Pro-survival and anti-apoptotic properties of androgen receptor signaling by oxidative stress promote treatment resistance in prostate cancer

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Abstract

Oxidative stress caused by an increase in reactive oxygen species levels or a decrease in the cellular antioxidant capacity can evoke modulation of various cellular events including androgen receptor (AR) signaling via direct or indirect interaction. In this review we summarize the mechanisms of AR activation by oxidative stress including: (1) AR overexpression; (2) AR activation by AR co-regulators or intracellular signal-transduction pathways; (3) generation of AR mutations or splice variants; and (4) de novo androgen synthesis. AR signaling augmented by oxidative stress appears to contribute to pro-survival and anti-apoptotic effects in prostate cancer cells in response to androgen-deprivation therapy. In addition, AR signaling suppresses anti-survival and pro-apoptotic effects in prostate cancer cells in response to various cytotoxic and tumor-suppressive interventions including taxanes and radiation through modulation of βIII-tubulin and ataxia telangiectasia mutated kinase expression, respectively. Taken together, AR signaling appears to render prostate cancer cells refractory to various therapeutic interventions including castration, taxanes and radiation, indicating AR signaling is a comprehensive resistant factor and crucial target for prostate cancer treatment.
1. Introduction

Prostate cancer is the most common non-cutaneous cancer and the second leading cause of male cancer-related mortality in Western countries. A special characteristic of prostate tumors is their dependence on androgen receptor (AR) signaling for their carcinogenesis, development and progression (Basu & Tindall 2010). Inversely, androgen-deprivation therapy, which reduces androgen production, or anti-androgen agents, which interfere with AR function, are the gold-standard treatments for recurrent or advanced prostate cancer (Miyamoto et al. 2004). However, androgen-dependent prostate cancer eventually develops to castration-resistant prostate cancer (CRPC), which can be attributable to augmented pro-survival and anti-apoptotic properties by AR signaling and others (Niraula et al. 2012). Against CRPC, few therapeutics including taxane chemotherapy are not curative, only ameliorating cancer-caused symptoms and prolonging survival for a few months. Recently, novel AR-targeting agents such as cytochrome P17 inhibitor abiraterone acetate and second-generation anti-androgen MDV3100 have been proved to reduce tumor burden and improve overall survival in CRPC patients although their efficiencies are also limited, prolonging survival for only 3-5 months (de Bono et al. 2011, Scher et al. 2012).
Reactive oxygen species (ROS) include superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals (HO•), and are produced by the partial reduction of oxygen. Cellular ROS are generated endogenously, mainly during the process of mitochondrial oxidative phosphorylation, or can arise exogenously from xenobiotic compounds. Oxidative stress is caused when the cellular antioxidant defense system is overwhelmed by an increase in ROS levels or a decrease in the cellular antioxidant capacity. This stress leads to damage of nucleic acids, proteins, and lipids, and has been implicated in various disorders including carcinogenesis (Trachootham et al. 2009), neurodegenerative diseases (Andersen 2004), atherosclerosis, diabetes (Paravicini & Touyz 2006), and aging (Haigis & Yankner 2010). Oxidative stress also has effects on the redox regulation of redox-reactive cysteine (Cys) residues in redox-sensitive proteins. Oxidation of these residues forms reactive sulfenic acid (−SOH) that can form disulfide bonds with nearby cysteine residues (−S–S−) or undergo further oxidation to sulfinic (−SO$_2$H) or sulfonic (−SO$_3$H) acid. These oxidative modifications change protein structure and thus affect their function. Except where −SO$_3$H is involved, these redox modifications can be reversed by reducing systems such as thioredoxin and peroxiredoxin (Roos & Messens 2011). Thus, oxidative stress can modulate various
cellular actions, including AR signaling, via direct or indirect interactions (Ray et al. 2012).

Oxidative stress has been shown to play an important role in the tumorigenesis and progression of prostate cancer (Bostwick et al. 2000, Khandrika et al. 2009, Sharifi et al. 2008), as well as the conversion of androgen-dependent prostate cancer into CRPC (Sharifi et al. 2008, Shiota et al. 2010, 2011a). Together, these results suggest intimate cross-talk between oxidative stress and AR signaling. In this review, we summarize the effects of oxidative stress, which plays pro-survival and anti-apoptotic roles against various prostate cancer treatments, on AR signaling.

2. Oxidative stress by treatment in prostate cancer

Several experiments in both in vitro and in vivo have indicated that castration induced oxidative stress through redox imbalance by up-regulating ROS production via NADPH oxidases and down-regulating ROS-detoxifying enzymes such as manganese superoxide dismutase (SOD2) (Best et al. 2005, Pang et al. 2002, Shan et al. 2010, Tam et al. 2003). Although there are several conflicting studies showing that androgens induces oxidative stress (Pathak et al. 2008, Pinthus et al. 2007), they may reflect physiological or non-physiological condition. Ripple et al. reported physiological level
of androgens deceased oxidative stress while overloading of androgens induced oxidative stress, suggesting non-specific stress under non-physiological condition (Ripple et al. 1997). In human, it was found that androgen-deprivation therapy decreases SOD2 expression in biopsy tissues of prostate cancer (Best et al. 2005), and increased oxidative stress in prostate cancer cells as well as surgically resected tissue of prostate cancer tissues (Shiota et al. 2010, 2012). In addition to androgen deprivation, several treatments against prostate cancer including taxane chemotherapy and radiotherapy are known to induce oxidative stress (Acharya et al. 2010). Thus, various treatments induce oxidative stress in prostate cancer cells, leading to cellular damages as well as modulations of cellular signaling including AR signaling.

3. Effects of oxidative stress on AR signaling

In 2008, oxidative stress was reported to be implicated in AR signaling in prostate cancer (Sharifi et al. 2008). SOD2, which regulates ROS levels by converting superoxide to a less reactive species, is reduced by androgen deprivation (Best et al. 2005, Pang et al. 2002) and is down-regulated in CRPC (Best et al. 2005, Quirós et al. 2009). Sharifi and colleagues showed that suppression of SOD2 induced activation of AR signaling by ROS production via the following pathways (Sharifi et al. 2008). First,
several genes involved in steroid metabolism, including AKR1C3, were induced by SOD2 knockdown, and this effect was reversed by treatment with the antioxidant, N-acetyl-cysteine (NAC). Changes in the expression of genes related to steroid metabolism can lead to an increase of local *de novo* androgen synthesis in CRPC, thus contributing to castration resistance via AR reactivation (Titus *et al.* 2005). Second, five nuclear receptor co-regulators, including NCOA4 (ARA70), were induced by repressing SOD2 in a ROS-dependent manner. AR reactivation can be induced by altering the balance of such steroid receptor co-regulators (Heemers & Tindall 2007). Last, the receptor for interleukin-6 (IL-6R) was induced by SOD2 down-regulation, and this effect was reversed by NAC. IL-6 activates AR in a STAT3-dependent manner, while antibodies to IL-6 reverse castration resistance (Lee *et al.* 2003, Wallner *et al.* 2006). Furthermore, levels of IL-6R are predictive of biochemical recurrence of prostate cancer and metastasis (Kattan *et al.* 2003, Shariat *et al.* 2001). Thus, SOD2 repression is found to contribute to castration resistance via AR reactivation by several mechanisms. Inversely, it has been reported that SOD mimetics reduce oxidative stress and exert a suppressive effect on AR expression, including expression of AR splice variants, and have a therapeutic effect on prostate cancer cells (Thomas & Sharifi 2012). These results suggest that antioxidant therapy is feasible and promising for the treatment of
prostate cancer, including CRPC. We have also independently found that oxidative stress plays a crucial role in AR signaling, leading to development of CRPC (Shiota et al. 2010, 2011a).

Since the findings of Sharifi and collaborators in 2008, further evidence supporting a role of oxidative stress in AR signaling has been acquired. AR signaling in CRPC is aberrantly augmented by the low androgen milieu, via various mechanisms including: (1) AR overexpression; (2) AR activation by AR co-regulators or intracellular signal-transduction pathways; (3) AR mutations or splice variants; and (4) de novo androgen synthesis. The effects of oxidative stress on AR signaling are reviewed in the following sections.

3.1. AR overexpression

AR overexpression is thought to be one of the major causes of CRPC (Shiota et al. 2011b). Many studies have shown that the progression of CRPC is associated with increased AR expression (Chen et al. 2004, Gregory et al. 1998, Scher & Sawyers 2005, Zegarra-Moro et al. 2002), which can be attributed to gene amplification, transcriptional up-regulation, translational up-regulation, and decreased degradation. As we summarized previously (Shiota et al. 2011b), various transcription factors activated by
oxidative stress, including Twist1 (Shiota et al. 2010), YB-1 (Shiota et al. 2011c), NFκB (Zhang et al. 2009), Sp1 (Faber et al. 1993, Yuan et al. 2005), Myc (Grad et al. 1999, Lee et al. 2009), CREB (Mizokami et al. 1994) and Foxo3a (Yang et al. 2005), regulate AR expression. In addition, it has recently been shown that the SREBP-1 transcription factor regulates AR expression (Huang et al. 2010), is overexpressed during progression to castration resistance (Ettinger et al. 2004), regulates lipogenesis, and induces oxidative stress via NADPH oxidase 5 (Nox5) expression that can be reversed by the Nox inhibitor, diphenyliodonium (DPI). Intriguingly, AR expression was shown to be repressed by DPI indicating that AR expression by SREBP-1 may be mediated by the Nox pathway (Huang et al. 2012). Lipogenesis by SREBP-1 may also be involved in AR expression as our findings demonstrated that statin suppresses AR expression by promoting degradation of AR protein (Yokomizo et al. 2011).

As described above, several transcription factors regulate AR expression. In addition, the above-mentioned transcription factors may involve the Twist1/YB-1 signaling pathway. NFκB (Pham et al. 2007) and Sp1 (Ohkuma et al. 2007) have been shown to promote Twist1 transactivation. Myc (Uramoto et al. 2002) and Twist1 (Shiota et al. 2008a, 2008b, 2009) were shown to up-regulate YB-1 expression, and YB-1 to increase Twist1 expression via a translational mechanism (Evdokimova et al. 2009),
suggesting mutual regulation between Twist1 and YB-1. Taken together, these results indicate that the above-mentioned transcription factors regulate AR expression by mutual interactions, suggesting that Twist1 and YB-1 may be nodal transcription factors in AR expression (Fig. 1).

In addition to transcription factors, other molecules have been reported to be involved in regulating AR expression, likely through intracellular signaling pathways and transcription factors. BLT2 is a receptor for leukotriene B4 and 12-HETE, and plays a critical role in tumor progression, as indicated by the finding that BLT2 is overexpressed in various cancers (Choi et al. 2010, Hennig et al. 2008, Yoo et al. 2004). Recently, it was reported that a BLT2-linked pathway evokes ROS production and up-regulates AR expression via the Nox4 pathway, while the Nox inhibitor, DPI, reduces AR expression (Lee et al. 2012). Furthermore, DPI was shown to reduce cell proliferation in prostate cancer cells, including LNCaP cells which express mutated AR protein and are dependent on AR for growth, but respond to other steroids than androgens and can be driven to castration-resistant phenotype. In addition to oxidative stress induced by H₂O₂, cadmium and zinc chloride, which are known to induce oxidative stress, were reported to increase AR expression in dysplastic glands of rat prostate (Arriazu et al. 2005). The synthetic antimicrobial chemical, mequindox, was
found to induce oxidative stress and AR overexpression in rat testes, indicating a
positive connection between oxidative stress and AR expression (Ihsan et al. 2011).
These data suggest that oxidative stress induced by internal and external stimuli induces
AR overexpression via various cellular processes.

Contrasting reports suggest that inducers of oxidative stress suppress AR
eexpression. The inducer of oxidative stress, \( t\)-butyl hydroperoxide, suppresses AR
eexpression in H4IIE rat hepatoma cells, indicating that the effect of oxidative stress on
AR expression may differ among cell types, and/or may be derived from differences in
concentration and/or pharmacological action among oxidants. Additionally, a curcumin
analog shown to induce oxidative stress was reported to partially down-regulate AR
eexpression at the transcriptional level, but not by proteasomal degradation (Fajardo et al.
2012). The effect of the curcumin analog was attenuated by the antioxidant NAC,
suggesting that AR down-regulation resulted from oxidative stress mediated by the
curcumin analog. However, the oxidative stress-inducing effect of the curcumin analog
did not appear to be significant. Furthermore, NAC alone down-regulated \( AR\) transcript
expression although NAC reduces oxidative stress, which is inconsistent with the
authors’ proposal that oxidative stress down-regulates AR expression. Similarly,
thymoquinone was shown to induce ROS production and down-regulate AR expression
(Koka et al. 2010). However, its effect on AR down-regulation was not reversed by NAC, suggesting that either AR down-regulation by thymoquinone induced oxidative stress, or that the effect of thymoquinone on AR expression was independent from its ability to induce oxidative stress.

3.2. AR activation by AR co-regulators and intracellular signal-transduction pathways

AR co-regulators modulate the transactivation of AR through interactions with AR (Shiota et al. 2011d). Several AR co-regulators, including peroxiredoxin, Hsp27 and EGR-1, have been reported to be activated by oxidative stress, and thus contribute to AR transactivation (Shiota et al. 2011a). In particular, oxidative stress modulates the redox-sensitive molecule, peroxiredoxin, via its Cys residues. We previously showed that Cys residues in peroxiredoxin are critical in its function as an AR co-regulator (Shiota et al. 2011e), suggesting that protein modification of AR co-regulators by ROS affects AR signaling.

Several molecules and intracellular signaling pathways play a role in AR transactivation. As previously summarized, cytokines such as insulin-like growth factor, fibroblast growth factor, epidermal growth factor and IL-6, and signal-transduction
pathways such as mitogen-activated protein kinase, JAK/STAT, protein kinase A, phosphatidylinositol-3-kinase/Akt, and protein kinase C, which may be activated by oxidative stress, can augment AR function (Shiota et al. 2011a). Thus, oxidative stress can activate AR signaling through intracellular signaling pathways that interact with various transcription factors and co-regulators of transcription factors.

3.3. AR mutations and splice variants

Although oxidative stress is known to evoke DNA mutations, the implications of oxidative stress-induced mutations of the AR gene are unknown (Khandrika et al. 2009). Mutations in the AR gene may change its ligand binding characteristics or its transcriptional activity, resulting in modulation of its target gene expression (Brooke et al. 2008, Brooke & Bevan 2009). In addition to AR mutations, several AR splice variants which exhibit transcriptional activity even in the absence of androgen and contribute to the promotion of CRPC have recently been identified (Dehm et al. 2008, Hu et al. 2009, Guo et al. 2009, Sun et al. 2010, Watson et al. 2010). Although a relationship between AR splice variants and oxidative stress has not been reported to date, it is possible that oxidative stress may be implicated in the expression of AR splice variants as it is for splice variants of other genes (Soliman et al. 2009, Takeo et al. 2009,
Xu & Chu 2007). Therefore, future studies should examine the effects of oxidative stress-induced mutations of the AR gene or expression of AR splice variants.

3.4. De novo androgen synthesis

De novo synthesis of androgen in adrenal glands and tumors has recently been recognized as a potential cause of CRPC (Locke et al. 2008, Montgomery et al. 2008, Stanbrough et al. 2006), which hypothesis was proved by the result of cytochrome P17 inhibitor abiraterone acetate in clinical trial (de Bono et al. 2011). Although there is no evidence at present that shows that oxidative stress promotes androgen synthesis in prostate cancer, several studies indicate a relationship between oxidative stress and steroidogenesis. For instance, H$_2$O$_2$ was recently shown to biphasically regulate androgen synthesis in rat Leydig cells, indicating that oxidative stress within physiological levels promotes steroidogenesis (Zhao et al. 2012). These data suggest the possibility that oxidative stress promotes de novo androgen synthesis in prostate cancer cells.

4. Oxidative stress and resistance to androgen deprivation

There is a close relationship between oxidative stress and castration resistance in
prostate cancer. Oxidative stress activates AR signaling, which promotes a conversion from androgen-dependent to CRPC through pro-survival and anti-apoptotic roles. We found that H$_2$O$_2$-resistant LNCaP cell derivatives of androgen-dependent prostate cancer cells have a high level of AR protein expression and exhibit a castration-resistant phenotype (Shiota et al. 2010). In addition, evidence has shown that oxidative stress is increased in CRPC cells, as indicated by higher intracellular ROS levels in castration-resistant LNCaP derivatives C4-2 cells compared with LNCaP cells (Shigemura et al. 2007), and greater antioxidant protein levels (Kuruma et al. 2005, Shiota et al. 2011e) and ability to scavenge ROS (Wu et al. 2007) in castration-resistant LNCaP cells and tumors. Thus, AR activation by oxidative stress is thought to render prostate cancer cells resistant to castration.

5. Pro-survival and anti-apoptotic roles of AR signaling in prostate cancer cells in response to therapeutic interventions other than androgen deprivation

Similarly to castration resistance by activation of AR signaling, AR signaling may contribute to the survival and anti-apoptotic effects of other insults on cancer cells. Recently it was shown that the heart of AR knockout mice is sensitive to oxidative stress induced by doxorubicin (Ikeda et al. 2010). Similarly, we have shown that AR
knockdown sensitizes bladder cancer cells to doxorubicin (Shiota et al. 2012). In addition, AR signaling may be involved in cellular resistance to taxanes including paclitaxel, docetaxel and cabazitaxel which are key cytotoxic anticancer drugs for prostate cancer, as androgen regulates the expression of the taxane resistance-promoting factor, βIII-tubulin (Butler et al. 2001, Mariani et al. 2012). In fact, CRPC cells in which AR was overexpressed developed cross-resistance to taxanes (Kosaka et al. 2011). Furthermore, concurrent therapy with paclitaxel and castration was found to improve suppression of tumor growth and overall survival compared with sequential therapy with paclitaxel and castration using Shionogi and LNCaP tumor models (Eigl et al. 2005). Similarly to these in vitro studies, administration of docetaxel plus estramustine in addition to androgen deprivation in a clinical setting was shown to improve prostate-specific antigen response after 3 months (Fizazi et al. 2012). Long-term results and effects of this treatment regime on overall survival have not yet been obtained. Taken together, these results suggest a favorable outcome from taxane and androgen deprivation combination therapy, and pro-survival and anti-apoptotic roles of AR signaling in response to treatment with taxanes. In addition, androgen and AR expression rendered prostate cancer cells resistant to TGF-β-induced apoptosis (Zhu et al. 2008). Similarly, administration of androgen inhibited apoptosis induced by the
Akt inhibitor, LY294002, while the anti-androgen drug, flutamide, abolished the anti-apoptotic effect of androgen (Kumar et al. 2011). Like cytotoxic agents, radiation cytotoxicity is known to be augmented by suppression of AR signaling. Numerous lines of evidence have demonstrated that androgen deprivation augments the therapeutic effect of radiation (Granfors et al. 1997, Kaminski et al. 2003, Nishiyama 2012, Zietman et al. 1997). In addition, clinical studies also showed favorable effects of castration in combination with radiation on locally advanced prostate cancer with intermediate and high risk (D’Amico et al. 2004, 2008, Denham et al. 2005, 2011, Jones et al. 2011). Interestingly, it has recently been reported that the cell-cycle and DNA repair regulator, ataxia telangiectasia mutated kinase (ATM), contributes to radiation resistance through up-regulation of AR phosphorylation and activation, which explains the molecular mechanism mediating radiation resistance by AR signaling (Mahajan et al. 2012). In fact, where AR signaling was aberrantly activated in CRPC, cells were found to be cross-resistant to radiation (Wu et al. 2007). Taken together, these findings suggest that AR signaling in prostate cancer cells suppresses anti-survival and pro-apoptotic effects of various commonly utilized therapeutic cytotoxic and tumor-suppressive interventions, including taxanes or radiation in combination with castration (Fig. 2).
6. Conclusions and future directions

Oxidative stress can activate AR signaling via the following pathways: (1) AR overexpression; and (2) AR activation by AR co-regulators or intracellular signal-transduction pathways, thus contributing to the tumorigenesis and progression of prostate cancer, as well as the acquisition of castration resistance. In addition, AR signaling promotes anti-apoptotic effects and survival of prostate cancer cells in the face of oxidative and cytotoxic stressors, including taxanes and radiation, through transcriptional modulation of βIII-tubulin and ATM. Taken together, AR signaling appears to render prostate cancer cells refractory to various therapeutic interventions such as castration, radiation and taxanes, indicating AR is a comprehensive resistance factor and crucial target for prostate cancer treatment. Furthermore, administration of therapeutic interventions such as taxanes and radiation concurrent or before androgen-deprivation therapy may exert improved outcome in recurrent or advanced prostate cancer. So far, numerous studies revealed the usefulness of antioxidants including natural compounds such as vitamin D, vitamin E, carotenoids, lycopene, green tea catechins and isoflavone, in addition to synthetic antioxidants such as NAC and DPI as a suppressor of AR signaling and prostate cancer growth (Gupta-Elera et al.)
Thus, suppression of AR signaling by antioxidants may be a promising strategy to overcome treatment resistance of prostate cancer, which will be clarified in future.
Declaration of interest

There are no conflicts of interest.

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Figure Legends

Figure 1. Schematic representation of the links between transcription factors that induce AR expression and promote the development of CRPC.

Figure 2. Schematic representation of pro-survival and anti-apoptotic properties of AR signaling that promote therapeutic resistance to androgen-deprivation therapy, chemotherapeutic agents (taxanes) and radiation.
Figure 1 Shiota et al.
Figure 2 Shiota et al.

Anticancer treatment
- Androgen-deprivation therapy
- Chemotherapeutic agent (taxanes)
- Irradiation

Anti-survival

Pro-apoptosis

AR
AR/βIII-tubulin
AR/ATM