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Oxidative stress and androgen receptor signaling in the development and progression of castration-resistant prostate cancer

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Abstract

Aberrant androgen receptor (AR) signaling plays a critical role in androgen-dependent prostate cancer (PCa), as well as castration-resistant PCa (CRPC). Oxidative stress appears to contribute to the tumorigenesis and progression of PCa, as well as the development of CRPC, via activation of AR signaling. This notion is supported by the fact that there is an aberrant or improper regulation of the redox status in these disorders. Additionally, androgen deprivation-induced oxidative stress appears to be involved in the pathogenesis of several disorders caused by androgen deprivation therapy (ADT), including osteoporosis, neurodegenerative disease and cardiovascular disease. Oxidative stress can be suppressed with antioxidants or via a reduction in reactive oxygen species production. Thus, developing new therapeutic agents that reduce oxidative stress might be useful in preventing the conversion of androgen-dependent PCa into CRPC, as well as reducing the adverse effects associated with ADT. The objective of the present review was to provide an overview regarding the relationship between oxidative stress and AR signaling in the context of PCa, and especially CRPC. Additionally, we discuss the potential use of antioxidant therapies in the treatment of PCa.

Keywords: androgen receptor; castration-resistant prostate cancer; oxidative stress; prostate cancer; reactive oxygen species
I. Introduction

Androgens, the male sex steroids, play a key role in the development of the male phenotype during embryogenesis, sexual maturation at puberty, and male reproductive function and behavior in adulthood. Androgens also play a role in various non-reproductive tissues including bones, muscle, brain, skin, heart, blood vessels, blood and adipose tissue. However, androgens are also implicated in various pathological disorders, including prostate cancer (PCa). Testosterone, the most abundant androgen present in blood serum, is synthesized by Leydig cells of the testes. Other androgens, including dehydroepiandrosterone, androstenediol and androstenedione, are produced by the adrenal glands and may be converted into testosterone in peripheral tissues [1,2]. Free and lipophilic testosterone then diffuses throughout the cells of its target tissues and organs, where it may be converted into its (about 10-fold) more potent metabolite, dihydrotestosterone (DHT), via 5α-reductase (type I or II) [3]. Both testosterone and DHT exert their actions by binding to the androgen receptor (AR), a 110-kDa member of the nuclear receptor superfamily. Prior to its activation, the AR is primarily located in the cytoplasm, and makes up a complex with heat shock proteins. Upon ligand binding, the AR undergoes conformational rearrangement, homodimerizes, and translocates into the nucleus [4]. After translocating into the nucleus, the AR binds to specific recognition sequences, known as androgen response elements, in the promoter and enhancer regions of its target genes, and modulates their gene expression.
PCa is the most common type of non-cutaneous cancer and the second leading cause of male cancer-related mortality in developed countries. The AR signaling pathway is known to play a critical role in prostate carcinogenesis and PCa progression. Androgen deprivation therapy (ADT) is commonly used in the treatment of PCa, and involves either a reduction in the production of androgens via surgical or medical castration or an interference in AR function with the use of anti-androgen agents [5]. Although ADT is initially effective in approximately 90% of PCa cases, most cases eventually become resistant to ADT and develop castration-resistant PCa (CRPC) [5]. In CRPC, the AR signaling pathway still plays a key role in cell proliferation despite low androgen levels being achieved with ADT [6]. Activation of the AR signaling pathway in CRPC has been attributed to a number of mechanisms, including AR hypersensitivity, de novo intraprostatic androgen production, promiscuous AR activation via adrenal androgens, non-androgenic steroids and even anti-androgens, and AR activation via growth factors and cytokines through intracellular signal-transduction pathways [7]. These phenomena may result from abnormalities in the AR (i.e. mutation and overexpression) and/or its related molecules (e.g. AR co-regulators). Furthermore, it has been recently reported that some AR splice variants may exert significant constitutive effects in the absence of ligands [8–12].

Hydroxyl radicals, peroxides and superoxides are reactive oxygen species (ROS) generated during metabolic processes. ROS generated either from endogenous or external sources play a key
role in regulating a wide range of biological mechanisms [13]. Although increased ROS production has been traditionally associated with tissue injury or DNA damage, an increase in ROS production in several cellular processes is also associated with neoplastic transformation and aberrant cellular proliferation [14,15]. In addition, processes associated with proliferation, apoptosis and senescence may be due to the activation of various signaling pathways in response to intracellular changes in ROS levels [16]. Thus, excessive ROS production or impairment of antioxidant defense systems can induce oxidative stress. This increase in ROS levels may contribute to the initiation and development of various cancers, including PCa, since oxidative stress regulates cellular fate in various systems.

Oxidative stress has been shown to play a key role in prostate carcinogenesis and PCa progression [17,18]. It has been recently reported that oxidative stress is implicated in the conversion of androgen-dependent PCa into CRPC via regulation of AR expression [19]. However, the mechanisms by which oxidative stress alters AR signaling and thereby induces CRPC are not fully understood and need to be further explored. Thus, in the present review, we will summarize the currently available research on the role of oxidative stress in AR signaling and CRPC pathology, as well as several disorders caused by ADT, including osteoporosis, neurodegenerative disease and cardiovascular disease (Fig. 1). Additionally, we will discuss the potential role of antioxidant therapy in the treatment of PCa, especially in preventing the conversion of androgen-dependent PCa into CRPC.
II. Effects of AR signaling on oxidative stress

Several reports have suggested that blockade of AR signaling may induce oxidative stress in various systems. It has also been shown that castration induces oxidative stress in the rat prostate by significantly upregulating ROS-generating NADPH oxidases and downregulating ROS-detoxifying enzymes [20]. Additionally, it was found that ADT decreases the mRNA expression levels of a major ROS scavenger, manganese superoxide dismutase (MnSOD), in biopsy tissues of PCa [21]. Furthermore, gene expression of ROS-detoxifying enzymes induced by oxidative stress, such as thioredoxin 1, peroxiredoxin (Prx) 5, and MnSOD, is reduced in the rat prostate following castration [22]. In addition, it was recently reported that thioredoxin 1 was reduced and oxidative stress was increased by androgen deprivation compared with those by androgen replacement [23]. MnSOD is located in mitochondria and implicated in protecting mitochondrial DNA from damage induced via oxidative stress. Recently, mitochondrial gene mutations were shown to upregulate intracellular ROS levels and lead to the development of malignancies [24]. These results were corroborated by previous observations, where increased oxidative cellular damage accompanied by declining testosterone levels was associated with the development of malignancies [17,25] and aging [26–28]. Additionally, ROS levels in myocardial cells of AR-knockout mice were found to be higher than those of wild-type mice when the anticancer drug, doxorubicin, was administered. Doxorubicin is
known to cause cardiotoxicity through oxidative stress and thereby result in greater
doxorubicin-induced cardiotoxicity among the AR-knockout mice [29]. Also, it was found that
castration of male mice evoked an increase in oxidative stress within their skeletal system [30,31].

Conversely, there are several reports suggesting that androgen may increase oxidative stress
[32–34]. Ripple et al. reported there was an increase in oxidative stress, and lipid peroxidation, in
androgen-dependent PCa LNCaP cells following exposure to androgens (i.e. DHT and R1881) [32].
Similarly, both Pinthus et al. and Pathak et al. reported that androgen exposure induces oxidative
stress in AR-positive PCa cells [33,34]. Furthermore, it was recently reported that androgen-induced
oxidative stress, achieved with 10 nM of R1881 in 22Rv1, was due to the activation of NADPH
oxidase [35]. Despite the equivocal evidence with respect to the interaction between androgen and
oxidative stress, it may be that both androgen deprivation and androgen exposure induce oxidative
stress via different mechanisms. For example, androgen deprivation in an androgen-positive milieu
or androgen exposure in an androgen-negative milieu may both evoke various stresses in PCa cells.
Thus, given that all the above-mentioned studies had added androgen to an androgen-negative milieu,
their findings may not reflect the clinical situation of ADT. Nevertheless, androgen deprivation,
which mimics clinical ADT, induces oxidative stress, suggesting that ADT may also induce
oxidative stress in human tissues and tumors, including PCa.
III. Effects of oxidative stress on AR signaling

Given that androgen deprivation induces oxidative stress in PCa cells, the effects of oxidative stress on AR signaling are reviewed in this section (Fig. 2). AR signaling is augmented in CRPC, or a low-androgen milieu, by the various mechanisms, including: (1) AR overexpression, (2) AR mutations or splice variants, (3) AR co-regulators, (4) AR activation by growth factors and cytokines through intracellular signal-transduction pathways, and (5) de novo intraprostatic androgen synthesis.

(1) AR overexpression

AR overexpression is thought to be one of the major causes of CRPC. AR overexpression can be attributed to gene amplification, transcriptional upregulation, translational upregulation and decreased degradation. Many studies have shown that the progression of CRPC is associated with increased AR expression [36–39]. AR gene overexpression occurs in most cases of CRPC, where in the majority of CRPC cases, the AR gene undergoes transcriptional upregulation, and in approximately 10–20% of these cases, the AR gene is amplified [40].

We previously reported that Twist1 was upregulated by oxidative stress, and in turn, Twist1 upregulated AR transcription directly by binding to the AR promoter region. This observation was
consistent with the finding that CRPC cells expressed higher levels of AR transcript and protein, as well as Twist1 protein, than androgen-dependent PCa cells [19]. Moreover, we recently reported that Y-box binding protein-1 (YB-1) was also involved in AR transcription [41]. YB-1 is also a stress-related protein. YB-1 translocates into nucleus in response to various stressors, including ultraviolet radiation and the anticancer agent, paclitaxel [42,43]. In addition, YB-1 is a major target gene of Twist1 [44–46], and vice versa [47]. These findings suggest that both YB-1 and Twist1 may promote AR transcription.

Of the transcription factors that regulate AR transcription [48], several transcription factors appear to be also implicated in oxidative stress. The transcription factor, NFκB, is well known to be induced by cytokines and inflammation, as well as various stressors, including oxidative stress. c-Myc may also induced by certain stressors, such as ultraviolet radiation [49]. These transcription factors are shown to positively regulate AR transcription [50–52]. Also, CREB and Sp1 have been suggested to be involved in the oxidative stress signaling pathway [53,54], as well as in regulating AR transcription [55–57]. Moreover, Foxo3a, which protects cells from oxidative stress, also regulates AR transcription [58]. Lastly, the above-mentioned transcription factors involved in oxidative stress/AR signaling pathway via the modulation of AR transcription may also involve the Twist1/YB-1 signaling pathway. These findings suggest that there is a close relationship between oxidative stress and AR expression.
(2) AR mutations or splice variants

Mutations in the AR gene may create a promiscuous receptor, and thereby alter the interactions between its co-regulating proteins and intramolecular NH$_2$-COOH moieties [59]. Approximately 70 different missense mutations have been documented in clinical samples, with varying consequences on AR activity. These mutations and their effects on the AR have been previously reviewed in [59,60]. Mutations in the ligand-binding domain of the AR, such as H874Y, T877A, and T877S, increase the binding capacity of the AR to motifs associated with its co-regulatory proteins, stimulate the expression of AR target genes, and make it susceptible to being activated by other hormones and even anti-androgens [61]. Although oxidative stress is well known to induce DNA mutation through the oxidation of nucleotides, to the best of our knowledge, no reports exist on oxidative stress-induced mutations in the AR gene.

In addition to AR mutations, several AR splice variants have been recently identified. Their role in promoting castration-resistant growth in PCa cells has been of much interest, since such splice variants exhibit transcriptional activity in the absence of androgen [8–12]. It is thought that AR splice variants may contribute to the promotion of CRPC, as there is no need for a ligand in its activation. However, so far, to the best of our knowledge, there are no reports on the relationship between AR splice variants and oxidative stress.
(3) AR co-regulators

The importance of AR co-regulators in the activation of AR signaling has been previously recognized [62]. Throughout the development and progression of PCa, a subset of AR co-activators have been shown to be overexpressed or over activated. Additionally, the deregulation of AR co-activators tends to increase with tumor progression, correlate with the aggressiveness and poor prognosis of PCa, and contribute to the development of CRPC [62,63].

Previously, Prx1 was reported to be one of the co-activators involved in facilitating the binding of androgen to the AR [64,65]. The Prx family consists of six members (i.e. typical 2-Cys, Prx1-4; atypical 2-Cys, Prx5; and 1-Cys, Prx6), and plays a critical role in the redox-dependent signal transduction pathway, as well as protecting cells from cytotoxicity induced via oxidative stress [66]. Generally, Prxs are thought to be upregulated by oxidative stress. Specifically, Prx1 is regulated by a transcription factor induced by oxidative stress, known as NF-E2-related factor 2 (Nrf2) [67]. Additionally, we have found that Ets, a transcription factor also induced by oxidative stress, is upregulated by high-mobility group protein B1, a protein implicated in the oxidative stress-induced regulation of Prx1 and Prx5 expression [68]. These findings suggest that Prx1 expression is regulated by oxidative stress. Recent findings also suggest that Prx2 expression is regulated by oxidative stress through the Foxo3a transcription factor [69], which has been implicated in AR
transcription and cellular responses to oxidative stress [58,70]. We found that of all the members of the Prx family, Prx2 was the most overexpressed in CRPC and hydrogen peroxide-resistant cells. Prx2 was also found to augment AR transactivation by acting as an AR co-activator. Moreover, it was found that cytoplasmic Prx2 enhances AR transactivation, while nuclear Prx2 decreases it, suggesting that the redox status of the nucleus and cytoplasm might affect AR signaling through Prx2 [71]. Similarly, it was reported that the oxidized forms of nuclear thioredoxin 1 were higher in prostate cancer cell lines compared with benign prostate epithelial cells, suggesting that nuclear redox imbalance occurred [23]. These notions are supported by the report that AR binding to DNA was inhibited by oxidizing reagent prior to AR DNA binding \textit{in vitro} whereas AR dissociation with DNA was also mitigated by oxidizing reagent after AR DNA binding, these phenomena likely result from cross-linking of cysteine residues in the DNA-binding domain of AR [72].

There are other AR co-regulators are implicated in oxidative stress. Hsp27 is a cytoprotective chaperone that is induced in response to various stressors, including oxidative stress. Hsp27 regulates AR transactivation by increasing AR stability [73]. Additionally, signal transducers and activators of transcription (STAT) 3, a downstream protein in the Janus-activated protein kinase (JAK)/STAT pathway, is activated by oxidative stress, and is a well-known AR co-activator [74–76]. Early growth response-1 (Egr-1) is another AR co-activator [77], which is induced by injury, mitogens, and cytokines, as well as various stressors [78]. Thus, oxidative stress regulates AR
signaling by regulating the expression levels of various AR co-regulators, and thereby inducing transcription of AR target genes.

(4) AR activation by growth factors and cytokines through intracellular signal-transduction pathways

Androgen-induced prostate epithelial and PCa cell proliferation is regulated by an indirect pathway involving paracrine mediators produced by stromal cells, such as insulin-like growth factor, fibroblast growth factor, and epidermal growth factor [79,80]. These growth factors and cytokines interact with AR signaling through their downstream intracellular signal transduction pathways.

AR signaling is influenced by a complex web of signal cascades, such as mitogen-activated protein kinase (MAPK), JAK/STAT, phosphatidylinositol-3-kinase (PI3K)/Akt, protein kinase C (PKC) and protein kinase A (PKA) [81–86]. The most significant effects of oxidative stress have been observed in pathways involving MAPK [87,88]. Activation of the extracellular-regulated kinase [87,88], c-Jun N-terminal kinase [89], and p38 [90] subfamilies of the MAPK pathways occur in response to changes in the cellular redox balance [91]. In addition, the JAK/STAT and PKC pathways are activated by oxidative stress [92,93]. Both the PKA [53] and PI3K/Akt [94] pathways may also be involved in mechanisms that target oxidative stress. Taken together, these findings suggest that oxidative stress affects AR signaling via various intracellular signal-transduction
pathways.

(5) De novo intraprostatic androgen synthesis

Another mechanism by which AR signaling may be augmented in CRPC is via intratumoral repletion of endogenous AR agonists. This was first suggested in studies assessing the prostate tissues of patients that had demonstrated the progression of tumors following castration and DHT concentrations similar to untreated tumors [95]. Other studies found that while DHT levels may be somewhat depleted in CRPC tumors, intratumoral testosterone concentrations are similar to those of untreated PCa [96,97]. Recently, intratumoral conversion of adrenal androgens and de novo steroid synthesis have been brought forward as potential causes of tumor progression [97–99]. The presence of active AR in CRPC samples and high intratumoral testosterone and DHT concentrations among CRPC patients with castrate levels of serum androgen support the concept of intratumoral conversion of steroidal precursors [97,100]. Recent publications have re-emphasized that intratumoral de novo steroidogenesis can occur by demonstrating that expression of steroidogenic enzymes occurs in both normal prostate and PCa tissue [97,99,101,102], and that there is a differential expression pattern between the various tumor types and the normal prostate gland [97,99]. Furthermore, a radio-labeled steroid precursor, acetic acid, was shown to be converted into DHT in androgen-dependent PCa and CRPC cells [98]. The potential upregulation of steroidogenic
enzymes in CRPC and the resulting production of local testosterone and DHT may explain the observed increase in intratumoral androgen levels, which are sufficient to activate the AR [97,98,100,103]. However, to the best of our knowledge, there are no reports to date on the relationship between androgen synthesis and oxidative stress. Future research on the interaction between androgen synthesis and oxidative stress is thus warranted.

IV. Oxidative stress and the progression of castration-resistant PCa

It is well known that MnSOD expression is markedly decreased in CRPC. MnSOD converts superoxides into less reactive species, and thereby decreases oxidative stress. Conversely, a decrease in MnSOD expression causes an increase in oxidative stress. Recently, it was reported that there was an increase in AR activity in PCa following inhibition of MnSOD expression. Specifically, a knockdown in MnSOD expression induced a similar change in androgen gene expression, and augmented the DNA-binding ability and transactivation of the AR, which was reversed by N-acetyl-cysteine (NAC) [104].

We also found that there is a close relationship between oxidative stress and castration resistance in PCa. Hydrogen peroxide-resistant LNCaP derivatives of androgen-dependent PCa cells exhibit a castration-resistant phenotype [19]. As described in the section above, oxidative stress can
activate AR signaling via the interaction with various pathways. Since dysregulated AR signaling leads to castration-resistant growth in PCa, oxidative stress also induces castration resistance through the activation of AR signaling.

V. Interactions between oxidative stress and AR signaling in other conditions

ADT is known to lead to numerous adverse effects, such as osteoporosis, obesity, cognitive disorders, lipid alterations, insulin resistance, and increased risk for diabetes and cardiovascular morbidity [105–107]. The pathogenesis of these disorders is closely associated with oxidative stress. As described previously, oxidative stress can affect AR signaling in PCa. Since oxidative stress also plays a critical role in other conditions, oxidative stress induced via ADT may be implicated in the various adverse effects caused by ADT.

1) Interaction between oxidative stress and AR signaling in osteoporosis

Osteoporosis and bone fractures accompany ADT. Osteoporosis is a skeletal disorder that involves the micro-architectural deterioration of bone tissue, which results in bone fractures [108,109]. Age, lifestyle, genetics, endocrine disorders, and oxidative stress collectively influence and contribute to the development of osteoporosis [110]. A number of studies have suggested that
oxidative stress can exacerbate age-related bone loss [111,112]. Bone-resorbing osteoclasts generate high levels of superoxide anions and hydrogen peroxide [113]. These free radicals modulate intra- and inter-cellular signaling responsible for bone loss [113]. Castrated rats are more likely to develop osteoporosis, and this can be prevented with antioxidants, such as orange and grapefruit pulps [114,115]. Similarly, citrus bioactive compounds have been shown to decrease oxidative stress and improve bone quality in castrated rats [116]. These findings suggest that antioxidants can be used to ameliorate osteoporosis induced by ADT.

The anti-apoptotic effects of sex steroids on osteocytes have been well documented in mice, rats, and humans [117–119], and may contribute to their anti-fracture properties independent of their effects on bone mineral density [120]. Almeida et al. reported that gonadectomy of mice induced a phosphorylation of p53 and p66shc in their bones, which were activated by oxidative stress and reversed by androgen or estrogen [30]. Thus, these findings suggest that androgen and estrogen deficiency induces oxidative stress in osteoclasts, thereby leading to increased bone resorption and osteoporosis. Furthermore, it was found that castration of male mice induced an increase of oxidative stress with their bones [30,31].

(2) Interaction between oxidative stress and AR signaling in neurodegenerative diseases

Recently, adverse effects of ADT on cognitive function have also been recognized [106]. It
has been demonstrated that testosterone and related androgens can attenuate neuronal loss caused by certain insults [121–123]. Additionally, Lewis et al. found that castration reduces the density of pyramidal cells in male rat spines [124]. Furthermore, previous studies have demonstrated that patients with neurodegenerative diseases display lower levels of androgens [125–130]. Taken together, these findings suggest that androgens play an important role in neuron protection. Huntington's disease, an autosomal dominant inherited neurodegenerative disease, is characterized by progressive motor and cognitive deterioration [131,132]. Oxidative damage is thought to play an important role in the striatal cell loss observed in Huntington's disease [133]. A study by Túnez et al. has demonstrated the neuron protective effect of sex steroid hormones (i.e. 17β-estradiol) against cellular injury and oxidative damage induced on the striatum of ovariectomized rats [134]. This protective action was characterized by a reduction of oxidative stress and biomarkers of cellular damage. Similarly, they reported that castration triggered oxidative damage and cellular death, which were blocked by testosterone administration [135].

(3) Interaction between oxidative stress and AR signaling in blood coagulation

Cardiovascular complications are major side effects of ADT [107]. Platelets are intimately involved in the pathogenesis of thromboembolic disorders, especially in arterial forms of thrombosis. Defective regulation of platelet activation/aggregation is a predominant cause for arterial thrombosis.
Thromboxane-dependent platelet activation is associated with cardiovascular risk factors, such as cigarette smoking [136,137] and diabetes mellitus [138], and may contribute to the increased risk of myocardial infarction and stroke, as suggested by the aspirin trials [139]. These risk factors are also associated with low-grade inflammation [140] and enhanced oxidative stress [141]. Oxidative stress impairs endothelial function and promotes platelet activation and aggregation, which may play an important role in the pathogenesis of acute cardiovascular diseases.

Within the hematopoietic system, testosterone regulates fibrinogen, plasminogen activator inhibitor-1 and platelet aggregability. Previous findings indicated that testosterone downregulated fibrinogen and plasminogen activator inhibitor-1 [142]. Li et al. demonstrated that androgen inhibited experimental arterial thrombosis at physiological doses, and that its receptor was mediated via the modulation of platelet activation [143]. It was demonstrated that an addition of DHT inhibits platelet aggregation induced by hydrogen peroxide. Moreover, platelet aggregation induced by hydrogen peroxide was found to be increased in castrated rats, which was reversed by androgen replacement. These findings suggest that physiological doses of androgen and its receptor may play an important role in regulating platelet aggregation, in particular in counteracting oxidative injury [144].

(4) Interaction between oxidative stress and AR signaling in the heart
Substantial evidence suggests that oxidative stress may play a crucial role in the pathogenesis of cardiovascular disease [145,146], such as ischemic heart disease, hypertension, atherosclerosis, hypertrophy, cardiomyopathies, and congestive heart failure. ROS are capable of not only inducing oxidative damage to various cellular components and impairing cellular energetics, but also of modulating redox signaling, and thereby inducing highly specific acute or chronic changes to the cellular environment [147]. Conversely, sex differences in cardiovascular responses to a variety of experimental interventions and the presence of specific receptors for androgens and estrogens in the myocardium of rats suggest that sex hormones play a physiological role in cardiac function [148]. Klapcińska et al. found that castration significantly worsened the antioxidant status of the left ventricle, as evidenced by a significant decline in the activities of antioxidant enzymes (i.e. superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase), and by the increase in lipid peroxidation and nitrotyrosine concentrations [149]. These results are further supported by the fact that ADT may increase the risk of death from cardiovascular disease [150].

VI. Clinical implications of antioxidant therapy in PCa

Given the accumulating evidence suggesting that ADT induces oxidative stress in PCa, we speculate that antioxidant therapy may play a role in the treatment of PCa in patients receiving ADT.

NAC is an electrophile supporting the production of glutathione, a major intracellular
antioxidant and functions as an antioxidant. NAC has been shown to inhibit the mitogenic activity of v-H-Ras in NIH3T3 [151], and prevent chronic ulcerative colitis-associated colorectal adenocarcinoma in mice [152]. Additionally, NAC has been shown to induce p53-dependent apoptosis in transformed mouse embryo fibroblasts (MEF), but not in normal MEF [153]. Furthermore, NAC promotes angiostatin production and vascular collapse in a breast cancer orthotopic model [154]. NAC was also shown to have a chemo-preventive effect on cancer progression due to its protective effects against UV-induced cellular damage [155,156] and angiogenesis [157,158]. Several investigators have shown that NAC prevents the induction and maintenance of DNA damage and progression of cancer in smokers [159]. In fact, NAC has reduced staining against 8-hydroxy-2’-deoxyguanosine (8-OHdG), nitrotyrosine and 4-HNE in the prostate of TRAMP mice [160]. Therefore, taken together, antioxidant therapy with NAC appears to be promising for the treatment of PCa. In a large randomized intervention trial, EUROSCAN, it was found that both vitamin A and NAC (i.e. 600 mg daily for 2 years) did not have any benefit in preventing tumour recurrence or the occurrence of second primary tumours in patients with head and neck or lung cancer [161]. Despite these findings, the effects of NAC may be dependent on the cancer type and route of administration.

Previously, it was found that lycopene, a carotenoid with antioxidant properties and a role in preventing oxidative damage to cellular protein, lipid and DNA, augmented the therapeutic effects of
orchietomy on advanced PCa [162]. Additionally, serum prostate-specific antigen levels and disease-associated symptoms in a CRPC patient were reported to be alleviated by intake of saw palmetto supplements with lycopene [163]. Although the number of enrolled patients in these clinical studies was relatively small, their findings may also suggest the potential use of lycopene combined with castration in the treatment of PCa. Similarly, vitamin E, or α-tocopherol, also an antioxidant, has been implicated in decreasing risk of PCa mortality, suggesting that it may also be used as a therapeutic agent for preventing the progression of PCa [164]. Also, NADPH oxidase inhibitor diphenyleneiodonium chloride which functions as antioxidant by inhibiting ROS production by NADPH oxidases suppressed prostate cancer cell viability including LNCaP cells [165]. In addition, another NADPH oxidase inhibitor apocynin suppressed prostate cancer cell invasion [166].

Additionally, antioxidants may play a favorable role in reducing the adverse effects induced by ADT. As previously mentioned, ADT may cause several adverse effects, including osteoporosis, neurodegenerative disease and cardiovascular disease. Several preclinical studies have suggested that the unfavorable effects of ADT are ameliorated by antioxidants, which act to suppress oxidative stress. Therefore, antioxidant therapy with ADT may not only augment the therapeutic effects of ADT, but also suppress the adverse effects associated with ADT.
VII. Conclusions and future directions

Oxidative stress appears to contribute to the tumorigenesis and progression of PCa, as well as
the development of CRPC through the activation of the AR signaling pathway. This notion is
supported by the fact that there is an aberrant or improper regulation of the redox status implicated in
these disorders. Given that oxidative stress can be suppressed by antioxidants or via a reduction in
ROS production, developing new therapeutic agents that ameliorate oxidative stress may prevent the
progressive conversion of androgen-dependent PCa into CRPC, as well as reduce the adverse effects
associated with ADT (i.e. osteoporosis, neurodegenerative disease and cardiovascular disease).
However, little is known regarding the relationship between oxidative stress and AR signaling, the
progression of PCa into CRPC, and the use of antioxidants with ADT in PCa. Thus, future research
in the above-mentioned areas is warranted to shed some light in this field.
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**Figure Legends**

**Fig. 1.** Schematic representation of the links among androgen-deprivation therapy, oxidative stress, androgen receptor signaling, and castration-resistant prostate cancer.

**Fig. 2.** Schematic representation of the signaling pathways on androgen receptor signaling in prostate cancer by oxidative stress. Oxidative stress activates the signaling pathways circled (transcription factors, AR co-regulators and intracellular signal-transduction pathways), resulting in an activation of AR signaling pathway.
Figure 1 Shiota et al.

- Pro-oxidant properties (NADPH oxidases)
- Anti-oxidant properties (MnSOD, thioredoxin 1, peroxiredoxin 5)

Oxidative stress

- Androgen receptor signaling
- Castration-resistant prostate cancer

Androgen-deprivation therapy

Adverse effects
- osteoporosis
- neurodegenerative disease
- cardiovascular disease
- etc
Figure 2 Shiota et al.

Prostate cancer growth and progression

NF-κB Gene Expression of AR target

Intracellular signal-transduction pathways

PI3K/Akt PKA PKC MAPKs JAK/STAT

Transcription factors

AR co-regulators

AR Gene Expression

Prostate cancer growth and progression

Gene Expression of AR target

AR Gene Expression