New Approaches Targeting Androgen Receptor Signal Pathways for Treatment of Castration-Resistant Prostate Cancer

Donkena and Young
*Mayo Clinic College of Medicine*
USA

1. Introduction

Even orchiectomy androgen depletion was proposed for treating prostate cancer in the middle of the last century (Attar et al., 2009a; Ross et al., 2008; Vis & Schroder, 2009), androgen deprivation remains to be the mainstay for advanced prostate cancer treatment. However, androgen depletion is usually effective for a limited duration (i.e., a median time of 2-3 years) and majority of the treated prostate cancer patients will develop unresponsiveness to the initial androgen depletion treatment and then evolves to regain the ability to grow despite low levels of androgens in the circulation (Chen et al., 2008; Mostaghel et al., 2009). These so called “castration-resistant prostate cancer (CRPC)” is almost incurable and becomes insignificantly responsive or resistant to most of anti-cancer, cytotoxic drugs except docetaxel and cabazitaxel that seem to show modest but significant improvement of patient survival time (de Bono et al., 2010; Petrylak et al., 2004; Vaishampayan et al., 2009). Evidence strongly suggests that the nuclear androgen receptor (AR) still plays an important role in CRPC and may be, in part, attributed to the resistance to cytotoxic anti-cancer drugs. Intriguingly, these CRPC patients may still respond to second line of AR-related treatment. Thus, it has been urgent to develop novel and more effective AR-related treatments for CRPC.

Acquired resistance has been the main limitation of efficacy of cytotoxic drug chemotherapy and hormonal therapy for CRPC. Recently, new molecules or agents aiming at local angiogenesis, immunotherapy, apoptosis, chaperone proteins, the insulin-like growth factor (IGF) pathway, mammalian target of rapamycin (mTOR), RANK ligand, endothelin receptors, and the Src family kinases in CRPC have been developed for treating CRPC, some of which have been either approved by US Food and Drug Administration (FDA) or entering into different phases of clinical trial (Aggarwal & Ryan, 2011; Leo et al., 2011; Macfarlane & Chi, 2010; Seruga et al., 2011). This chapter will only focus on AR related targeting approaches for CRPC treatments.

2. AR mediated actions in prostatic cells

Wild type of AR is an approximately 110 kDa protein encoded by the gene on Chromosome Xq11-12 containing 8 exons. It is a ligand (e.g., dihydrotestosterone or DHT) dependent transcription factor belonging to the nuclear steroid hormone receptor superfamily (Balk &
Knudsen, 2008; Li & Al-Azzawi, 2009; He & Young, 2009). AR protein has three main functional domains consisting of an N-terminal (NTD) transactivation-1 domain (AF-1; exon 1), a central DNA binding domain (DBD; exons 2 and 3), and a C-terminal ligand binding domain (LDB) with a ligand dependent activation function-2 (AF-2) (exons 4-8) and a short hinge region (exon 4). Normally, androgen binding to LDB in cells will induce an AR conformational change that facilitates its nuclear translocation and specific genomic DNA binding, and therefore, the consequence of the regulation of AR target gene expression.

The functions of AR can vary depending on the cellular context of cell types. In the adult prostate, both stromal and secretary epithelial cells express the same AR protein. It has been shown that castration can induce secretory epithelial cells regress due to apoptosis in rodent and human prostates (Cunha & Chung, 1981; Cunha & Donjacour, 1989; Cunha & Lung, 1978; Kurita, et al., 2001; Niu et al., 2008a; Niu & Xia, 2009; Placencio et al., 2008), but stromal or epithelial basal cell components remain unaffected. After replenishing with exogenous androgens, secretory epithelial cells will re-grow from stem cells in the basal cell layer (Heer et al., 2007; Niu & Xia, 2009; Vander Griend et al., 2008). It has been suggested that the AR in stromal cells, upon androgen stimulation, controls the out growth of the epithelial cells from basal cells by secreting a number of growth factors (Heer et al., 2007; Niu & Xia, 2009; Vander Griend et al.). The basal cells normally do not express detectable AR protein. The prostatic stem cells stimulated by surrounding stromal cells may out grow and differentiate into mature secretory epithelial cells which express AR protein. There are many known prostate-specific differentiation proteins, including prostatic specific antigen (PSA), human kallikrein-2 (hK2), prostatic alkaline phosphatase and others found to be expressed directly or indirectly through regulation by androgens via AR in these secretory epithelial cells (Niu et al., 2008b; Young et al., 2004). Thus, in the normal prostate, the role of AR in the epithelial cells seems to maintain a normal, terminal differentiation state of the cells. The results of recent studies using transgenic mouse models appear to be consistent with the above notion that the AR in prostatic lumar epithelial cells may function, partly, as a tumor suppressor. Furthermore, there are reports (Olshavsky et al., 2008; Balk & Knudsen, 2008) demonstrating that cyclin D3 is an intriguing cell cycle regulatory molecule that interacts with AR in prostatic epithelial cells and represses cell growth stimulatory activities of AR. This event may actually occur in in vivo, because it was found that cyclin D3 was expressed in a higher level in normal or benign prostate tissues than that in prostate cancer tissues. There are also other cell cycle regulators such as p53 and RB tumor suppressor proteins (Balk & Knudsen, 2008; Chen et al., 2008; Goo et al., 2004; Shenk et al., 2001; Vis & Schroder, 2009) that have been shown to be able to interact with and regulate AR. However, their roles in regulation of AR functions in normal prostatic epithelial cells still require further clarification (see below).

Even both prostatic epithelial and stromal cells express the same AR protein, the functions of AR in stromal cells may eventually be different from that in luminal epithelial cells. In addition, studies have shown that the stromal AR in some conditions may act to promote the formation of prostate cancer (Cunha & Chung, 1981; Cunha & Donjacour, 1989; Cunha & Lung, 1978; Kurita, et al., 2001; Niu et al., 2009a; Niu & Xia, 2009; Placencio et al., 2008). For example, the mouse renal capsule tissue recombination experiments were used to demonstrate that only stromal AR is required for prostate epithelial development, therefore, the presence of epithelial AR is not required for the development of prostatic epithelia. Further, transgenic prostate cancer mouse models with cell-specific AR knock-out were used to demonstrate that the prostate stromal AR plays a more prevalent role than the
epithelial AR for the promotion of prostate cancer and early stages of cancer progression. Importantly, prostatic stroma play an important role in inducing epithelial apoptosis during castration or androgen deprivation through stromal transforming growth factor-β (TGFβ) action (Kurita, et al., 2001; Niu & Xia, 2009; Placencio et al., 2008). Early studies showed that an increase of TGFβ is associated with androgen ablation in normal/benign and cancer tissues (Lee et al., 1999; Kyprianou & Isaacs, 1999; Hsing et al., 1996; Brodin et al., 1999). In more recent studies, rodent prostate models were used to show that stromal TGFβ was upregulated by androgen ablation which then antagonized stromal Wnt expression and reduced the paracrine effect of Wnt on neighboring epithelia (Placencio et al., 2008; Li et al., 2008). TGFβ can also inhibit proliferation of malignant prostatic epithelial cells, however, it has been shown that the loss of TGFβ receptors I and II was associated with high Gleason grades and with a reduce survival time in prostate cancer patients. Loss or reduction of expression of TGFβ receptors I and II in prostate cancer cells has been suggested to be partially attributed to TGFβ resistance in prostate cancer (Cardillo et al., 2000; Li et al., 2008).

In transgenic TRAMP mouse models, the dominant-negative mutant TGF-beta receptor II expressed in mouse prostate epithelial cell seems to increase prostate tumor initiation and progression (Pu et al., 2009). Interestingly, the dysfunctional TGF-beta receptor II expression significantly enhanced AR expression at mRNA and protein levels. However, another study (Turley et al., 2007) found that the decreased or lost expression of the type III TGF-beta receptor (TbetaRIII) could be largely accounted for TGFβ resistance of this cancer and its progression because the loss or reduction of this receptor was found frequently in the cancer tissues. In vitro and in vivo studies showed that TbetaRIII mediates the decrease of cell migration and invasion as well as of tumor growth in xenografts. The results of the study conclude that the lost or reduced expression of this receptor correlates well with increased clinical stages, metastatic status, and PSA recurrence of the cancer. Further studies showed the reduced expression of the receptor is probably due to the combination of the loss of heterozygosity at the TGFBR3 genomic locus around chromosome 1p32 and epigenetic control of the TbetaRIII promoter.

Increasing angiogenesis in prostate tumor areas is another important function of the AR in prostate stroma in order to support sufficient nutrients for cancerous prostate cells to grow. Reducing the expression and secretion of VEGF with subsequently decreasing micro-blood vessels by androgen deprivation can be attributable to another mechanism for castration-mediated apoptosis (Cheng et al., 2004). Interestingly, it was found that, when injected into an AR expressing stromal environment, the androgen-independent/AR-null prostate cancer cells can benefit from the presence of androgens owing to its effects on angiogenesis (Cano et al., 2007; Halin et al., 2007). On the other hand, castration of the rodent host can induce partial apoptosis of orthotopically injected AR-less prostate cancer cells due to the inhibition of expression of VEGF from the stroma and angiogenesis of tumor (Cano et al., 2007; Hammarsten et al., 2006). Castration plus administration of an inhibitor of vascular endothelial growth factor receptor 2 and epidermal growth factor receptor signaling, N-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine (ZD6474), enhanced apoptosis of the prostate cancer tumors, suggesting a potential new way to treat CRPC (Cano et al., 2007; Hammarsten et al., 2006; Johansson et al., 2007). Moreover, studies also showed that, by unknown mechanisms, the AR could re-appear in stroma and epithelial areas of human prostate cancer tissues after castration therapy, therefore, this means that stromal AR could be important to the progression of CRPC and be a highly feasible target for CRPC treatment (Henshall et al., 2001; Ricciardelli et al., 2005; Wikström et al., 2007).
3. AR in malignant prostatic cells

AR in malignant prostate cells shows cell proliferative stimulation and cell survival functions that are apparently different from that of the AR in normal epithelial cells as described above. In primary prostate tumor AR responds to castration or androgen deprivation and cause tumor regression by apoptotic cell death. AR expression may be reduced during the hormonal treatments. In fact during the malignant progression, both stromal and epithelial cells in tumor areas become resistant to hormonal deprivation (Henshall et al., 2001; Wikström et al., 2007). However, AR may re-appear and has been shown to be still expressed in the majority of CRPC as well as in a number of androgen-independent prostate cancer cell lines. The AR in the prostate tumor cells draws a great deal of attention from clinicians and researchers, because it mediates not only cell growth and survival but also cell death in response to new hormonal treatments for CRPC cells (Attar et al., 2009a; Chen et al., 2008; Mostaghel et al., 2009; Ross et al., 2008; Vis & Schroder, 2009).

Survival and proliferation of CRPC cells have been suggested as mediated by gain-of-function changes in the AR and AR reactivation (Attar et al., 2009a; Chen et al., 2008; Mostaghel et al., 2009; Ross et al., 2008; Vis & Schroder, 2009). Importantly CRPC seems to be able to respond to second line hormonal therapy. Experimentally knocking-down of AR expression showing the induction of cell death in CRPC cells in cell culture and xenograft systems provides strong evidence that AR target therapy for CRPC is highly feasible. There are several mechanisms that have been widely suggested that AR still plays an important functioning role in promoting CRPC and can be used as therapeutic target for treating the cancer (Attar et al., 2009a; Chen et al., 2008; Mostaghel et al., 2009; Ross et al., 2008; Vis & Schroder, 2009). These include (1) AR mutations and splicing variations; (2) AR amplification and/or overexpression; (3) de novo androgen production; (4) overexpression of AR co-regulators; and (5) AR activation by non-steroidal growth factors, cytokines, or aberrant AR phosphorylation [please see Attar et al., 2009a; Chen et al., 2008; Mostaghel et al., 2009; Ross et al., 2008; Vis & Schroder, 2009; Steinkamp et al., 2009 for review]. Although we will not further discuss all of these mechanisms, some of them with the recent new findings will be selected for discussion in this article in order to further enlighten the point of views of these mechanisms as well as new derivative concepts of the mechanisms, by which more efficient treatments for CRPC may be developed.

4. AR and cell cycle in androgen dependent and CRPC cells

As mentioned, one major role of AR in prostate cancer cells is to promote cell proliferation, and the AR seems to be involved in the cell cycle process (Balk & Knudsen, 2008). It has been suggested that androgen activated AR may direct G1-S transition in androgen dependent prostate cancer cells by increasing motor mediated translational activities that result in cyclin D1 accumulation and CDK4/cyclinD1 assembly. In this process, S-phase progression is further enhanced by RB phosphorylation and de-repression of cyclin A expression. It has been demonstrated that AR may acts as a DNA licensing factor in androgen responsive prostate cancer cells (D’Antonio et al., 2009). “DNA licensing” process occurs in a critical period of G1 phase by a temporally coordinated binding of a number of licensing factors including origin recognition complex (Orc1–6), cell division cycle 6 homolog (Cdc6), chromatin licensing and DNA replication factor 1 (Cdt1), and mini chromosome maintenance proteins (Mcm2–7) at origins of DNA replication sites to form a pre-replication complex (pre-RC). Immuno-precipitation analysis showed that nuclear AR
was associated with the licensing proteins Orc2, Cdc-6, Cdt-1 and Mcm2. AR protein levels may be fluctuated along with cell cycle phases because the AR protein is degraded by proteasome in mitotic phase and increases in G1 phase in order to ensure next cell cycle re-entry and re-licensing. This study (D’Antonio et al., 2009) points out the critical role of AR in G1 through S phase for promoting cell growth of androgen dependent prostate cancer cells that is different from its functions in normal prostatic cells.

Nonetheless, a delicate study (Wang et al., 2009) reported recently the identification of a number of androgen regulated genes using a number of sophisticated assays including a global gene expression profiling and other analyses on an androgen-independent LNCaP sub-line to address the AR mediated proliferative function in CRPC cells. The authors suggested that, instead of promoting G1/S transition by androgen activated AR in androgen responsive prostate cancer (ARPC) cells, ligand-free AR may play a role to drive M-phase transition in androgen-independent prostate cancer (AIPC) cells. This study discovered that many of these AR up-regulated genes are M-phase related genes. Clinical tissue samples from AIPC and ARPC patients were also used for gene profiling analyses which strongly support the conclusion obtained from the above in vitro AIPC cell model. To further prove the differences of AR bound genes between AIPC and ARPC cells, chromatin immunoprecipitation (ChIP) plus genome-wide tiled oligonucleotide microarrays (ChIP-on-chip) was performed. The authors found that there was more AR occupancy in AIPC upregulated cell cycle and M-phase genes in AIPC cells than that in ARPC cells. The M-phase related regulatory genes including CDC20, UBE2c CDK1 and ANAPC10 were indeed AR binding genes in androgen-independent (AI) LNCaP cells when a direct ChIP assays was performed. These M-phase genes were shown to be up-regulated by AR in the absence of androgens and anti-sense knock down of these genes can affect cell proliferation activity. This study strongly suggested that AR without exogenous androgens can regulate M phase gene expression for cell proliferation in AIPC/CRPC cells. M-phase regulatory genes become AR’s new targets, whose elevation of expression seems to be assisted with alteration of epigenetic marks in AIPC/CRPC cells. This also raises the possibilities that in addition to G1/S transition, M phase regulated by AR is also important in AIPC cells and the observed changes in epigenetic marks could increase the previously inaccessible AR binding sites in ARPC cells to become accessible by AR in AIPC/CRPC cells.

Another possible explanation of cell cycle deregulation by AR in AIPC/CRPC cells could be because of alterations of the RB pathway. Although the RB tumor suppressor alterations are not the major events in primary prostate cancer, the alterations were observed frequently in prostate cancer tissues after androgen blockade treatments (Mack et al., 1989). A study also showed that RB-deficient prostate cancer cells can increase susceptibility to certain anticancer cytotoxic agents by inducing cell death. The authors suggested that RB depletion can be developed to enhance conventional cytotoxic therapeutic intervention for a subset of prostate cancer (Sharma et al., 2007). Recently, examination of 44 CRPC tissues with both RB mRNA expression levels and gene expression signature profiling of RB function loss indicated the frequent alterations of RB coincidence with CRPC development (Sharma et al., 2010). Further analyzing 156 CRPC tissue samples from the 44 patients showed 115 of 156 (73.7%) of the specimens with negative for nuclear RB immunodetection. The authors concluded that loss of the RB1 locus itself may be a major mechanism of RB inactivation in CRPC which was infrequently observed in primary disease (Sharma et al., 2010).

Experimental evidence then demonstrated that loss of RB may enhance AR expression and functions even in the absence of androgens or the presence of anti-androgens via enhanced...
E2F transcription factor 1 activity. Interestingly, RB loss/depletion can also enhance AR regulated M-phase genes like CDK1 described above, indicating the AR activities may be broadened in CRPC cells (Sharma et al., 2010; Macleod, 2010). Furthermore, the study demonstrated that RB loss/depletion enhanced expression and activities of E2F-1. The authors (Sharma et al., 2010) concluded that the RB/E2F/AR network may provide potential new avenues for developing effective therapeutic intervention for CRPC as suggested elsewhere (Knudsen & Wang, 2010).

Contrast to the above report (Sharma et al., 2010), an earlier study (Davis et al., 2006) indicated that by examining the expressions of E2F1 and AR proteins in 667 prostate tissue microarray cores E2F1 protein levels are low in benign and localized prostate cancer, moderate high in hormone native metastatic lymph nodes and significantly increased in CRPC metastatic tissues. However, the AR protein levels are strong in 83% benign prostate, 100% localized prostate cancer and 80% lymph node metastasis tissues and decreased to 40% in metastatic CRPC tissues. Additionally, forced expression of E2F1 showed inhibition of expression of AR mRNA and protein. The authors also used chromatin immunoprecipitation assays to demonstrate that the AR promoter is the target for E2F1 and the pocket protein family members p107 and p130 to bind and repress the expression of the AR gene. The reasons for the different conclusions of the two studies (i.e., Davis et al., 2006; Sharma et al., 2010) are not very clear. However, this study (Davis et al., 2006) did not examine RB status in the tissues studied.

5. AR variants or splicing isoforms in CRPC

Through previous structural and functional analyses, AR NTD that comprises nearly 60% of the coding region has been shown to exhibit constitutively active, transactivation activity (Dehm & Tindall, 2006; Dehm & Tindall, 2007). AF-1 embedded within NTD functions as a ligand-independent transcriptional activator, and there are two transactivation units TAU-1 and TAU-5 located within the AF-1. The AF-1a (a. a.101-211) and AF-1b (a. a. 253-361) domains in the TAU-1 (Dehm & Tindall, 2006) and WHTLF (a. a. 435-439) motif in the TAU-5 are the main sub-regions in mediating the ligand-independent NTD transcriptional activity (Dehm et al., 2007). AR protein becomes active without androgens in response to stimulation of several molecules such as interleukin 1 beta (IL-1β), IL-6, bone-derived factors (Blaszczyk et al 2004; Ueda et al., 2002a; Wang & Sadar, 2006) and others via crosstalking mechanism in activating AR NTD. For example, TAB2, a sensor for inflammatory signals, in response to IL-1beta treatment was shown to interact with the AR NTD at residues 179-188 and then recruit MEKK1, which in turn mediates dismissal of the N-CoR/HDAC co-repressor complex from AR and allows derepression of AR target genes. Additionally, IL-1beta induces a switch whereby anti-androgens were able to activate the AR through AR NTD (Ping et al., 2006). Steroid receptor coactivator-1 was also shown to respond to IL-6 stimulation and bind the AR NTD in order to induce ligand-independent activation of the AR (Ueda et al 2002b). Interestingly, calpain, a calcium-dependent proteinase and highly expressed in prostate cancer cells (Libertini et al., 2007), was shown to be able to cleave the native AR into an androgen independent isoform. In vitro and in vivo analyses demonstrated that calpain removes the COOH-terminal ligand binding domain from an intact AR molecule generating a constitutively active molecule which would be highly potentially attributable to androgen independent growth of prostate cancer cells mediated by the AR isoform. However the study did not show how often this truncated AR occurs in CRPC.
Recently three splicing variants of AR have been discovered in a castration resistant human CWR22Rv1 (22Rv1) prostate cancer cell line (Dehm et al 2008). One of the variants produced a full-length AR with a duplicated exon 3 (AR 1/2/3/CE3) and other two resulted in two shorter isoforms (AR 1/2/2b and AR 1/2/3/2b) devoid of ligand binding domain but with a novel exon 2b. All three isoforms are constitutively active transcription factors and able to bind an androgen responsive element as well as to promote cell growth of 22Rv1 cells in an androgen-independent manner. One of these variants may be detectable in other commonly studied cell lines. More recent study (Li et al., 2011) from the same group of the investigators indicated these aberrant variant expressions were derived from an intragenic rearrangement of an approximately 35-kb AR genomic segment containing a cluster of the above mentioned alternative AR exons. Genomic analysis of tissues from 14 CRPC patients revealed similar AR intragenic rearrangement in conjunction with AR amplification. Another study (Guo et al., 2009) reported the identification of over 20 splicing variants, many of which were derived from intronic cryptic exon splicing, in prostate cancer cells. AR3 is a major variant and encodes an about 80 kDa protein containing an intact NTD and a DBD domain but lacking a hinge region and a LBD. Some of these variants including the AR3 can be found in 22Rv1 prostate cancer cells. However, AR3 is different from the variants described in the above studies. Moreover, an AR3 specific antibody was generated, which was able to detect AR3 protein in benign prostatic stromal and basal epithelial cells but little expression in normal luminal epithelia. On the other hand, AR3 protein was expressed at higher levels in malignant prostatic epithelia and suggested to be a significant predictor for prostate cancer recurrence after prostatectomy. The study also indicated that androgen depletion forced the overexpression of AR3. Interestingly, AR3 protein was demonstrated largely in cytoplasm, however, experimental evidence showed that AR3 could act as a transcription factor via an ARE of AR regulated genes and promote cell proliferation in the absence of androgens. In addition to the variants identified in the above studies, a group of investigators (Marcia et al., 2010) recently also identified several novel constitutively active AR variants in this same CWR22R cell line, which were generated by aberrant pre-mRNA splicing or nonsense mutations. It will be interesting to determine if these new variants are frequently expressed in clinical specimens.

Another new AR variant, AR^{567es}, initially identified from a series of human prostate cancer xenografts was also reported (Sun et al., 2010). This variant is produced by alternative mRNA splicing and contains an exact full amino acid sequence from exons 1-4 and a 10 amino acid sequence from exon 8 by skipping exons 5-7. By examining 13 CRPC patients, 10 of the patients had at least one metastasis showing positive for AR^{567es}. The study demonstrated that AR^{567es} protein is constitutively active and capable of forming CRPC in a xenograft model. Interestingly, this variant can bind wild type AR and therefore stabilize and increase the activities of wild type AR with or without androgens. Moreover, in a different report (Watson et al., 2010) a number of AR variants including the previously reported ones like AR3 and new ones were examined for their functions and regulation. It was found the expression of these variants was repressed by androgens and de-repressed by androgen deprivation. Some of these variants including AR3 and AR^{567es} as described above confer CRPC growth activity but other variants such as AR-V1 may show dominant interfering activity. Unexpected finding in this study was that the CRPC growth activity of the constitutively active AR variants requires the presence of the wild type AR, because an antiandrogen (i.e., MDV 3100; also see below) or specific siRNA for only silencing wild type
AR can abolish CRPC growth activity of the AR variants tested. The authors of this study suggested that the roles of AR variants in CRPC appear to be highly complex and the requirement of wild type AR for the function of AR variants indicates the need of developing better antiandrogens for treating CRPC.

6. Other mechanisms for overexpression of AR in CRPC

As already mentioned above, there are several mechanisms that have been reported to manifest aberrant AR functions during CRPC progression. It has been considered that AR over-expression and re-expression are the major mechanisms for the progression of CRPC after hormonal therapy (Edwards et al., 2003; Holzbeierlein et al., 2004; Linja et al., 2001). Although AR gene amplification is recognized as an important way for overexpression of AR, the amplification only occurs in 20 -30% of CRPC tumors. Therefore there are other mechanisms, in addition to those being already described above, as discussed below that could be contributed to the AR overexpression in CRPC.

Immunohistostaining assays previously showed heterogeneity in the expression of AR protein increases with progression of high grade prostate cancer (Magi-Galluzzi et al., 1997; Pertschuk et al., 1995). Examining the methylation status of the 5' CpG of the AR gene promoter showed that that approximately 20-30% of CRPC tumors losing or reducing AR expression, partly, by DNA hypermethylation in the AR promoter (Jarrard et al., 1998; Kinoshita et al., 2000; Nakayama et al.,2000; Sasaki et al., 2002; Takahashi et al., 2002). DNA methylation and histone modification are two major epigenetic mechanisms that can affect gene expression in normal and malignant cells. The above studies indicated that DNA methylation could be one possibility for causing heterogeneity of AR expression CRPC. However, it is not clear whether aberrant DNA hypomethylation in the AR gene can be a mechanism for overexpression and re-expression of AR. This would be a research area of interest to pursue. Moreover, aberrant DNA methylation in other genes may affect AR function. For example, it has been demonstrated that melanoma antigen gene protein-A11 (MAGE-11) as a transcriptional regulator can enhance AR transcriptional activity by interacting through FXXLF related motifs in both NH(2)-terminal domains of AR and p300 (Askew et al., 2010; Karpf et al., 2009). This study showed that an increased DNA hypomethylation in a CpG island of the MAGE-11 5' promoter in CRPC tissues and cell lines may cause increased expression of MAGE-11 mRNA and protein which, in turn, enhances AR signal activities in CRPC.

AR mutation may not be frequent events in CRPC, however, it becomes apparent that hormone therapy such as the use of anti-androgens could be a potential selection force for AR mutations that can affect its stability, promoter preference, or ligand specificity (Attar et al., 2009; Chen et al., 2008; Mostaghel et al., 2009; Ross et al., 2008; Vis & Schroder, 2009; Steinkamp et al., 2009). Increased stability of mutated receptor may be a factor for the apparent AR overexpression.

The signal transducer and activator of transcription 5 (Stat5) has been shown to be involved in prostate cancer progression including development of CRPC (Dagvadorj et al., 2008; Tan et al., 2008; Thomas et al., 2011). Stat5 protein is capable of interacting with and increasing AR activity without the presence of androgens (Tan et al., 2008). It has been found that Stat5 expression levels correlated with high grades of prostate cancer and its overexpression
could be used to predict early prostate cancer recurrence in patients treated with radical prostatectomy (Li et al., 2004, Li et al., 2005). Moreover, Stat5 immunostaining on tissue microarray containing 143 primary prostate cancer and 20 CRPC presented significant overexpression of Stat5 in short-term and long-term androgen deprivation tissues as well as CRPC tissues compared with untreated primary prostate cancer tissues (Thomas et al., 2011). A Stat5 antisense oligonucleotide (ASO) was used for specific Stat5 knockdown and showed diminished nuclear translocation of AR and decreased AR protein stability by triggering its degradation via the proteasome pathway. The result of an in vivo study using Stat5 antisense oligonucleotide via ip injection showed that the Stat5 knockdown treatment significantly delayed CRPC tumor progression in a xenograft model. The study did not address whether Stat5 indeed increases AR expression in CRPC cells. However, it is conceivable that AR expression as well as functions is enhanced through its interaction with Stat5, thus, a potential mechanism for AR overexpression. Stat5 should be an effective therapeutic target by a number of means including a specific prolactin receptor antagonist G129R-Prl, small-molecule inhibitors for Jak2, and oligonucleotides or RNA interference, etc., as suggested to remove or reduce AR from CRPC cells (Liao et al., 2010).

It has been shown that NF-κB proteins as transcription factors play critical roles in the development and progression of several types of human cancer (Baud & Karin, 2009; Shen & Tergaonkar, 2009). NF-κB can be constitutively activated with a consequence of increasing cell proliferation and survival, reducing programmed cell death and enhancing angiogenesis and metastasis in many tumors. Because NF-κB/p65 expression has been found to be higher in prostatic intraepithelial neoplasia and cancer tissues when compared with benign tissues, its expression levels were suggested as a predictor of PSA biochemical recurrence in certain prostate cancer patients (Chi et al., 2009; Jin et al., 2008; Ko et al., 2008; Ross et al., 2004; Sweeney et al., 2004; Zhang et al., 2009). In addition, evidence showed that activation of NF-κB may be highly associated with the development of CRPC. NF-κB activation can respond to several proinflammatory cytokines such as interleukin 6 and tumor necrosis factor alpha (TNFα). Intriguingly, androgen responsive and androgen independent PCa cells may respond to TNFα treatment differently. TNFα induces apoptosis in androgen responsive LNCaP cell line by activating NF-κB with subsequent down-regulation of AR mRNA and protein. It was shown that the 5'-untranslated region of the AR gene contains a composite response element for NF-kappaB and B-myb transcription factors (Ko et al., 2008). With TNFα treatment, NF-kappaB and B-myb were enriched in this composite site with a number of co-repressors including the histone deacetylase 1, corepressor silencing mediator of retinoid and thyroid hormone receptor and the corepressor-associated scaffold protein mSin3A. On the other hand, the above complex formation did not appear at the composite site of the AR gene in two androgen independent LNCaP subline lines, C4-2 and C4-2B, which are resistant to TNFα treatment (Zhang et al., 2009). Indeed, the activated NF-κB binds its responsive element in the 5′ promoter of the AR gene and enhances AR expression in especially CRPC cells. In fact, there were eight putative NF-κB binding sites within the –3.6 kb 5′ promoter region of the AR gene detected by ChIP assays (Jin et al., 2008). This seems to suggest there may be a sub set of CRPC progressed during androgen deprivation therapy that is associated with overexpression and constitutive activation of NF-κB as well as AR overexpression. NF-κB would be an important therapeutic target for reducing AR in CRPC cells.
7. Overexpressed AR and AR variants in CRPC as therapeutic targets

The failure of hormonal therapy for prostate cancer has been largely attributed to overexpression of AR in recurrence of the cancer (Chi et al., 2009; Joseph et al., 2009; Norris et al., 2009; Singh et al., 2008; Tran et al., 2009; Zhou et al., 2008). Development of more effective hormonal therapeutic agents targeting overexpressed AR becomes an important research area. Many AR antagonists previously developed are mainly ligand competitors for androgens and can not overcome the over-expressed AR in CRPC.

Newer approaches have been used to develop agents that can target potential sites in the AR to abrogate the function of the mutated or over-expressed receptor in cancer cells (Chi et al., 2009; Joseph et al., 2009; Norris et al., 2009; Singh et al., 2008; Tran et al., 2009; Zhou et al., 2008; Shen & Balk, 2009). For example, it was reported that a conformation-based assay was used to screen a diverse small molecule library of ≈10,000 compounds in order to select chemicals that can inhibit the AR-gelsolin interaction (Joseph et al., 2009). Among the 87 compounds that scored positive in the primary mammalian 2-hybrid-based assay with no demonstrable cell toxicity, two chemicals, D36 and D80, were selected based on their ability to inhibit agonist-mediated transcription of an MMTV-luciferase reporter in LNCaP cells. These two AR binding compounds were shown to be different from currently used AR antagonists such as casodex and hydroxyflutamide, because they do not compete with androgens for binding and do not affect AR nuclear localization. Experimental evidence showed they affect recruitments of co-activators and RNA pol II enzyme to AR and AR DNA binding ability, and subsequently loss of its ligand-induced transcription activity as well as inhibition of cell proliferation in AR overexpressing LNCaP cells.

In a study shAR lentiviral vector directed AR knockout CWR22R cells which ectopically re-express a truncated AR lacking of LBD (i.e., ARΔLBD) were used to screen the DiverSet Chemical Library consisting of 34,000 small molecules (Gioeli, 2010; Narizhneva et al., 2009). The ARΔLBD is a constitutively active AR variant capable of supporting androgen independent proliferation of CWR22R cells. The genome of the cells also contained integrated ARE linked luciferase reporter. In this cell based assay, compounds showing inhibited luciferase activity at least two-fold after 24 hr incubation were selected for further studies. Focusing on AR mediated cell proliferation, there were two types of compounds selected, one type caused cell death, and other reduced cell growth in cell cultures. Although the actual mechanisms by which these compounds act on AR, obviously not via AR LBD, are not known, both types of compounds can diminish in vivo prostate tumor growth in a xenograft model. There is no doubt that clinically relevant studies of these compounds will decide their potential clinical utility.

Also, there are two new classes of small molecules isolated from sponge (Dysidea sp.) extract that exhibit anti-AR activity (Andersen et al., 2010; Quayle et al., 2007; Sadar, 2011; Sadar et al., 2008). One type of these sponge compounds are small chlorinated peptide called sintokamides, and the other is EPI-001 (i.e., bisphenol A diglycidic ether.HCl.H2O). The study showed that isolated sintokamides were able to inhibit androgen dependent AR activation in cells trasfected with a native androgen response element (ARE) linked luciferase reporter plasmid. Further studies were performed in LNCaP cells transected with plasmids for the AR NTD-Gal4DBD chimera protein and the Gal4-luciferase reporter. This system allows transactivation of AR NTD by forskolin without the presence of androgens showed that sintokamides indeed interfere with transactivation of AR NTD. Although both sintokamides and EPI-001 target AR NTD activities, the latter has been studied more
extensively using cell based assays to measure anti AR/AR NTD activities. The studies provided evidence that EPI-001 may directly bind the AR NTD not the AR LBD in test tubes. Moreover, in cells EPI-001 seems to be able to interact with the NTD of AR and inhibit both the interaction of CREB binding protein (CBP) with AR AF1 and the interaction of the AR NTD and AR C-terminal domain. EPI-001 does not inhibit AR nucleus translocation and DNA binding and does not reduce AR protein levels in cells or xenograft tumors. This compound was shown to inhibit in vitro and/or in vivo prostate cancer cells growth mediated by either wild type AR or constitutively active AR variants mentioned above but not inhibit in vitro and/or in vivo proliferation of AR null cells like PC-3.

The problem with currently clinically used AR antagonists such as bicalutamide (also known as casodex) is their low affinity to AR that can not effectively suppress AR in CRPC, especially at overexpressed states. This also seems to explain, in part, why the antagonists can become a weak agonist for CRPC that overexpresses AR. Another new approach was used to improve antagonist affinity to AR in order to effectively repress AR function at high levels (Tran et al., 2009). The investigators used a relatively high affinity and specific nonsteroidal AR agonist RU59063 as a starting compound for developing potential high affinity antagonists (Tran et al., 2009). By screening about 200 thiohydantoin derivatives of RU59063 for AR antagonism in human prostate cancer cells expressing elevated AR, two diarylthiohydantoins RD162 and MDV3100 were chosen for further biological and clinical studies. It was shown that in addition to their higher affinities than bicalutamide, these two compounds can reduce AR nuclear translocation, DNA binding to androgen response elements, and recruitment of coactivators. Oral treatment of RD162 and MDV3100 demonstrated tumor regression in mouse xenografts of human CRPC. Furthermore, MDV3100 was used in a Phase I/II clinical trial in 30 CRPC patients. Thirteen of 30 (43%) showed sustained declines (by >50%) in serum biomarker prostate-specific antigen concentrations. The second phase1-2 study (Scher et al., 2010) was conducted with 140 CRPC patients in 5 US centers. The goals were to determine the safety and tolerability profile of MDV3100 and to establish the maximum tolerated dose. The study concluded that the drug had a well tolerable toxicity and was able to reduce prostate-specific antigen levels and circulating tumor cell counts as well as to stabilize prostate cancer metastases. It seems that this compound is underway for further clinical trials for advanced prostate cancer (Schmidt, 2011).

Other new AR LBD antagonists have also been described. For instance, similar to the above studies, a new series of thiohydantoin derivatives were generated for screening AR antagonistic activities and led to the identification of a metabolic stable compound, CH5137291 (Kawata et al., 2011; Yoshino et al., 2010). This compound also showed to repress AR nuclear translocation and AR mediated tumor growth in xenografts. It would warrant further clinical studies on human patients. BMS-641988 was, an another example, identified as a promising AR antagonist, with more than ten times AR binding affinity compared to bicalutamide, in the initial preclinical studies (Attar et al., 2009b) However, phase 1 clinical trial with 61 CRPC patients showed a limited anti-cancer activity with occurrence of seizure activity in patients, eventually leading to closure of the study (Rathkopf et al., 2011).

8. Androgen synthesizing enzymes in CRPC as therapeutic targets

Hormonal therapies can effectively reduce circulating androgens by surgical or chemical castration plus adrenal steroidogenic enzyme inhibitors (e.g., ketoconazole, liarozole, and
aminoglutethamide), however, AR can still be functionally active inside CRPC tissues. In fact one of the main reasons is that androgens can be *de novo* synthesized in tumor cells of CRPC patient tissues and cell lines (Attard et al., 2009a; Cai et al., 2009; Dillard et al., 2008; Locke et al., 2009a; Locke et al., 2009b; Locke et al., 2008; Locke et al., 2010; Mohler, 2008; Mohler et al., 2011; Montgomery et al., 2008; Stanbrough et al., 2006). This relatively wide spread phenomenon is due to up-regulation of expression of certain steroidogenic/androgen synthesizing enzymes in prostate cancer cells, including FASN, CYP17A1, HSD3B1, HSD17B3, HSD17B6, CYP19A1, and UBT2B17. It is highly critical that the up-regulated steroidogenic enzymes can produce sufficient intracellular androgens to act as intracrine or paracrine ligands for AR in order to facilitate progression of CRPC.

Because of the inefficiency of suppression of adrenal androgen production by using ketoconazole and aminoglutethamide, circulating adrenal androgens, dehydroepiandrosterone (DHEA) and 5'-androstenediol (ADE) could be a source for making high affinity androgens testosterone (T) and DHT in prostate cancer tissues by HSD3B, HSD17B6, AKR1C and SRD5A enzymes. Intratumor androgens can also be generated by the de novo or intracrine synthesis from cholesterol or other steroid precursor biosynthetic pathways in especially CRPC cells. It was found that in a xenograft CRPC progression model, the expression of proteins for cholesterol regulation including LDL-r, SR-B1, HMG-CoA reductase, ACAT1,2, and ABCA1) were deregulated, and influx and synthesis of cholesterol were increased as sources for intratumor DHT synthesis (Leon et al; 2010). A net increase of intra-tumor androgens can be achieved by either increasing reductive enzymes such as HSD17B3 and HSD17B5 or decreasing those enzymes that catalyze the reverse oxidative reaction (e.g., HSD17B2) or both. Also, pregnenolone and progesterone may be used as the initial substrates catalyzed by CYP17A in the so called "backdoor" pathway to produce DHEA and ADE, and DHT can be formed from the above products by HSD17B and SRD5A enzymes. When examining 19 local CRPC tissues, a recent study concluded that intratumoral steroid biosynthesis mentioned above may be less important than the production of intraprostatic T and DHT from adrenal androstenedione by overexpressed AKR1C3 and SRD5A1 in prostate cancer tissues for CRPC progression (Hofland et al., 2010).

How the steroidogenic related-enzymes become deregulated remains unclear presently. It is possible that as discussed above, CRPC cells could adapt to have different epigenetic responses from that of androgen dependent prostate cancer cells. For example one could speculate that because chromatin structures of the steroidogenic related-enzyme genes become more accessible for their expression in CRPC cell, however, there is no experimental evidence to support it yet. Nonetheless, a recent study showed that a proinflammatory cytokine, IL-6, increased expression of several steroidogenic enzymes including H SD3B2 and AKR1C3 at mRNA and protein levels in LNCaP cells or xenografts (Chun et al., 2009). The up-regulation appeared to be mediated through IL-6 receptor signaling pathway, as shown by abolishing the up-regulation by knocking down IL-6 receptor with a specific small interfering RNA. The study also convincingly showed that the upregulated steroidogenic enzymes by IL-6 treatment indeed increased T production in LNCaP cells.

Although an adrenal steroidogenic enzyme CYP17 inhibitor like ketoconazole has been widely used to repress adrenal and testicular androgen production as a second line hormone therapy for CRPC patients, its efficacies are not high and side-effects including hepatotoxicity, gastrointestinal toxicity, and adrenal insufficiency could be severe (Mostagel et al., 2009; Shah & Ryan, 2010). Corticosteroid replacement has to be used with
New Approaches Targeting Androgen Receptor Signal Pathways for Treatment of Castration-Resistant Prostate Cancer

ketoconazole treatment. A recently developed, highly selective irreversible CYP17 inhibitor, abiraterone, presents higher potency and selectivity, and lower, manageable toxicity compared to ketoconazole (Attard et al., 2008; Shah & Ryan, 2010; Yap et al., 2008). Note, even combined with prednisone, abiraterone still showed certain degrees of adverse effects (e.g., fatigue, hypertension, headache, nausea, and diarrhea). A phase I clinical trial of 21 CRPC patients with continuous administration of abiraterone showed safety of its use and its antitumor activity in up to 70% of the patients (Attard et al., 2008). A phase II study by the same group of investigators seemed to be able to confirm the previous result (Attard et al., 2009b; Reid et al., 2010). Another phase II clinical trial study (Danila et al., 2010) was performed to determine the safety and efficacy of abiraterone when used with prednisone in order to reduce the secondary symptoms such as hyperaldosteronism. The study had 58 CRPC patients who had previous hormonal therapy including antiandrogens (91%), ketoconazole (47%) and estrogens (16%) and failed on previous docetaxel treatment. The inclusion of prednisone in the abiraterone treatment seems to reduce significantly incidence of hypokalemia, hypertension, and fluid retention and the efficacy of abiraterone remain similar to previously observed. A phase III clinical trial on post-docetaxel, castration-resistant prostate cancer patients presented a significantly increased overall survival by about 3.9 months compared with the placebo group (i.e., 14.8 months vs. 10.9 months). The clinical use of this drug seems to be much closer to an approval by U.S. FDA.

There are also other new CYP17 inhibitors such as TAX700 and VN124-1 (Vasaitis et al., 2008; Vasaitis et al., 2010), currently undergoing early clinical trial evaluation. VN/124-1 (3 β-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene), also known as TOK-001, shows an IC50 value of 300 nM in an assay system using intact CYP17 expressing Escherichia coli in comparison with abiraterone using the same assay system had an IC50 value of 800 nM (Handratta et al., 2005; Vasaitis et al., 2008). However, the inhibitory effect of VN/124-1 is not strictly specific for CYP17 because it was found this compound has multiple targets in androgen signaling pathways (Chan et al., 1996; Schayowitz et al., 2008; Vasaitis et al., 2008). For example, VN/124-1 can act as a ligand antagonist for wild type and a mutant AR (in LNCaP cell) for the binding of a synthetic androgen R1881 as well as in transactivation assay with an ARE linked luciferase reporter and has similar anti-AR potency compared to bicalutamide in LNCaP cells (Chan et al., 1996; Danila et al., 2010). VN/124-1 also reduces AR protein levels. Interestingly, VN/124-1 can inhibit proliferation of CRPC cell lines exhibiting overexpression levels of AR and no longer responding to antiandrogen bicalutamide. In preclinical tests, VN/124-1 at a dose of 0.13 mmol/Kg twice daily showed 93.8% reduction of LAPC-4 tumor volume which seemed to be more effective than castration (Chan et al., 1996, Handratta et al., 2005). The results of all these studies together seem to indicate that VN/124-1 acts as an AR antagonist as well as a CYP inhibitor. It would be very useful if the authors demonstrated that serum testosterone levels can be reduced by this compound.

TAK-700 [(1S)-1-(6, 7-dimethoxy-2-naphthyl)-1-(1H-imidazol-4-yl)-2-methylpropan-1-ol] is an non-steroidalimidazole CYP17 inhibitor with IC50 = 28 nM. This is a selective inhibitor for CYP17 over 11 β-hydroxylase (Vasaitis et al., 2010). In animal studies, TAK-700 showed inhibitory effects on testosterone production and reduction of prostate gland and seminal vesicles in rats. In addition, results of testing on monkeys showed a single TAK-700 oral administration at 1 mg/kg body weight was sufficient to decrease serum testosterone
levels to castration levels after 8 hours of the treatment (Matsunaga, et al., 2004). Clearly, clinical trials in humans will determine if this CYP inhibitor has values to treat CRPC.

9. Conclusions

As discussed above, surgical or medical androgen deprivation appears to be a major force to cause AR overexpression and gain of function with the consequence of developing CRPC. Intriguingly, recent studies found that androgen ablation can induce leukocytes infiltration into treated tumour areas. Particularly, infiltrated B cells may produce lymphotoxin which in turn causes IKK-β activation results in production of cytokines that activate IKK-α and STAT3 in prostate cancer cells (Ammirante et al., 2010). The activation of STAT3 or IKK-α could activate AR and enhance survival of hormone-deprivation prostate cancer (Ammirante et al., 2010; Jin et al., 2008). Mainly due to overexpression and reactivation of the AR, the resistance to the hormonal deprivation treatments may be overcome by developing new, high affinity AR antagonists. Since recent findings indicated that CRPC tumor can gain the ability by up-regulating genes in androgen synthesis pathways to produce sufficient androgens to activate AR, searching and developing more safe and effective inhibitors, in addition to abiraterone, to the key enzymes in the pathways would also be important approaches for CRPC therapy.

<table>
<thead>
<tr>
<th>Target molecules</th>
<th>Potential drug development</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AR including wild type, mutants and variants</strong></td>
<td>AR ligand antagonists (Norris et al., 2009; Tran et al., 2009; Shen et al., 2009)</td>
</tr>
<tr>
<td><strong>AR co-activators</strong></td>
<td>Anti-sense DNA or RNA or protein interaction disrupting agents (Joseph et al., 2009; Sadar, 2011; Charlier et al., 2000)</td>
</tr>
<tr>
<td><strong>Androgen synthesizing enzymes</strong></td>
<td>Enzyme inhibitors (Attard et al., 2009b; Reid et al., 2010; Vasaitis et al., 2010)</td>
</tr>
<tr>
<td><strong>Androgen independent AR activating molecules (e.g., EGF, interleukin 6, etc.)</strong></td>
<td>Signaling pathway inhibitors (Lee et al., 2002; Wallner et al., 2006; Yu)</td>
</tr>
</tbody>
</table>

Table 1. Drug development in Targeting AR and AR related molecules in CRPC

Although it has been shown that the expression of the constitutively active AR variants may be increased by androgen deprivation and overexpressed in CPRC tissues, whether these AR isoforms can become prevalent in CRPC without the presence of wild type AR is currently unknown and should be an interesting topic for further investigation. However, if this will be the case, more proactive strategy for generating new types of non-ligand AR antagonists will be needed, because the oncogenic activities of this type of AR variants will no longer be inhibited by conventional antagonists. Also, AR in microenvironmental stromal areas that surround CRPC cells would be a useful target site. Clearly, nucleotides like
antisense molecules being suggested as drugs for targeting AR will have to overcome numerous obstacles in order to become effective and safe for CRPC treatment. Of course, further understanding AR activities in CRPC will help develop better ways to treat the advanced prostate cancer including CRPC. Finally, many laboratories have attempted to develop drugs targeting AR and/or AR signaling related molecules as listed in Table 1 for CRPC treatment, many of these potential drugs face site specificity, toxicity, efficacy, and delivery challenges/problems.

10. Acknowledgements

The authors are partly supported by an NIH grant 91956 (CYY), an ACS grant RSG-09-175-01-CCE (DKV), and DOD grant, W81XWH-09-1-0216 (DKV).

11. References


Attard, G.; Reid, A. H.; Olmos, D. & de Bono, J. S. (2009a) Antitumor activity with CYP17 blockade indicates that castration-resistant prostate cancer frequently remains hormone driven. Cancer Res. 69, 4937-4940.

inhibition of CYP17 with abiraterone acetate is highly active in the treatment of castration-resistant prostate cancer. J Clin Oncol. 27, 3742-3748.


transduction inhibitors in prostate cancer progression to hormone independence in vitro. Mol Cancer Ther. 7(1), 121-132.


Hofland, J.; van Weerden, W. M.; Dits, N. F.; Steenbergen, J.; van Leenders, G. J.; Jenster, G.;


New Approaches Targeting Androgen Receptor Signal Pathways for Treatment of Castration-Resistant Prostate Cancer


surfaces on the androgen receptor defines the pharmacological actions of bound ligands. Chem Biol. 16, 452-460.


Currently there have been many armamentaria to be used in cancer treatment. This indeed indicates that the final treatment has not yet been found. It seems this will take a long period of time to achieve. Thus, cancer treatment in general still seems to need new and more effective approaches. The book “Current Cancer Treatment - Novel Beyond Conventional Approaches”, consisting of 33 chapters, will help get us physicians as well as patients enlightened with new research and developments in this area. This book is a valuable contribution to this area mentioning various modalities in cancer treatment such as some rare classic treatment approaches: treatment of metastatic liver disease of colorectal origin, radiation treatment of skull and spine chordoma, changing the face of adjuvant therapy for early breast cancer; new therapeutic approaches of old techniques: laser-driven radiation therapy, laser photo-chemotherapy, new approaches targeting androgen receptor and many more emerging techniques.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:
