprehensive characterization of prostate cancer cells under hypoxic condition, which seems to be crucial in the light of the intensive search for novel cancer therapies.

Keywords: Hypoxia; Prostate cancer; Cancer stem cells.

1. INTRODUCTION

Prostate cancer (PC) is among the most common malignancies in men [1, 2]. Metastatic PCs still represent the second leading cause of cancer-related deaths. Although important advances have led to an earlier diagnosis and effective therapeutic intervention by prostatectomy and/or radiation therapy in patients with localized PCs, the disease progression to locally invasive and metastatic castration-resistant prostate cancers is generally associated with treatment resistance and disease relapse [3]. Moreover, current anti-hormonal treatments against metastatic PCs are only palliative and lead to death in most patients after approx. 12–19 months [4, 5]. In the tumor microenvironment, hypoxia is one of the crucial factors which promote an
aggressive phenotype of tumor cells and decrease the effectiveness of standard treatment. Hypoxia leads to the selection of more malignant cells by inducing a series of cellular adaptation processes that sustain and foster tumor invasion [6-8].

It is accepted that hypoxia induces selection of stress-resistant tumor cells with more malignant features. It implies that tumor cells surviving hypoxic stress are likely to be a significant source of viable clonogens that can repopulate tumors with more malignant/metastatic cells. Despite the many studies of tumor hypoxia, there is a considerable degree of confusion with this term. “Normoxia” is almost universally used to describe the “normal” oxygen levels in the gaseous phase within the tissue culture flasks, i.e. approx. 20–21% oxygen. Despite the widespread usage of “normoxia”, it is far from being an accurate comparator for the oxygenation of peripheral tissue in which median oxygen levels range from 3.4% to 6.8%, with an average of approx. 6.1%. It is proposed that 5% oxygen is a more accurate approximation of tissue oxygenation and that this value should be recognized as “physoxia”. In turn, “physiological hypoxia” can be defined as the oxygen level at which tissues respond to maintain their preferred oxygen level. Since normal tissues are usually maintained at 3–7% oxygen, physiological hypoxia is likely to be in the range of 2–6% oxygen. Angiogenesis in tumors is often enhanced, yet the oxygen levels (even in untreated tumors) are significantly lower, ranging from 0.3% to 4.2%, with almost all decreasing to under 2% [3]. Once tumor cells become pathologically hypoxic, the proportion of cells in this fraction depends on their hypoxia tolerance. The more tolerant they are, the longer they will remain quiescent, yet still viable, resulting in a proportionally more hypoxic tumor with a larger hypoxic fraction. Conversely, a hypoxia-sensitive tumor cell will die more quickly, so the hypoxic fraction will be smaller. Well-adapted tumor cells acquire significantly reduced requirements for oxygen; this leads to a markedly improved ability to survive in hypoxic conditions. Genetic changes caused by hypoxia are often measured in vitro and compared with “normoxia” which 20% oxygen is most frequently [3, 9]. These are non-physiological conditions which expose cells to a roughly 10 times higher oxygen concentration than that encountered in their natural niches. It would be more relevant to normal tissue if control cells were maintained in physoxia, i.e. 5% oxygen, and compared with physiological hypoxia (1–3%) and pathological hypoxia (0.1–0.5%).

There are two types of hypoxia: chronic (uninterrupted) hypoxia, associated with an increasing distance of proliferating cells from the vessels, and cycling (acute, interrupted) hypoxia, mainly caused by fluctuations in the blood flow rate [8, 9]. In the actual intratumoral microenvironment, continuous hypoxia is rare, and hypoxia and reoxygenation are more frequent conditions due to the irregular blood flow during ischemia–reperfusion [8]. The existence of cycling hypoxia has been directly observed in human tumors. The presence of cycling hypoxia in tumors has direct consequences on the tumor behavior. It promotes spontaneous metastasis, and cells exposed to such conditions have an even greater metastatic potential than cells exposed to chronic hypoxia. Cycling hypoxia also affects the effectiveness of anti-cancer therapies, predominantly radiotherapy (RT) [9]. Recent studies have shown that cycling hypoxia can also be a factor in selecting and promoting cells with stem-cell like phenotype, presenting increased tumor-initiating capabilities and metastatic potential [8, 10]. An increasing amount of evidence demonstrates that alterations in microenvironmental oxygen levels and activation of hypoxic signaling through hypoxia-inducible factors (HIF) are emerging as important triggers and modulators of the epithelial–mesenchymal transition (EMT) which is now thought to play a key role as the convergence point between hypoxia and cancer.

2. PROSTATE CANCER STEM CELLS

Cellular heterogeneity is characteristic for most human tumors, also for prostate cancer. Prostate cancers contain multiple independent clones [11]. Considering tumor heterogeneity, cancer stem cells (CSCs) are a small subpopulation with a self-renewal potential, clonal tumor initiation capacity and clonal long-term repopulation ability [12] (Fig.1).
CSCs are also able to escape cell death and metastasize, although they may be inactive for long periods of time [13]. A lot of clinical studies indicate that these cells survive treatment with many cancer therapeutics. CSCs reside in niches, which are specialized microenvironments regulating stem cell fate and, in the case of cancer stem cells, are part of the tumor microenvironment [14]. As aforementioned, CSCs represent only a small subpopulation of cancer cells within a tumor with the potential to regenerate the tumor. This population shares a number of characteristics with normal stem cells such as self-renewal or high proliferative potential. CSCs express many surface markers and transcription factors, including CD24, CD44, CD133, OCT-4, SOX-2 and others. These cells also possess multilineage differentiation potential. Due to all features mentioned above, CSCs in prostate cancer are thought to be responsible for tumor progression, metastasis and therapeutic resistance [15]. The main biomarker which have been identified on the surface of CSCs and distinguish these cells from the bulk of the tumor is CD133. CD133 (known as AC133 and prominin-1) is also the most common used cell surface antigen to detect and isolate cancer stem cells from different tumors, including prostate [16, 17]. The physiologic role of CD133 in the progression of cancer remains elusive. Some studies suggested a potential role of this biomarker in determining cellular fate or maintaining stem cell-like properties, however the molecular mechanism is still unclear.

Hypoxic niches are a preferred location of CSCs, and HIF-1 has an important role in controlling these cells. CSCs have characteristic functional features, which make CSCs different from the bulk tumor cells and enable them to initiate and maintain tumor development [18, 19]. Hypoxia in tumor microenvironment has also been shown to promote CD133 expression via hypoxia inducible factor-1α (HIF-1 α) upregulation [20-22]. CD133 has been postulated to identify CSCs population, also in prostate cancer. However, some controversies are related with this idea, e.g. it is also expressed in normal stem cells, so it is difficult to differentiate between CSCs and non-stem like cancer cells. What is more CD133pos CSCs population have only been shown to represent 1-5% of the total cell population in prostate cancer cell lines. However, this CD133pos populations can be enriched through chemotherapy and radiotherapy, claiming that these cells, at least at some level, are chemo/radioresistant [23]. Reyes et al. showed that CD133pos cells exhibit higher proliferative potential than CD133neg population. These results suggest that CD133pos cells do have enhanced potential for cell division despite chemo or radiotherapy. However, it is still unclear whether CD133 plays a direct role in prostate cancer stem cells maintenance or it is related with aggressive form of disease [24].

One important capability of CSCs is the epithelial-mesenchymal transition (EMT). This event plays an important role in promoting cell migration and the development of metastasis.
3. ROLE OF EPITHELIAL–MESENCHYMAL TRANSITION AND CANCER STEM CELLS

Epithelial-mesenchymal transition (EMT) is a cellular process in which cells lose epithelial markers and features and acquire mesenchymal characteristic [25] (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Characteristic of EMT.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EMT state</strong></td>
</tr>
<tr>
<td>Cell shape</td>
</tr>
<tr>
<td>Cell adhesion</td>
</tr>
<tr>
<td>Surface markers</td>
</tr>
</tbody>
</table>

Induction of the EMT has been demonstrated in various cell lines after hypoxia or constitutive expression of HIF, as shown by a shift from epithelial to mesenchymal markers and an increase in the ability of migration and invasion [26]. A hallmark of the EMT is the loss of E-cadherin expression, a very important caretaker of the epithelial phenotype [27, 28]. Repression of E-cadherin expression, often correlated with tumor grade and stage, results in the disruption of cell–cell adhesion and an increase in the level of beta-catenin in the nucleus. The EMT also includes downregulation of epithelial markers, such as desmplakin and plakoglobin, and upregulation of mesenchymal markers, such as vimentin, fibronectin and alpha-smooth muscle actin [29]. It has been widely accepted that the EMT has a central role in cancer progression and metastasis [30]. It has been associated with many tumor functions like tumor initiation, progression, tumor stemness and resistance to therapy [25]. It is the most important mechanism behind the initiation of cancer metastasis. By adopting a mesenchymal phenotype through the EMT, individual carcinoma cells obtain the ability to infiltrate adjacent tissues, cross endothelial barriers and enter the circulation through blood and lymphatic vessels [29, 31-33]. It is proved that isolation of different tumor cell populations based on EpCAM or E-cadherin are associated with increased tumor propagating potential [25]. There is now a considerable amount of clinical evidence that tumors with a higher proportion of hypoxic cells have a poor prognosis. Studies have shown that patients have a markedly poorer prognosis when their tumors are more hypoxic [3, 18]. It is likely that these patients will have a larger number of hypoxia-tolerant cells with more malignant features free in the circulation and/or already at secondary sites, with both factors having impact on recurrence and survival. Concluding, the basic understanding of the mechanisms controlling EMT should be used in developing new therapeutic strategies to control tumor progression, metastasis and resistance to common therapies.

4. SIGNIFICANCE OF NUCLEOTIDE SIGNALING IN PROSTATE CANCER

Elevated concentrations of ecto-adenosine (ecto-Ado) are associated with the increased in vitro proliferation capacity of different populations of stem cells and cancer cell lines. Ecto-5’-nucleotidase (ecto-5’-NT, CD73) is responsible for conversion of AMP to ecto-adenosine in the extracellular environment. The most recent data show that the ecto-5’-NT gene is the most increased gene in the TGF-β1-induced gallbladder carcinoma cell line (GBC-SD cells), as compared with normal GBC-SD cells. Transforming growth factor-β (TGF-β) modulates epithelial plasticity in the physiological contexts of the tumor by inducing epithelial–mesenchymal transitions (EMT) [34]. On the other hand, LaSOM 63, a monastrol derivative acting as a 5’-NT inhibitor, induces apoptotic cell death of glioma cell lines [35].
Moreover, the activity of ecto-5’-nucleotidase in normal endothelial and epithelial cells increases as an effect of their adaptation to hypoxia [36]. To date, there has been no comprehensive characterization of prostate cancer cells under hypoxic conditions, particularly in respect to their ecto-5’-NT activity. However, it cannot be excluded that the ecto-5’-NT activity in prostate cancer cells and/or prostate cancer stem cells, similarly as in endothelial cells, is upregulated. Adenosine receptors mediate the adenosine signaling and have been found on cell membranes of different human tumor cells, i.e. SH-SY5Y neuroblastoma, A375 melanoma, colon carcinoma HT29 and human breast cancer MCF-7 cells [37]. Full characterization of the role of adenosine in prostate cancer also requires addressing the question of whether adenosine receptors are present on the surface of cancer cells.

5. HYPOXIA AND RESISTANCE TO ANDROGEN DEPRIVATION?

Androgens and androgen receptor play an important role in the proliferation of human prostate cancer. Androgen action is a summed effect of bioactive androgens (such as DHT and testosterone) and the responsiveness of the androgen receptor (AR) in target cells. Free testosterone, after diffusion through the cell membrane, binds specifically to the AR in the cytoplasm causing nuclear translocation of the receptor. Critical function of the activated AR is to trigger the expression of other genes. This mechanism is not fully described, but it is known that the concentration of significant numbers of proteins may be regulated by the activated AR [38, 39]. Since the development and progression of prostate cancer depends on androgenic stimulation, treatment of this cancer relies on depriving the tumor of androgens or blocking their actions [40]. Defects in the AR can play a role in metastatic prostate cancer. The mutated receptor stimulates prostate growth and development of metastases despite androgen ablation. This treatment can reduce primary and metastatic lesions probably by inducing the apoptosis of tumor cells expressing the wild-type receptor. As the AR is of critical role in cell biology, it is important to measure the total amounts of the AR present in different types of cells and tissues [41].

Bicalutamide, a drug widely used in locally advanced prostate cancer (androgenic blockade) has a short-term efficacy with prostate cancer. Based on the literature data, the drug is suspected to cause hypoxia and can select for hypoxia-tolerant tumor cells that present more malignant features [2]. This can be the foremost reason for which vessel-targeting drugs, used as single agents, are not as successful as originally expected. It is possible that many treatments cause early antivascular effects and the associated increase in hypoxia. Tumors can adapt to this hypoxic insult and recover with a more proangiogenic and potentially malignant phenotype. There is no analysis of effectiveness of hormonotherapy against distinct populations of PC cells in different oxygen concentrations, which reflect the in vivo conditions.

5. CONCLUSION

The comprehensive biological analysis of different PC populations cultured in hypoxic conditions and undergoing standard treatment (radio- and hormonotherapy) will help to indicate cells that are responsible for the recurrence of the disease and factors that can be used as diagnostic and prognostic markers in patients with cancer. It seems to be a crucial aspect in the light of novel, personalized cancer therapies.

Author Contributions: PS: study design/planning; data collection; data interpretation; literature analysis/search, wrote the initial draft of the manuscript. KR: literature analysis, data interpretation, preparation of manuscript. The final manuscript has been read and approved by all authors.

Conflict of Interest: The authors declare that they have no conflict of interest.
Financial disclosures: No financial disclosures from any author.

REFERENCES

