Androgen Withdrawal in Patients Reduces Prostate Cancer Hypoxia: Implications for Disease Progression and Radiation Response

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Abstract

Hypoxia is a feature of many human malignancies, and leads to aggressive clinical behavior and recurrence after treatment. Here, we show for the first time that androgen withdrawal reduces prostate cancer hypoxia in patients. Oxygen measurements were done in 248 patients with clinically localized prostate cancer prior to radiotherapy, and showed hypoxia of potential biological and clinical significance. In 22 of these patients, prostate oxygen levels were measured both before and after 30 to 145 days of the androgen antagonist bicalutamide. There was a significant reduction in tumor hypoxia with androgen withdrawal ($P = 0.005$). The median $pO_2$ increased from 6.4 to 15 mm Hg, and the hypoxic proportion decreased from 40% to 31%. However, the response was heterogeneous, with improvement in 12 patients, stable oxygen readings in 9 patients and worsening hypoxia in 1 patient. Among the responding patients, the median $pO_2$ increased from 4.9 to 33 mm Hg, and the hypoxic proportion decreased from 51% to 23%. There was no apparent relationship between the change in oxygenation and baseline prostate volume, $T$ category, Gleason score, prostate-specific antigen levels, the duration of treatment with bicalutamide, or the change in prostate-specific antigen levels with bicalutamide. These results might, in part, explain the improved patient outcome that has been observed in clinical trials of radiotherapy and hormones, and suggest a role for novel therapeutic agents that block the molecular response to hypoxia in prostate cancer either alone or in combination with other established treatments. [Cancer Res 2007;67(13):6022–5]

Introduction

Prostate cancer is the most common malignancy among North American men. At diagnosis, most patients have tumors that are clinically confined to the prostate gland. Depending on comorbidities and individual preferences, they are candidates for potentially curative treatment with either radical prostatectomy or radiotherapy. Despite technical advances in both of these treatments, ~25% of radically treated patients will develop progressive disease either locally in the pelvis or at remote sites, most commonly in bone (1). This underscores the importance of better understanding the biological factors that are responsible for malignant progression, the development of metastases, and the failure of currently available treatments.

Androgens play an important role in the growth and progression of prostate cancer. Androgen withdrawal through surgical or medical castration causes tumor regression in most patients. Several phase III studies have shown improved tumor control and patient survival when radiotherapy is combined with androgen withdrawal to treat prostate cancer (2), and combined treatment is widely used in clinical practice. Despite this, the biological mechanisms responsible for the favorable interaction between radiotherapy and androgen deprivation remain ill-defined.

Hypoxia is a feature of many human malignancies. In general, patients with hypoxic primary tumors at diagnosis are at greater risk of developing progressive disease and dying regardless of whether initial treatment is with surgery or radiotherapy (3). These clinical observations are consistent with hypoxia-mediated changes in DNA repair, genomic instability, and abnormal expression of genes that promote both malignant progression and metastasis formation (4). Faulty DNA repair is an important determinant of genetic instability and contributes to chromosomal rearrangement, oncogene activation, and tumor suppressor gene inactivation (5). Repair-deficient cells are more likely to acquire a mutator phenotype characterized by greater biological and clinical aggressiveness. Indeed, preclinical data have shown that hypoxia could lead to the selection of aggressive cancer cells with decreased sensitivity to apoptotic and DNA repair pathways and increased genetic instability, angiogenesis, proliferation, and metastatic capability (4, 5).

We and others have previously reported that many prostate cancers are hypoxic (6–8). Here, we show for the first time that androgen withdrawal reduces prostate cancer hypoxia in patients, and discuss the implications of this for prostate cancer development and progression, metastasis formation, and response to radiotherapy.

Materials and Methods

A total of 248 patients with $cT_1c-T_2c$, $N_0$, $M_0$ (UICC-TNM Classification, 6th Edition, 2002) prostate cancer participated in this prospective study of tumor hypoxia prior to treatment with escalated-dose conformal or intensity-modulated radiotherapy. The characteristics of these patients are summarized in Table 1. The study was approved by the University Health Network Research Ethics Board, and informed consent was obtained in all cases.

Prostate cancer hypoxia was measured prior to any treatment using an ultrasound-guided transrectal needle-electrode technique, as previously described (7). Between 40 and 80 individual oxygen readings were obtained along two to four linear measurement tracks 1.5 to 2 cm in length through regions of the prostate gland likely to contain tumor based on information from previous diagnostic biopsies, digital rectal examination, and real-time
hypoxic proportion (HP5), defined as the percentage of the individual oxygen readings that were nearly identical. Readings are presented, as the results using the transformed data were relatively robust to departures of the data from a normal distribution. Nevertheless, all analyses were done both before and after logarithmic transformation of the oxygen data. Only the analyses using the raw oxygen readings were designated as arising from either the superficial (subcapsular) or deep prostate gland by dividing the measurements along each linear track into two equal groups. The deeper regions of the gland were more hypoxic than the superficial regions along each linear track. The individual oxygen readings varied from 6.2 to 9.8 mm Hg. The individual oxygen readings as the dependent variable. We previously described hypoxia in benign prostatic hypertrophy using tissue-based markers (6). These observations suggest a global reduction in prostate oxygenation in patients with prostate cancer that is not necessarily confined to tumor. Therefore, all oxygen measurements along all tracks were included in this analysis. Hypoxia was summarized using the median pO2 and the hypoxic proportion (HP5), defined as the percentage of the individual oxygen measurements <5 mm Hg. A threshold of 5 mm Hg was chosen to define the hypoxic proportion because it reflects the oxygen concentration that is required to produce approximately half-maximal radiosensitization. In addition, we have previously shown HP5 to be a strong, independent determinant of survival in patients with cervical cancer (3).

Some of these patients also participated in a separate phase III study of either escalated-dose radiotherapy alone, or the androgen antagonist bicalutamide (150 mg daily) for 3 months followed by concurrent radiotherapy and bicalutamide. High-dose bicalutamide has been shown to produce similar antitumour effects to castration, with greater preservation of erectile function (9). Twenty-two patients who agreed to both studies consented to have oxygen measurements done a second time using the same technique after 30 to 145 days of bicalutamide. As shown in Table 1, these are similar to previously reported results for prostate cancer (8) and other human tumors (3), and indicate hypoxia of potential clinical and biological significance. The pretreatment grand median pO2 and median HP5 values in the androgen withdrawal group were in the same range at 5.5 mm Hg (range, 1.8–61 mm Hg) and 46% (0–93%), respectively.

There were statistically significant (P < 0.0001) differences in pretreatment hypoxia among the four measurement tracks and as a function of depth in the gland. In the 226 patients who only had pretreatment assessment of hypoxia, the median pO2 values for the four tracks varied from 6.2 to 9.8 mm Hg. The individual oxygen readings were designated as arising from either the superficial (subcapsular) or deep prostate gland by dividing the measurements along each linear track into two equal groups. The deeper regions of the gland were more hypoxic than the superficial regions (median pO2 of 7 versus 9 mm Hg, respectively). Similar results were obtained when the analysis was repeated using the pretreatment oxygen values for the 22 patients in whom measurements were done both before and after androgen withdrawal. Therefore, measurement track number and depth were included as covariates in the analysis of androgen withdrawal to minimize the potential for bias in the results.

Androgen withdrawal with bicalutamide produced a significant (P = 0.005) reduction in prostate hypoxia among the 22 patients who had both pretreatment and posttreatment measurements. The median pO2 increased from 6.4 to 15 mm Hg and the HP5...

### Table 1. Characteristics of patients with prostate cancer oxygen measurement

<table>
<thead>
<tr>
<th></th>
<th>Patients with oxygen measurements prior to treatment only</th>
<th>Patients with oxygen measurements both pre- and post–androgen withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>226</td>
<td>22</td>
</tr>
<tr>
<td>Age*</td>
<td>71 (55–82)</td>
<td>71 (55–79)</td>
</tr>
<tr>
<td>T category†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>87 (39%)</td>
<td>12 (54%)</td>
</tr>
<tr>
<td>T2</td>
<td>135 (60%)</td>
<td>10 (46%)</td>
</tr>
<tr>
<td>T3</td>
<td>4 (2%)</td>
<td></td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>67 (30%)</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>7</td>
<td>146 (65%)</td>
<td>18 (82%)</td>
</tr>
<tr>
<td>8, 9</td>
<td>13 (6%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>PSA* (ng/mL)</td>
<td>8.0 (0.9–33)</td>
<td>7.8 (3.3–30)</td>
</tr>
<tr>
<td>Prostatic volume† (cm³)</td>
<td>40 (13–179)</td>
<td>37 (23–171)</td>
</tr>
<tr>
<td>Grand median pO2† (mm Hg)</td>
<td>6.8 (0–75)</td>
<td>5.5 (1.8–61)</td>
</tr>
<tr>
<td>Median HP5† (%)</td>
<td>33 (0–100)</td>
<td>46 (0–93)</td>
</tr>
</tbody>
</table>

*Median and range.
†Clinical T category. All patients were clinically N0, M0 (UICC-TNM Classification, 6th Edition, 2002).

Results and Discussion

This is the largest reported study of patients with prostate cancer who have undergone assessment of tumor hypoxia. A total of 14,038 individual oxygen measurements were done in 226 patients prior to treatment, and includes the cohort of 55 patients that we previously described (7). In addition, an additional 3,388 measurements were done in 22 patients who were evaluated both before and after androgen withdrawal. In the larger group, the pretreatment grand median pO2 was 6.8 mm Hg (range, 0–75 mm Hg) and the median HP5 was 33% (range, 0–100%), as indicated in Table 1. These are similar to previously reported results for prostate cancer (8) and other human tumors (3), and indicate hypoxia of potential clinical and biological significance. The pretreatment grand median pO2 and median HP5 values in the androgen withdrawal group were in the same range at 5.5 mm Hg (range, 1.8–61 mm Hg) and 46% (0–93%), respectively.

Doppler ultrasound during the procedure. Patients were awake throughout the procedure and local anesthetic was not used. We previously reported that tumor was present along 70% of the measurement tracks with this technique (7). We also showed hypoxia in both malignant and nonmalignant regions of the gland, based on a comparison of oxygen readings and histology from needle biopsies along the measurement tracks (7). Others have described hypoxia in benign prostatic hypertrophy using tissue-based markers (6). These observations suggest a global reduction in prostate oxygenation in patients with prostate cancer that is not necessarily confined to tumor. Therefore, all oxygen measurements along all tracks were included in this analysis. Hypoxia was summarized using the median pO2 and the hypoxic proportion (HP5), defined as the percentage of the individual oxygen measurements <5 mm Hg. A threshold of 5 mm Hg was chosen to define the hypoxic proportion because it reflects the oxygen concentration that is required to produce approximately half-maximal radiosensitization. In addition, we have previously shown HP5 to be a strong, independent determinant of survival in patients with cervical cancer (3).

Spatiotemporal variability of hypoxia in the prostate gland and the influence of androgen withdrawal were evaluated using a mixed model with the individual oxygen readings as the dependent variable. We previously reported, in a smaller cohort of patients with prostate cancer, that the oxygen readings varied from one measurement track to the next but were similar in the peripheral and central regions of the gland (7). This was reexamined here with the goal of identifying significant covariates that might influence the androgen withdrawal analysis. The mixed model is relatively robust to departures of the data from a normal distribution. Nevertheless, all analyses were done both before and after logarithmic transformation of the oxygen data. Only the analyses using the raw oxygen readings are presented, as the results using the transformed data were nearly identical.
Jain et al. (11) showed reduced transcription of vascular endothelial growth factor (VEGF) and tumor endothelial cell apoptosis through an antiangiogenic effect, with regression of immature vessels and remodeling leading to improved vascular efficiency (14).

Alternatively, there is increasing evidence that androgen withdrawal may reflect lower consumption, as hormone-sensitive malignant cells either die or enter quiescent phases of the cell cycle (13). Alternatively, there is increasing evidence that androgen withdrawal improves the delivery of oxygen and other nutrients to tumors through an antiangiogenic effect, with regression of immature vessels and remodeling leading to improved vascular efficiency (14). Jain et al. (11) showed reduced transcription of vascular endothelial growth factor (VEGF) and tumor endothelial cell apoptosis beginning less than 24 h after castration of mice bearing androgen-sensitive carcinomas, followed by changes in vascular architecture and organization towards a more normal-appearing phenotype. Similar molecular and morphologic changes have been reported following the inhibition of angiogenesis using a VEGF monoclonal antibody (10). Treatment with either castration or direct VEGF inhibition has been shown to reduce tumor hypoxia in a time-dependent fashion consistent with improved oxygen delivery (10, 12, 15). However, laboratory studies have also shown increased hypoxia with antiangiogenic treatment (16), possibly due to excessive pruning of the tumor vasculature (15). In our clinical study of patients with prostate cancer, the androgen antagonist bicalutamide produced an overall reduction in tumor hypoxia. The response was variable among patients, but there was no indication that this related to differences in the duration of bicalutamide treatment.

Androgens seem to directly stimulate angiogenesis in hormone-sensitive prostate cancer through activation of growth factor receptors and the phosphatidylinositol-3-kinase/AKT signaling pathway, leading to hypoxia-independent up-regulation of HIF-1α and VEGF expression (17–19). This probably occurs early in prostate cancer development (18), and triggers a cascade of increasing angiogenesis and hypoxia. Hypoxia in turn may sensitize tumor cells to very low levels of androgens through a receptor-mediated mechanism (20). The variable oxygen response to bicalutamide that we observed in this study may in part be due to differences in the relative importance of androgenic and hypoxic stimulation of angiogenesis in individual hormone-sensitive tumors. Our future research will focus on better understanding this heterogeneity among patients.

The reduction in prostate cancer hypoxia with androgen withdrawal shown in this study might in part explain the improved patient outcome that has been observed in many clinical trials of combined treatment with radiotherapy and hormones (2). However, there may be more far-reaching implications. We hypothesize that androgen-induced angiogenesis and hypoxia in nonmalignant regions of the prostate gland may be important in prostate carcinogenesis. Our group has observed aberrant DNA repair in hypoxic prostate cancer cells, which supports hypoxia as a mediator of genetic instability in this disease (5). Therefore, novel therapeutic strategies, which inhibit HIF-1α or other downstream molecular targets, might not only reduce morbidity and mortality in men with diagnosed prostate cancer but might also play an important role in prostate cancer prevention.

Acknowledgments


Grant support: U.S. Army Prostate Cancer Research Program and the National Cancer Institute of Canada with funds from the Terry Fox Run.

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We thank Ami Syed for invaluable administrative and technical support.

References


Figure 1. Posttreatment versus pretreatment marginal mean prostate cancer pO2 levels in 22 patients. Dark points, significant (P = 0.001) changes in oxygenation with androgen withdrawal; bars, SEs. The line of unity is also shown.
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