Hypoxia Increases Androgen Receptor Activity in Prostate Cancer Cells

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Abstract

Recent studies show that prostate cancer cells are able to survive in a hypoxic tumor environment, and the extent of tumor hypoxia correlates with poor clinical outcome. Androgen deprivation, the most common form of prostate cancer therapy, was itself shown to induce a state of transient hypoxia at the microenvironmental level. Because androgen receptor (AR) signaling plays a critical role in prostate cancer, we investigated the effect of hypoxia in regulating AR function. We found that in LNCaP prostate cancer cells, AR binding to the androgen-responsive element (ARE), prostate-specific antigen accumulation, and ARE-reporter gene activity were increased after hypoxia treatment. Hypoxia-enhanced AR function was also observed when AR was exogenously introduced into AR-null DU145 cells. Confocal microscopy and chromatin immunoprecipitation assays showed that AR translocation to the nucleus and AR recruitment to the prostate-specific antigen promoter were facilitated after hypoxia treatment. The AR stimulatory effect seemed to be ligand-dependent because it was abrogated when cells were cultured in an androgen-depleted medium, but was restored with the addition of R1881, a synthetic androgen. The sensitivity of AR activation to R1881 was also increased after hypoxia treatment. Although concentrations of <1 nmol/L R1881 did not induce ARE reporter activity under normoxic conditions, exposure to hypoxia greatly potentiated the AR response to low levels of R1881. Collectively, our results provide compelling evidence that changes in hypoxia/reoxygenation stimulate AR trans-activation and sensitization. The AR-stimulatory effect of an unstable tissue oxygenation milieu of a tumor is likely to contribute to treatment resistance and the emergence of recurrent prostate cancer. (Cancer Res 2006; 66(10): 5121-9)

Introduction

Every year, an estimated 230,000 new cases of prostate cancer are diagnosed, and approximately 30,000 men will die from prostate cancer in the U.S. (1). Eppendorf pO2 microelectrode studies of human prostate carcinoma showed that cancer cells can survive and grow in a hypoxic microenvironment (2, 3). Studies suggest that hypoxia in prostate cancer is one of the key factors in determining tumor progression and may also predict clinical outcome (4–7). Hypoxia is a reduction in the normal level of tissue oxygen tension, usually defined as an overall reduced oxygen availability or partial pressure below critical levels (8). Tissue oxygenation within a tumor, however, is highly unstable and heterogeneous as a consequence of the architectural and functional abnormalities of the vascular network (9, 10). Fluctuations in blood flow in the tumor microvasculature can lead to perfusion-limited hypoxia in the tumor parenchyma (11, 12). In terms of the ultrastructure, tumor vasculature has numerous "holes" or "openings," widened interendothelial junctions, and a discontinuous or absent basement membrane (9, 13–15). Aberrant blood vessels can be shut down locally at any time, but the same defects also allow closed vessels to be reopened, resulting in the localized and often acute reoxygenation within a tumor. In addition to the reopening of the temporarily closed/blocked vessels (9, 13–15), dynamic changes of hypoxia/reoxygenation may also occur as a result of regional angiogenesis. A heterogeneous and unstable tissue oxygenation/blood flow milieu of a tumor has been proposed to serve as a selection pressure for a subpopulation of cells with an increased survival advantage to preferentially proliferate (16).

Androgen receptor (AR) plays a critical role in the development, progression, and treatment response of prostate cancer (17, 18). Ligand binding to AR activates the receptor and induces the transcription of AR-regulated genes (17, 19). Because prostate cancer arises as an androgen-dependent cancer, androgen deprivation is the most common form of therapy. Although effective in blocking tumor growth at the beginning, the treatment eventually fails and most men will relapse due to adaptive progression of the surviving prostate cancer cells. The recurrent cancer is commonly referred to as "androgen-independent." However, it usually continues to express AR and AR-regulated genes (20), suggesting a functional role of AR in the survival and growth of recurrent prostate cancer cells. Recently, Mohler et al. (21) showed that androgens are still present in the recurrent prostate cancer tissue at levels that are sufficient to activate AR. These studies suggest that the state of "androgen-independence" may be a misnomer. Prostate cancer that recurs during androgen deprivation therapy may not be "androgen-independent" but continue to depend on AR androgen for survival and growth. Several other mechanisms have been proposed to explain the abnormal activation of AR in recurrent prostate cancer. These include mutation of AR, AR gene amplification (22–24), or the activation of AR by non-androgen ligands (25–28). Although alterations of the AR gene have been correlated with response to androgen deprivation therapy, the majority of patients do not have AR mutations or amplification, and retain active AR signaling (29, 30).

Studies with castrated rat, transgenic mouse, and xenograft models all suggest that androgen deprivation generates a state of...
transient hypoxia in the prostate tissue (31–33). Androgen deprivation causes rapid endothelial cell death, degeneration of capillaries, and vasocostriction in the prostate. These studies imply that recurrent prostate cancer cells must survive an acute transient hypoxia in order to survive and undergo clonal expansion. Considering the critical role of the AR in disease progression and treatment response, we hypothesize that unstable, dynamic changes of tissue oxygenation milieu of a tumor influence AR function in some prostate cancer cells, and in doing so, contribute to their survival and malignant progression.

Materials and Methods

Cell culture. LNCaP and DU145 human prostate cancer cells were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 mmol/L glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. In some experiments, cells were switched to a medium containing 2% charcoal-stripped FBS (CS-FBS; Hyclone, Logan, UT) 24 hours prior to hypoxia treatment.

Hypoxia treatment. The culture medium was replaced with deoxygenated RPMI 1640 before hypoxia treatment as reported previously (34). Deoxygenated medium was prepared prior to each experiment by equilibrating the medium with a hypoxic gas mixture containing 5% CO₂, 85% N₂, and 10% H₂ at 37°C. The oxygen concentration in the hypoxic chamber and the exposure medium was maintained at <0.05%, and was monitored by using an oxygen indicator (Forma Scientific, Marietta, OH). All experiments were done at 70% to 80% confluency at a pH between 7.2 and 7.4 for the duration of the experiment.

Western blot analysis. Equal amounts of protein were analyzed in duplicate by SDS-PAGE. Protein concentrations were measured by the method of Lowry et al. (35). One gel was stained with Coomassie blue, destained and dried for recordkeeping. A second gel was transferred to a nitrocellulose membrane in a Trans-Blot apparatus (Bio-Rad, Hercules, CA) for Western blot analysis. The following monoclonal antibodies were used: anti-AR (Santa Cruz Biotechnology, Santa Cruz, CA), anti–prostate-specific antigen (PSA; Lab Vision, Fremont, CA), anti-HIF-1α (BD Biosciences, San Jose, CA), and anti-β-actin (Sigma, St. Louis, MO). Immunoreactive proteins were detected with secondary antibody and visualized using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Piscataway, NJ).

Transfection and luciferase assay. An aliquot of 3 × 10⁵ cells was placed in a six-well plate and transfected with DNA using LipofectAMINE PLUS reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instruction. The pSG5hAR expression vector (36) was used to express AR. Equal amounts of protein were analyzed in duplicate by SDS-PAGE. Protein concentrations were measured by the method of Lowry et al. (35). One gel was stained with Coomassie blue, destained and dried for recordkeeping. A second gel was transferred to a nitrocellulose membrane in a Trans-Blot apparatus (Bio-Rad, Hercules, CA) for Western blot analysis. The following monoclonal antibodies were used: anti-AR (Santa Cruz Biotechnology, Santa Cruz, CA), anti–prostate-specific antigen (PSA; Lab Vision, Fremont, CA), anti-HIF-1α (BD Biosciences, San Jose, CA), and anti-β-actin (Sigma, St. Louis, MO). Immunoreactive proteins were detected with secondary antibody and visualized using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Piscataway, NJ).

PSA ELISA analysis. PSA secretion was quantified by ELISA following the manufacturer’s protocol (Beckman Coulter, Fullerton, CA). The experiments were repeated thrice.

Immunofluorescence and confocal microscopy. Cells were grown on six-well Teflon-coated microscope slides (Erie Scientific, Erie, PA), underwent hypoxia for 4 hours and returned to normoxic CO₂ incubator. After 2 hours of incubation in a CO₂ incubator, cells were fixed in 3.7% formaldehyde for 20 minutes and permeabilized in methanol for 15 minutes on ice. AR-specific polyclonal antibody (Santa Cruz Biotechnology) was diluted (1:500) in antibody dilution buffer [5% BSA, 0.005% saponin in PBS (pH 7.2)] and incubated with fixed cells for 1 hour at room temperature. Slides were immersed in washing buffer [0.005% saponin in PBS (pH 7.2)] for 1 hour. Samples were then incubated for 1 hour at room temperature with Alexa 488 anti–rabbit secondary antibody (Molecular Probes, Eugene, OR) and propidium iodide (50 μg/mL) for nuclear staining. Slides were washed and mounted with coverslips in Vectashield solution (Vector Laboratories, Burlingame, CA). Normal rabbit IgG (Santa Cruz Biotechnology) at 1:100 dilution was used as a negative control. Confocal microscopy was done by using a Bio-Rad MRC 1024 confocal laser scanning microscope. Control cells were processed identically except for the hypoxia treatment.

Chromatin immunoprecipitation assay. Chromatin immunoprecipitation (ChIP) assays were carried out following a previously described procedure (39). Briefly, formaldehyde was added directly to control and hypoxia-treated LNCaP cells. The cross-linking process was limited to <30 minutes because longer incubation with formaldehyde caused cells to aggregate, and prevented efficient sonication of the chromatin. After cross-linking, cells were lysed in lysis buffer containing protease inhibitors. Nuclei were isolated and chromatin was sheared to an average length of 250 to 500 bp using sonication. The sheared chromatin was preclared with salmon sperm DNA/protein A-sepharose and precipitated with an antibody specific to AR (2 μg/mL, Santa Cruz Biotechnology). Rabbit IgG control

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was used to monitor nonspecific interactions. Immune complexes were adsorbed onto salmon sperm DNA/protein A-sepharose beads. After an extensive wash to reduce background, Ab/AR/DNA complexes were eluted. After precipitation, DNA was resuspended in water and PCR was carried out to amplify the proximal and upstream ARE regions identified in the PSA gene (40, 41). The following primer sequences were used to amplify the proximal (ARE I/II) and upstream ARE regions: ARE I, forward 5\'-TCTGCCTTTGTCCCCTAGAT-3\', reverse 5\'-AACCTTCATTCCCCAGGACT-3\'; ARE II, forward 5\'-GTTGGGAGTGCAAGGAAAAG-3\', reverse 5\'-CGCCCAGGATGAAACAGAAA-3\'; and ARE III, forward 5\'-CCTCCAGGTTCAAGTGATT-3\', reverse 5\'-GCCTGTAATCCCAGCACTTT-3\'. The intervening region between ARE I/II and ARE III regions of the PSA gene that does not contain ARE was amplified as a negative control by using forward 5\'-CTGTGCTTGGAGTTTACCTGA-3\' and reverse 5\'-GCA-GAGGTGTGGCAGTGAGCC-3\' primer pair. Triplicate PCR reactions were conducted for each sample and the experiments were repeated at least thrice.

**Statistical analysis.** Statistical significance was examined using Student’s t tests. The two-sample t test was used for two-group comparisons. Values were reported as mean ± SD. P < 0.05 was considered significant and indicated by asterisks in the figures.

**Results**

ARE-binding activity of AR is increased after hypoxia treatment. LNCaP is a widely used cell model for human prostate cancer studies because it is one of the limited androgen-responsive prostate cancer cell lines. LNCaP cells express a functional AR, and
secrete PSA in vitro and in vivo. In order to examine whether dynamic changes of hypoxic conditions are capable of influencing AR activity, cells were exposed to hypoxia for 4 hours and returned to a normoxic CO₂ incubator. The ARE-binding activity of AR was analyzed at 0, 2, 6, or 24 hours during reoxygenation after hypoxia treatment. As shown in Fig. 1A, significant increases of ARE-binding activities were observed after 4 hours of hypoxia treatment. The increase was evident as soon as the cells were withdrawn from hypoxia, reached a maximum at 2 hours during reoxygenation, and gradually declined with time. The ARE-binding activity was specific because the DNA-binding complexes became undetectable when molar excess of the unlabeled competitor of the ARE probe was added. Supershift assays with an antibody specific to AR confirmed the binding of AR to the ARE-containing probe (Fig. 1B). When cells were exposed to a longer period of hypoxia (i.e., 8 or 16 hours) followed by 2 hours of oxygenation, the AR stimulatory effect was also consistently observed, although the maximal AR stimulatory effect was seen after 4 hours of hypoxia treatment (Fig. 1C). No appreciable changes of AR protein levels were detected at 2 hours of reoxygenation after 4, 8, or 16 hours of hypoxia treatment (data not shown).

We questioned whether the ability of hypoxia to induce HIF-1α signaling might be a contributing factor to AR activation. As shown in Fig. 1D, HIF-1α activities were decreased during reoxygenation after hypoxia treatment. Unlike AR, HIF-1α activities were increased progressively as a function of time of exposure to hypoxia. Consistent with these changes of HIF-1α activities, the levels of HIF-1α protein and VEGF mRNA accumulation were increased by longer duration of hypoxia, and decreased during reoxygenation. The decrease of HIF-1α protein and VEGF mRNA levels were pronounced at 6 hours of reoxygenation. These results suggest that the mechanisms of AR stimulation by hypoxia/reoxygenation are unique and unlikely to involve HIF-1α signaling. However, a possible cross-talk between the AR and HIF-1α pathways during continuous hypoxia cannot be excluded.

**Hypoxia increases AR trans-activation.** We wanted to validate the functional significance of the AR-ARE complex formation after transient hypoxia treatment. PSA is one of the best-characterized genes regulated by AR. Because LNCaP cells express and secrete PSA, we examined the changes of PSA regulation after 4 hours of hypoxia treatment. Our results showed an increased accumulation of PSA mRNA during reoxygenation following hypoxia treatment (Fig. 2A). Consistent with the increased PSA mRNA level, increased accumulation of cellular PSA protein and increased secretory PSA in the culture medium were confirmed by Western blot analysis (Fig. 2B) and ELISA assay (Fig. 2C), respectively.

Activated AR exerts its trans-activation function by binding to the DNA containing an ARE. We next examined the effect of hypoxia on AR trans-activation by transfecting LNCaP cells with an ARE-luciferase reporter construct. As shown in Fig. 2D, a significant increase of luciferase activity was observed after hypoxia treatment, confirming that increased trans-activation of AR after hypoxia treatment is not limited to the PSA gene. In order to rule out that the increased ARE-luciferase activity was due to increased AR protein level, we carried out Western blot analysis with an anti-AR antibody (bottom). We did not detect any change in AR protein levels up to 24 hours after reoxygenation.

To further verify that the effect of transient hypoxia on AR trans-activation was not unique to LNCaP cells, we turned to the DU145 human prostate cancer cells which are devoid of AR. We introduced exogenous wild-type AR together with the ARE-luciferase into the DU145 cells, exposed them to 4 hours of hypoxia, and analyzed the ARE-luciferase activity. As shown in Fig. 3A, transient exposure to hypoxia induced the trans-activation of exogenous AR and stimulated ARE-luciferase activity. The pattern of AR activity changes in the DU145 cells was similar to that observed in LNCaP.

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**Figure 2.** Hypoxia increases PSA expression and induces ARE-luciferase promoter activity. A, RT-PCR analysis was carried out at the indicated times after 4 hours of hypoxia treatment with PCR primer pairs specific for the PSA gene. β-Actin gene was amplified as a control. B, equal amounts of proteins obtained from cells treated as in (A) were subjected to Western blot analysis using anti-PSA antibody (Lab Vision). β-Actin antibody (Sigma) was used as a loading control. C, the amounts of the secreted PSA in the medium were determined using ELISA after 4 hours of exposure to hypoxia followed by 24 hours of reoxygenation. Columns, mean of triplicate samples; bars, ±SD. *, P < 0.05 compared with control. D, cells were transfected with the ARE-luciferase construct. At 24 hours after transfection, cells were exposed to 4 hours of hypoxia and the luciferase activity was analyzed at the indicated time points after hypoxia treatment. Normalized mean luciferase activity measured in ARE-luciferase transfected cells without hypoxia treatment (control) was set to 1.0. **, P < 0.01; *, P < 0.05 compared with controls. Aliquots of cells used in luciferase assays were processed for Western blot analysis and probed with AR-specific antibody (Santa Cruz Biotechnology). The β-actin antibody (Sigma) was used as a loading control.
cells. When the DU145 cells were exposed to longer periods of hypoxia, the maximal AR stimulatory effect of hypoxia was also seen after 4 hours of hypoxia treatment. Collectively, these data validate our conclusion that dynamic changes of hypoxia/reoxygenation enhance AR-ARE binding activity and AR target gene expression.

**Hypoxia stimulates the nuclear translocation of AR and its promoter occupancy.** Activated AR translocates from the cytosol to the nucleus to trans-activate its target genes. To examine the effect of hypoxia/reoxygenation on the subcellular distribution of AR, LNCaP cells were treated with 4 hours of hypoxia followed by 2 hours of reoxygenation. Nuclear and cytosolic extracts obtained from control and hypoxia-treated cells were subjected to Western blot analysis. As shown in Fig. 4A, transient hypoxia increased nuclear AR and decreased cytosolic AR, but did not affect total AR levels. Increased nuclear localization of AR was further confirmed by immunofluorescent cell staining and confocal microscopy (Fig. 4B). Propidium iodide staining of the same cells verified the location and integrity of the nuclei. Virtually identical results were obtained in DU145 cells after the exogenous introduction of AR (data not shown).

The next step was to examine whether hypoxia increased the recruitment of AR to the promoter of its target gene in the natural chromatin milieu. ARE I/II are in the proximal promoter region at ~ 163 and ~ 390 bp, respectively, whereas ARE III resides at ~ 4 kb upstream of the PSA transcription site (40). We carried out ChIP assays on these ARES with the AR-specific antibody. A schematic diagram of these three ARE regions is shown in Fig. 5A. To control for possible nonspecific interactions and DNA contaminations of the solutions, samples precipitated with rabbit IgG were included. As shown in Fig. 5B, our results showed that transient hypoxia clearly increased the AR occupancy of ARE regions in the PSA gene. The recruitment of AR to ARE I/II and ARE III was specific as no signal was detected in the rabbit IgG control samples. We did not find an increase of AR recruitment to the nonspecific intervening region located between the proximal and upstream ARES. The results obtained from the DNA that was PCR-amplified from chromatin extracts before immunoprecipitation (input) are shown for comparison.

**Increased AR trans-activation by hypoxia is ligand-dependent.** Although the effect of AR is known to be mediated through ligand binding, numerous studies have suggested that AR can also be activated by ligand-independent mechanisms. In order to determine whether hypoxia-enhanced AR activity is ligand-dependent or ligand-independent, we carried out the following experiment in a CS-FBS medium. LNCaP cells were switched to the CS-FBS medium 24 hours prior to hypoxia treatment. Cells were then exposed to 4 hours of hypoxia followed by 2 hours of reoxygenation. Nuclear extracts were isolated, and EMSA was done.

Our results showed that hypoxia induction of ARE-binding activity was abrogated completely in cells cultured in an androgen-depleted medium, suggesting that the binding of androgen to AR is necessary for the AR stimulatory effect (Fig. 6A). Not surprisingly, the steady state ARE-binding activity of AR was also lost under androgen deprivation. Subsequent AR ChIP assays corroborated that increased AR trans-activation by transient hypoxia is ligand-dependent because increased promoter occupancy was not observed in cells cultured in an androgen-depleted CS-FBS medium. Figure 6B shows the data from the ARE I region, although similar results were obtained from the other ARE regions.

When the synthetic androgen, R1881 (10 nmol/L), was supplemented to the CS-FBS medium, the AR stimulatory effect of hypoxia was restored and the increased promoter occupancy of AR was again observed (Fig. 6C). In those cells grown in a normal FBS medium, AR promoter occupancy was further increased when R1881 was combined with hypoxia. We were curious to find out whether hypoxia might sensitize the AR to androgen. To answer this question, we treated cells in a CS-FBS medium with increasing concentrations of R1881, either alone or in combination with hypoxia. As shown in Fig. 6D, R1881 alone at concentrations <1 nmol/L did not stimulate ARE-luciferase activity. However, hypoxia greatly enhanced the effectiveness of lower concentrations of R1881. The AR-sensitizing effect of hypoxia was most dramatic at subphysiologic R1881 concentrations (<1 nmol/L). The magnitude of AR sensitization by hypoxia was less pronounced at physiologic levels of R1881 (10 nmol/L) when AR might be saturated with the ligand. Our results showed that hypoxia enhances AR sensitivity without noticeably affecting AR levels at a given concentration of R1881 (bottom). Treatment with R1881 alone slightly increased the AR levels. Similar results were obtained when the exogenous AR was introduced into DU145 cells. The magnitude of AR sensitization was comparable to that observed in LNCaP cells (data not shown). These results suggest that hypoxia sensitizes AR trans-activating activity to subphysiologic levels of androgen.
Discussion

AR signaling plays an important role in prostate cancer. Despite our awareness of the effects of tumor hypoxia on the behavior of prostate cancer cells, the effect of hypoxia on AR activity has not been well characterized. In this study, we provide new insights into the action of AR in the context of unstable, dynamic, and often acute changes of tissue oxygenation of a tumor at the microenvironmental level. We showed that transient hypoxia clearly increases AR function in human prostate cancer cells. Our results show that the AR-ARE binding and AR-regulated gene expression are increased after hypoxia treatment. Confocal microscopy and ChIP assays confirmed that the AR translocation to the nucleus and the recruitment of AR to the PSA promoter are increased after hypoxia treatment. AR also sensitizes AR trans-activation to very low concentrations of androgen. The importance of our research finding is underlined by the realization that, in addition to the inherently abnormal and unstable vascular network of a tumor, changes of hypoxia/reoxygenation also emerge in a variety of clinical situations in prostate cancer. These situations include a localized and acute hypoxic microenvironment induced by...
vasculature destruction after castration, or during neoadjuvant androgen deprivation therapy prior to radiation therapy, or in recurrent tumors during androgen deprivation therapy.

A recent study by Mohler et al. (21) suggests that prostate cancer which recurs during androgen deprivation therapy is not necessarily androgen-independent but continues to depend on androgen for growth. The measurement of androgens revealed that there are significant amounts of dihydrotestosterone, in the order of $1.45 \text{nmol/L}$. The presence of AR-regulated gene products in these tissues suggests that AR is active in recurrent prostate cancers. For comparison, benign prostate contains $8.13 \text{nmol/L}$ of dihydrotestosterone (21). Our finding that transient hypoxia stimulates AR activity at very low levels of R1881 ($<1 \text{nmol/mL}$) suggests an intriguing possibility that hypoxia permits AR to function at subphysiologic levels of androgens, and may facilitate the survival and growth of some prostate cancer cells during androgen deprivation therapy.

Prostate cancer cells are known to be heterogeneous in their molecular makeup. Using autopsy samples from men who died of recurrent prostate cancer, Shah et al. (30) elegantly showed the evidence of molecular heterogeneity within the same patient as well as among patients. Our use of LNCaP and DU145 cells for the present study is meant to simulate the backdrop of heterogeneity. For example, LNCaP cells possess functional p53 and wild-type retinoblastoma protein (RB; ref. 42), whereas DU145 cells possess p53 mutation and are RB null (42, 43). The increased AR activation by hypoxia occurs not only in LNCaP cells, but also in DU145 cells when wild-type AR is exogenously introduced. We believe that the AR stimulatory effect of hypoxia may be generalizable to different subsets of prostate cancer cells.

During the course of our study, we noted an article by Ghafar et al. (44) that examined hypoxia-responsive gene expression in LNCaP cells. These authors reported a decrease in PSA and AR
protein levels during continuous exposure to hypoxia for 24 hours, as well as during reoxygenation after 24 hours of hypoxia treatment. We did not find any change in AR protein up to 24 hours of reoxygenation after 4 hours of hypoxia treatment. In our study, a treatment condition of 24 hours of hypoxia followed by >16 hours of reoxygenation was too severe for LNCaP cells; we were unable to perform the 24-hour time point experiment after >16 hours of hypoxia treatment because most cells did not survive. Both LNCaP and DU145 cells were ~30% growth-inhibited after 4 hours of hypoxia treatment based on the clonogenic survival assay (data not shown).

How does hypoxia increase AR function and what is the underlying molecular mechanism? Cumulative evidence seems to point to reactive oxygen species (ROS) as a second messenger. The role of ROS in stimulating the P13K/PTEN and mitogen-activated protein kinase pathways via protein tyrosine kinase was shown in cells treated with platelet-derived growth factor (PDGF) or epidermal growth factor (EGF) (45–47). Considering that mitogen-activated protein kinase signaling by EGF receptor HER-2/neu tyrosine kinase and other growth factors was shown to increase the activation of AR (27, 48), it is plausible that ROS may play a role in mediating the cross-talk between the peptide growth factors and the AR activation signaling pathways. Future studies are needed to clarify whether ROS acts as a second messenger, and if so, what specific ROS-mediated molecular pathways are linked to AR activation.

Oxidation-reduction (redox) regulation bound of transcription factors is fundamental to the control of cell function. Disulfide bond formation between distant cysteines has been suggested as a mechanism to induce conformational changes of proteins (49). An alternative possibility is that ROS generated by hypoxia might directly modify redox-sensitive residues contained in AR. Redox-dependent activation of recombinant AR prepared in insect cells has been reported (50). Lastly, changes in the balance between steroid receptor coactivators and corepressors may affect AR activation (51, 52). Although the prevalence of these events and their relationship to clinical androgen-independence remains unclear, it is tempting to speculate on the possible role of hypoxia in altering the equilibrium of coregulators. All of these are important biological and clinical issues that warrant more intensive future investigation. Delineating the molecular mechanisms by which dynamic changes of hypoxia/reoxygenation affect AR function will provide valuable insights into treatment resistance and malignant progression of prostate cancer cells. Novel therapeutic approaches should be developed to prevent hypoxia and/or its consequences to enhance the efficacy of androgen deprivation therapy, a treatment that has not been improved significantly since its introduction >50 years ago (53).

References
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