Vol 3, No 2, Jun, 2017, P 50-66
DOI, 10.15562/tcp.46

CANCER PRESS
Vol. 3, No. 2, jun, 2017

Review Article

Androgen Receptor (AR) Based Diagnosis of Prostate Cancer Metastasis: A Biological Perspective

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ABSTRACT

Androgen receptor (AR) is a nuclear transcription factor and a member of the steroid hormone receptor superfamily of genes, which is abundantly expressed in neuroendocrine and musculoskeletal tissues and the male genitourinary system. Neoplastic cells have to perpetuate the androgen receptor (AR)-mediated dynamics by a wide range of integrated pathways that crosstalk at various levels and it ensures the robust expression and activation of AR-mediated genes. There are multidirectional pathways opted for by the AR to meet the demands of a desperate cancer-prone environment. Interference with these histone demethylases with siRNA or pharmacological inhibitors leads to distinct changes in histone marks at AR target promoters. Several inhibitors such as CaMKII inhibitor have been found to have a broader effect on apoptosis than just their inhibition which also resulted in the inhibition of AR activity and induces p53-independent apoptosis, inhibits anti-apoptotic protein Mcl-1, upregulates pro-apoptotic protein PUMA and generates ROS.

Keywords: Androgen Receptor, Prostate Cancer, Metastasis

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Submitted: 23-04-2017
Accepted: 23-05-2017
Published : 29-06-2017

Historical Background

Prostate cancer encompasses a heterogeneous assembly of tumors, with distinctive risk factors, clinical presentation, histopathological features and molecular uniqueness. Currently, cure of metastatic or recurrent disease is dependent on conventional chemotherapy combination regimens. It is a matter of deep concern that efficient clinical management of prostate cancer has been relentlessly challenged by considerable intratumoral multiplicity at genomic and pathological levels and a limited understanding of the genetic elements governing disease development. Prostate cancer (PCa) cells remarkably express androgen receptor (AR) and need androgens to survive. Androgen suppression is doubtlessly, front-line therapy for metastatic disease. Almost all PCa patients at the start show responsiveness to hormonal therapy but gradually show refractoriness to castration. There is an accumulating confirmation that these tumors are relying on AR signaling. Numerous mechanisms that augment AR signaling in an androgen-depleted environment have been explicated. Normal development of prostate gland is directly related to normal functioning of androgen receptor (AR) as its critically important in prostate carcinogenesis and androgen-dependent (AD) or androgen independent (AI) progression of the disease. Functional AR is expressed during various stages of prostate carcinogenesis from the very early stage of prostate intraepithelial neoplasia to organ-confined or locally invasive primary tumors, in metastatic tumor and before or after androgen-deprivation therapy (ADT) (1-3). When activated by the endogenous androgenic ligands, testosterone (T)

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and dihydrotestosterone (DHT), AR becomes phosphorylated and the ligand-receptor complex translocates into the nucleus, and in association with coregulatory factors, binds to specific genomic DNA (gDNA) sequences in the regulatory regions of AR target genes.

Prostate carcinogenesis and the molecular events involved in it are essentially to be known before developing any therapeutic strategies to prevent AR-dependent signaling and progression of PCa. It also has been observed that AR target genes such as PSA expression remains high in spite of AR blockage in hormone-refractory PCa (HRPCa) patients (4). Lack of precise information regarding the molecular mechanisms of AI-PCa development is the challenging task while developing a therapeutic strategy, but while studying the literature we came to know that physical presence and activity of AR are critical factors. In a relatively large number of AI-PCa patients, AR is expressed, over expressed, mutated or amplified (5, 6). It also has been observed that cytokines, polypeptide growth factors, neuropeptides, non-androgenic steroid hormones, antiandrogens and other trophic agents activate the AR-dependent and AI-signaling pathways (7). These non-androgenic factors can regulate AR expression and/or activity through establishment of downstream cytoplasmic signaling crosstalk or cross-modulation by other transcription factors (8). The net effect of these events could potentially contribute to AI progression of PCa. It also has been believed that genomic mutations and amplifications could be the major cause of aberrant AR activation in AI-PCa. Collectively, advanced PCa will acquire the phenotype of oncogenic addiction to AR and continue to grow and resist available therapeutic regimens. More than 60 years ago, Huggins and Hodges (9) showed the effectiveness of surgical castration in men with prostate cancer (PCa). Since that time, hormonal therapy remains as the most effective and widely used palliative method for advanced and/or metastatic PCa. This method leads to a biochemical response in the majority of patients for up to 3 years, but eventually and almost exclusively during therapy, an incurable highly aggressive AI- or hormone-refractory disease will

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will emerge (10). Current comprehensive introduction and critical review of literature will give a snapshot of AR involvement in prostatic carcinogenesis.

AR Structure and Signaling

AR Structure

The AR is a nuclear transcription factor and a member of the steroid hormone receptor superfamily of genes, which includes but is not limited to the receptors for estrogen, progesterone, glucocorticoids, mineralocorticoids, vitamin D, retinoic acid and retinoid X. With the exception of the spleen, the AR is abundantly expressed in neuroendocrine and musculoskeletal tissues and the male genitourinary system (11). The AR gene is located on the X-chromosome at position Xq11-12 and spans ~90 kb containing eight exons that code for a ~2757 bp open reading frame and ~919 amino acids within a 10.6 kb mRNA. AR expression is expressed in two isoforms: the predominant isoform B with 110 kDa mass and the less dominant isoform A with ~80 kDa. In addition to these two isoforms, a recent report described additional novel AR splice variants designated as AR3, AR4 and AR5 in androgen-insensitive PCa cell lines (see reference (12) for detailed description). A highly conserved AR genomic has been observed throughout evolutionary lineage of mammals.

Similar to many other steroid receptors, the AR consists of distinct functional motifs organized as the amino-terminal domain (NTD; 555 amino acids coded by exon 1), DNA binding domain (DBD; 68-amino acid coded by exon 2 and 3), ligand-binding domain (LBD; 295 amino acids coded by exons 4–8), nuclear localization (amino acid 628–657) and AF-1, AF-5 and AF-2 transactivation units encoded by exon 1 and 8. A hinge region separates LBD from DBD. On the contrary to the very high evolutionary conservation for LBD, DBD and the N-terminal of the hinge fragment, the most variable region is the NTD sequence. This domain is encoded by several regions of highly repetitive DNA sequences, such as CAG and GGC repeats. Similar to other nuclear receptors, the DBD region of AR contains nine cysteines, of which eight are linked to two zinc ions, and through the sulfohydryl groups they are organized in two zinc finger domains. The AR–DNA recognition specificity is determined by the first zinc finger and stabilization of the DNA–receptor complex, and receptor dimerization is determined by the second zinc finger.

AR coregulators

After the discovery of first steroid receptor coactivator (SRC-1), over 170 potential AR coregulators have been identified (13). This growing list of coregulators has been functionally classified as coactivators or corepressors, or, on the basis of their best recognized primary function, as components of the chromatin remodeling complex, as histone modifiers such as acetyl-transferases and deacetylases, methyltransferases or demethylases, as components of the ubiquitination and proteasomal pathways, as components of the sumoylation pathway, as proteins involved in endocytosis, DNA repair system, splicing and RNA metabolism, or as chaperones and cochaperones, cytoskeletal proteins,

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signal integrators, transducers, scaffolds and adaptors, or cell cycle or apoptosis regulators (14). On activation by ligand and nuclear translocation, AR binds to the androgen-response elements (AREs) that may also involve or recruit coregulators to assemble a functional transcriptional complex regulating AR target gene transcription.

In addition, AR coregulators may at least partially contribute to differences in AR ligand specificity or its transcriptional activity (15). It has been shown that the expression of SRC-1, TIF-2 and SRC-3, the three members of the SRC or p160 family of coactivators, is increased in PCa (16). SRC-1 expression is increased in 50% of AD-PCa samples, as compared with the benign or normal prostate tissues. However, SRC-1 and TIF-2 expression are increased in 63% of HRPCa (17). In another study, an increased expression of SRC-3 was associated with increased PCa grade and stage and decreased disease-free survival. The AR coactivator ARA70 is also overexpressed in PCa samples (18) and after castration in the hormone-refractory CWR22 xenografts. The Cdc25B (cdk-activating phosphatase) was also identified as an AR coactivator, and found not only to be overexpressed in PCa but also with the highest expression in advanced-stage tumors with high Gleason score (19). The AR coactivator, Tat interactive protein, 60 kDa (Tip60), was found to be overexpressed on androgen deprivation in the LNCaP cells and CWR22 tumor xenograft (20). Overall, these studies support that PCa is associated with overexpression of multiple AR coactivators and may contribute to PCa progression. Taking into consideration of the simultaneous involvement of multiple coregulators and their overlapping interaction, additional translational studies are required to determine the contribution of AR coregulators in prostate carcinogenesis and PCa progression in experimental settings.

Coactivators

To date, several hundred putative AR coactivators have been identified that enhance ligand dependent AR activity in model systems. These coactivators serve pleiotropic functions at the chromatin level, including recruitment of basal transcriptional machinery, modulation of chromatin remodeling

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changes. A subset of AR coactivators appears to be enhanced in human disease, including SRC1, SRC2, SRC3, or ARA70 (21-23). The importance of deregulated coactivator expression may be significant, as excessive coactivator expression may not only sensitize cells to a low hormone environment but also convert nuclear receptor antagonists into partial or full agonists. As AR is known to regulate a distinct transcriptional program in hormone sensitive versus castrate-resistant models of disease (24), an attractive hypothesis is that altered cofactor expression and/or regulation assists in eliciting the CRPC-specific transcriptional program.

Corepressors

Loss of AR corepressor function can convert therapeutic antagonists into agonists or promote agonist sensitization (reviewed in (25)). Such events can occur through downregulation of the corepressor itself (such as occurs with prohibitin) (26), through dismissal of the corepressors from the AR complex (as seen with NCoR in the presence of macrophage induced TAB2 signaling), and/or through aberrant corepressor mislocalization (such as observed with Hey1) (27). In addition to AR modulation, corepressors perturbed in prostate cancer may crosstalk with pathways directly associated with prostate cancer growth. For example, reduction of the AR corepressor Ebp1 is not only associated with resistance to hormone therapy, but also alters the proliferative response to heregulin (28). Similarly, crosstalk between the AR and cell cycle machinery is mediated by cyclin D1, which acts through cyclin-dependent kinase-independent functions to suppress AR activity; this function of cyclin D1 is abrogated in human disease through downregulation, mislocalization, or alternative splicing events (29, 30).

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enzymes function or recruitment (e.g. histone acetylase), and/or altered AR conformationalAs a result of such growth factor and cell cycle crosstalk functions embedded within selected AR cofactors, alterations therein may impinge both on AR signaling and connected pathways to yield a powerful pro-tumorigenic signal. Challenges remain with regard to discerning which of the several hundred co-repressors identified to date play critical roles in recurrent AR activity, and prioritizing those which could be developed as viable therapeutic targets.

AR cofactors

AR cofactors are cis-acting transcriptional modulatory proteins that substantially influence AR function. Given the prevailing posit that agonists induce recruitment of coactivators and antagonists promote conformational changes that promote recruitment of corepressors, two hypotheses emerge. First, it would be predicted that deregulation of coactivators or loss of corepressors promote unchecked AR activity and disease progression. Second, it is predicted that changes in overall AR levels alter the stoichiometry of assembled complexes. Both predictions appear to be correct and have disease relevance.

AR Signaling

Post translational modifications of AR

Steroid receptors can be modified by a variety of posttranslational modifications such as phosphorylation, acetylation, ubiquitinylation and sumoylation. These changes have the potential to affect the receptor stability, subcellular localization, interaction with other proteins within the transcription machinery complex or activity in a cell type- or genespecific manner. Interestingly, the net effect of AR activity could be affected by crosstalk among different types of posttranslational modifications such as acetylaton and phosphorylation (31). It is noteworthy that the consequences of posttranslational modifications of AR in prostate carcinogenesis and progression remain to be understood.

AR phosphorylation and dephosphorylation

AR is a nuclear phosphoprotein/transcription factor and in order to exert its transcriptional role after synthesis, it should translocate to the nucleus and remain at a hyperphosphorylated level (11). Constitutive AR phosphorylation at serine 94 and ligand-induced phosphorylation are reported for serines 16, 81, 256, 309, 424 and 650 (32). In addition, mitogen-activated protein kinases (MAPK) and phosphatidyl inositol-3 kinase/Akt (PI3K/ Akt), as the two very important core-signal transduction pathways, are also able to induce AR phosphorylation. On the contrary, AR dephosphorylation will be associated with loss of AR transcriptional activity and inability for nuclear translocation. Protein phosphatase 2A can dephosphorylate AR at NTD, which leads to loss of AR activity (33).

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AR acetylation

Protein acetylation has a central regulatory role in transcriptional activity of genes. Activity of transcription factors can be also regulated by acetylation. For AR, the KXKK motif of the hinge region is the site of acetylation. Lysine to alanine mutation of the KXKK motif significantly reduces AR activity by dysregulation of stimulation of coactivators in favor of the N-CoR corepressors (34). In addition, mutations that mimic acetylation increase AR-target gene expression and PCa cell proliferation. A clear example of the crosstalk between acetylation and phosphorylation is provided by the facts that AR acetylation mutants present with decreased phosphorylation and the finding that AR (S94A) phosphorylation mutant is less responsive to p300 stimulation. Another report showed that histone deacetylase inhibitors increase AR activity level without affecting its sub-cellular localization (35).

AR ubiquitylation

AR turnover is not fully understood. Timely degradation of transcription factors is necessary as a control step to sustain transcriptional activity or eliminate it by rapidly degrading the protein. Similar to many other proteins, steroid receptors are also subjected to ubiquitylation. It has been shown that the E3 ubiquitin ligase Mdm2, which promotes polyubiquitylation of AR and its proteasomal degradation, recognizes Akt-dependent phosphorylated serine. In addition, it has been shown that by recruiting the histone deacetylase, Mdm2/AR complex decreases AR-dependent transcriptional activity (36). On the contrary, it was shown that inhibition of ubiquitylation process and proteasomal degradation of AR by a protease, USP10, functions as an AR coactivator (37).

AR sumoylation

This type of posttranslational modification usually affects a small portion of a given protein and leads to covalent binding of a small ubiquitin-like modifier chain on lysine residues embedded in the consensus ΨKxE motif. Sumoylation can affect at different levels such as subcellular localization and DNA binding. There is also a possibility for crosstalk between sumoylation and MAP kinase phosphorylation of AR (38). Sumoylation of AR is hormone dependent and its effect is mainly repressive, but still context dependent. Unlike ubiquitination, sumoylation does not promote protein degradation. In some instances, sumoylation competes with ubquitylation on the lysine residues and functions as an ubiquitin (39).

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ARs are sumoylated at lysine 386 and 520 in vivo and mutation of these residues increases AR transactivation, that suggests a role for sumoylation in suppressing AR activity (40).

Androgen Receptor Infrastructure: Marching to a different drummer

The biological multiplicity of prostate cancer is the major stumbling block in the standardization of therapy. Currently a paradigm shift has occurred in the clinical management of prostate cancer. Instead of long term androgen ablation/deprivation therapy (ADT), scientists have strengthened the concept of intermittent ADT (Fig. 2). Progress in molecular profiling suggests that divergence in the genetic profile of tumors considerably participate to the intricacy of the disease. Alternative pre-mRNA splicing is principal genetic process involved in biological diversity. During alternative splicing, coding and noncoding regions of a single gene undergo rearrangement to generate several messenger RNA transcripts yielding discrete protein isoforms with multifarious biological functions. Dysregulation of the splicing machinery influence splicing of cancer-relevant genes. It is interesting to note that androgen receptor undertakes aberrant and alternative splicing and gives rise to proteins that influence cell phenotypes and survival of patients. Splicing mechanisms must be manipulated at clinical level. In view of the fact that splicing is concerned with information transfer from the genome to the proteome, it incorporates another vital dimension to '-omics'-based molecular signatures utilized to individualize clinical management of patients.

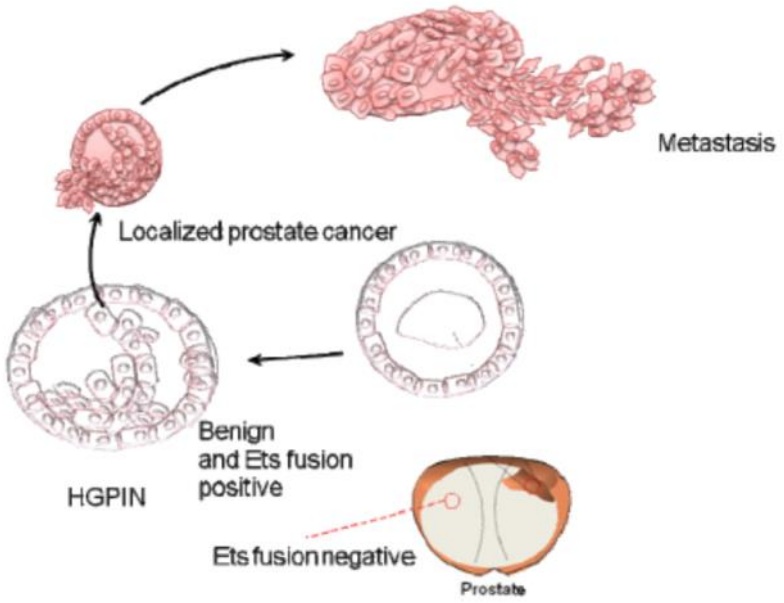

Figure 1: An overview of the transition from localized to metastatic stage. Short term (Androgen Deprivation Therapy) ADT seems to be more effective than long term ADT with hazardous outcomes. An intermittent ADT is more reasonable in terms of clinical management.

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Variant are able to register full-length version of AR with duplicated exon 3 and two truncated versions lacking the COOH terminal domain (CTD). AR isoforms also exist in CTD-truncated version and are encoded by mRNAs that have a novel exon 2b at their 3' end. In a ligand-independent manner, AR isoforms promote the expression of endogenous AR-dependent genes, as well as the proliferation of 22Rv1 cells. However discordant finding was given by Marcias et al. (41), who documented a variant. 22Rv1 cells also express a mutant AR lacking exon 3 tandem duplication, doubtlessly, a major feature of this cell line.

Shi et al. (42), registered ligand-binding domain deficient AR splice variants in hormone-insensitive PCA cells. AR3, one of the major splice variants expressed in human prostate tissues, is constitutively active and transcriptome was not triggered by androgens. At present available anti androgen drugs are unable to drug novel AR splice variants are not inhibited by. A rational drug design targeting these AR isoforms may potentially be successful for treatment of ablation-resistant PCA. Another novel human AR splice variant deficient in exons 5, 6, and 7 (ARv567es). This accumulating data marks these variants as candidates for therapies directly targeting the AR rather than ligand. CRPC cells express variant ARs which included truncated ARs (tARs), siRNA-mediated knockdown efficiently suppressed the androgen-independent cell growth. Nigericin-like compounds suppress AR expression at the mRNA level. This could be an approach for the clinical management of prostate cancer (43). It is a matter of deep concern for the cell to prolong the signaling of the cancer driving genes via AR. It ensures the robust expression and activation of androgen receptor mediated genes somehow or other. There are multidirectional pathways opted by the androgen receptor to meet the demands of desperate cancer prone environment. In the hypersensitive pathway, there is a robust quantitative expression of androgen receptor (AR) usually by gene amplification or AR has enhanced sensitivity to pay compensation for low levels of androgen or more testosterone is converted to the more potent androgen, dihydrotestosterone (DHT) by reductase. In the promiscuous pathway, criticalities are replaced by generalized patterns. The “specificity landscape” of the AR is broadened to encompass more non-specific ligands so that it can be activated by non-androgenic molecules normally present in the circulation. In the outlaw pathway, receptor tyrosine kinases (RTKs) are switched on and the AR is phosphorylated by either the AKT (protein kinase B) or the mitogen-activated protein kinase (MAPK) pathway, producing a ligand-independent AR. In the bypass pathway, parallel survival pathways, such as that involving the anti-apoptotic protein BCL2 (B-cell lymphoma 2), prevent the need for AR or its ligand. Compromising AR expression by siRNA induced PI3K-independent activation of Akt, which was triggered by calcium/calmodulin-dependent kinase II (CaMKII). Expression of CaMKII genes is tightly controlled by AR.

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Vol. 3, No. 2, jun, 2017

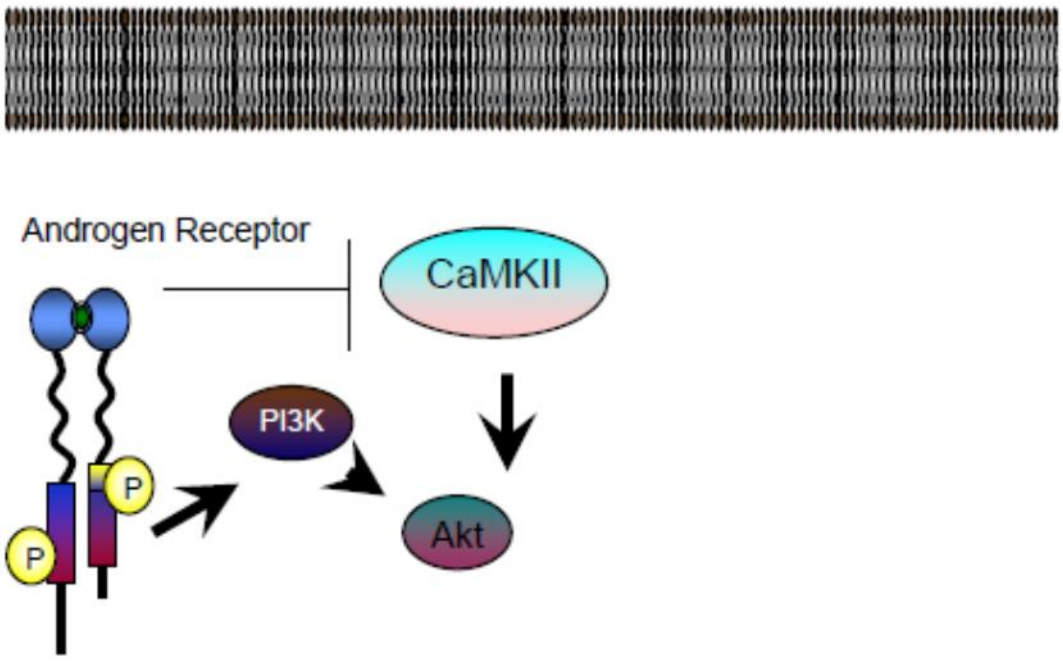

Figure 2: Androgen receptor suppresses the crosstalk of CaMKII with Akt. However if there is an inhibition of AR, it de-represses CaMKII.

It dampens CaMKII gene expression whereas abolition of AR activity results in an elevated level of kinase activity and in enhanced expression of CaMKII genes that activate anti-apoptotic PI3K/Akt pathways. Refractoriness to apoptosis is faced as overexpression of CaMKII genes desensitizes cells to apoptosis induced by KN-93, a CaMKII inhibitor, or wortmannin, a PI3K/Akt inhibitor, in terms of combinatorial drug use with doxorubicin, thapsigargin and TRAIL. Moreover, overexpression of CaMKII augments secretion of prostate specific antigen and promotes cell growth of LNCaP in steroid-free condition (illustrated in Fig. 3). There is an integration of two distinct transduction pathways including AR- and CaMKII-mediated pathways. CaMKII is an imperative performer in prostate cancer cells ability to escape apoptosis under androgen ablation and facilitate the progression of prostate cancer cells to an androgen independent state (44, 45). KN-93 (CaMKII inhibitor) has a broader effect on apoptosis than just inhibition of CaMKII: It inhibits AR activity and induces p53-independent apoptosis, inhibits anti-apoptotic protein Mcl-1, upregulates pro-apoptotic protein PUMA and generates ROS. Phenotype of prostate cancer cells undergoes transition from TRAIL-

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resistant to -sensitive in combinatorial drug treatment. This is suggestive of the fact that KN-93 could be used for novel therapeutic approaches when hormonal therapy has failed (46).

Expression Dynamics of Androgen Receptor: 'Heads', It Wins, and 'Tails', Therapeutics Lose

Neoplastic cells have to perpetuate the androgen receptor (AR)-mediated dynamics by a wide range of integrated pathways that crosstalk at various levels. It ensures the robust expression and activation of AR-mediated genes. There are multidirectional pathways opted for by the AR to meet the demands of a desperate cancer-prone environment. These pathways have been studied elsewhere (47, 48).

The growth-promoting effects of androgens are mediated mostly through the AR. Although PCa is heterogeneous in its etiology and progression, androgen signaling through the AR seems to be involved in all aspects of the disease. The binding of the androgen–AR complex to AREs involves recruiting coactivators and corepressors to regulate transcription of androgen-targeted genes such as PSA. Various members of the steroid receptor superfamily can recognize the same ARE. However, each receptor activates tissue-specific target genes under specific physiological conditions. This receptor-specific tissue response is due to a complex DNA–protein and protein–protein interplay among non-receptor coregulatory factors and/or cis-regulatory sequences. After the binding of native ligands, T and DHT, to the AR and in association with coregulators, the AR will be phosphorylated and translocated to the nucleus, and binds to AREs of the AR target gene promoters that induce their transcriptional activities. Although androgens are important in the maintenance of normal prostate homeostasis, complex interactions between peptide growth factors and other growth modulators regulated either by androgens or by other factors are also required. The transcriptional activity of the AR is important in prostate development, as well as in PCa progression. Androgen binding is the most important stimulus to the AR activity; thus, the PCa hormonal therapy aims to abolish this stimulus. Although hormonal therapy is partly effective, other factors can influence downstream AR-mediated transcription activity.

Whether at the level of the AR activation, downstream cytoplasmic signaling crosstalk or nuclear protein cross-modulation, the net effect in patients who have undergone androgen ablation and who have relapsed, is the retention of some androgen-regulated gene activity. Therefore, it is not surprising that much research into signaling and AR biology in the prostate is directed at addressing this issue. Blockade of peptide growth (trophic) factors or their receptors or the blocking of mutated ARs that are capable of responding to various stimuli may prove effective, and modulation of intermediate signaling factors such as MAPKs or coactivators may allow the suppression of multiple pathways to common DNA targets. G-protein-coupled receptors serve as the major receptor family for neuropeptides, neurotransmitters and other bioactive peptides (49).

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G-proteins share a common pathway for a multiplicity of neurotransmitter receptor functions and have an important role in the regulation of post-receptor levels including the activation of a variety of enzymes, that is, adenylyl cyclase and phospholipase C, which then regulate cell function through the production of second messengers. These second messengers include cyclic AMP (cAMP), diacylglycerol and inositol polyphosphates among others. Then, these molecules will activate the MAPK signaling pathway and several related protein kinases such as PKA that lead to the phosphorylation of various membrane and cytosolic proteins and to the regulation of gene expression by activating a variety of transcription factors (50). In addition, PKA pathway activation leads to phosphorylation of the nuclear transcription factor, cAMP response element (CRE)-binding protein (CREB) at Ser 133. The CREB binds the CRE of its target genes. It has also been shown that CREB-binding protein (CBP) enhances AR dependent transcription and this AR coactivator integrates androgen-mediated and other signaling pathways (51). In addition, in androgen-sensitive LNCaP cells, a putative ARCRE site forms specific and competable protein interactions with CREB (52). Li et al. (53) registered a robust expression of truncated AR isoforms in 22Rv1 cells along with intragenic reconstitution of an

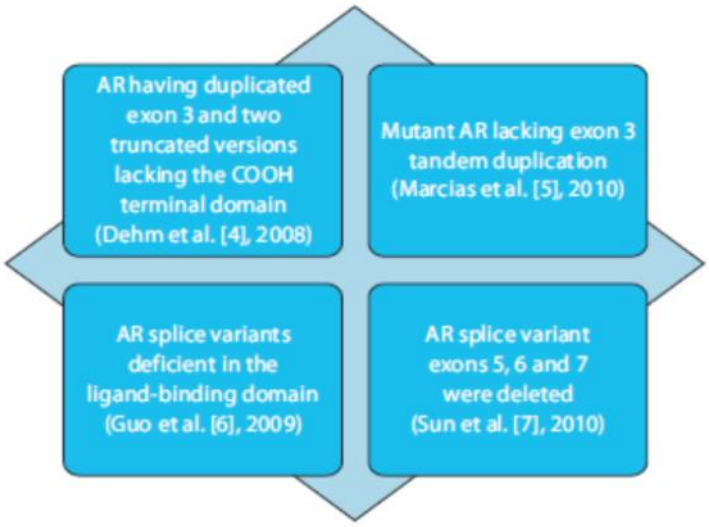

Figure 3: AR variants documented by different research groups

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approximately 35-kb AR genomic segment harboring a cluster of alternative AR exons. Cloning of the break fusion junction in 22Rv1 cells displayed long interspersed nuclear elements (LINE-1) encompassing the rearranged segment and a microhomology-mediated break-induced replication. Henceforth, intragenic rearrangements have emerged as new mediators in cancer progression. Some other variants documented by other research groups are mentioned in figure 1 (12, 41, 54, 55). Arachidonic acid pathway members PLA2G7, HPGD, EPHX2 and CYP4F8 were identified as putative novel therapeutic targets in prostate cancer. EPHX2 and PLA2G7 correlated with AR and their inhibition reduced AR signaling. It is obvious that inhibition of these enzymes may be effective when combined with other treatments, such as androgen deprivation (56). Lately, Kim et al. (57) have found that HOXB13 is a protein that is involved in suppression of AR-mediated signal transduction. This protein is downregulated in prostate carcinogenesis. Future research might converge upon mechanisms of restoration of the negative regulators of AR signaling. There are some other proteins which coexist at the promoter region and are involved in activation of target genes. H2A.Z and ubiquitin-specific protease 10 are each required for transcriptional activation of the AR regulated prostate-specific antigen and KLK3 genes (58).

A combinatorial drug design seems to dampen the key regulators which augment disease progression. Pharmacological targeting of STAT5 retards carcinogenesis because STAT5 cooperates with AR and favors cancer progression and aggressiveness (59). Yang et al. (60) stated that HDAC4 enhances AR SUMOylation, raising the likelihood that deacetylase may act as an E3 ligase for AR SUMOylation. Knockdown of HDAC4 increases the activity of endogenous AR. Further studies are important to explore antineoplastic detailed mechanistic insights. Another tumor suppressor gene, BTG2, is downregulated in prostate cancer, and the re-establishment of this gene in BTG2-deficient cells has been shown to inhibit prostate

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cancer cell growth (61). On a similar note, GLI1, important for Sonic hedgehog signal transduction, can act as a corepressor to considerably inhibit AR-mediated transactivation (62). Tan et al. (63) stated that treatment of AR by dihydrotestosterone potently activated GRP78 expression, and there was a coexistence of GRP78 with Hsp70-Hsp90 client proteins. All these proteins limit the efficacy of drugs. A multipronged drug design is necessary to checkmate this group of negative regulators. Accumulating data mark these AR variants as candidates for therapies directly targeting the AR rather than the ligand. Castration-resistant prostate cancer cells express variant ARs which included truncated ARs lacking the carboxyterminal ligand-binding domain, and small interfering RNA-mediated knockdown efficiently suppressed androgen-independent cell growth. Nigericin like compounds suppress AR expression at the messenger RNA level. These could be applied as new-type therapeutic agents that inhibit a broad spectrum of AR variants in hormone-refractory prostate cancer (45). However, there are some compensatory pathways which are switched on impairment of AR. Ablation of AR in in vitro cells induced PI3K-independent activation of Akt, which was triggered by calcium/calmodulin-dependent kinase II (CaMKII) (43, 64). It has lately been found that grape seed extract potently inhibits histone acetyl transferase, leading to decreased AR-mediated transcription and cancer cell growth (65). Similarly, procyanidin B3, an inhibitor of histone acetyltransferase, has broader implications in the inhibition of p300-dependent acetylation of ARs (66). After having outlined the current approaches for treating prostate cancer, we now focus on emerging therapeutic opportunities for prostate cancer aggressiveness that are based on recent insights into molecular off-track activities of AR. Targeted therapeutics, particularly those that restrain the activity of ARs that are mutated and/or overexpressed in cancer, have revolutionized the treatment. Hsp90-based therapy has so far shown inadequate activity in the clinic. In accordance with the same interpretation, efficacy of a novel mitochondrial-targeted, small-molecule Hsp90 inhibitor, gamitrinib (GA mitochondrial matrix inhibitor), was evaluated by Kang et al. (67) in the Transgenic Adenocarcinoma of the Mouse Prostate model.

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of localized and metastatic prostate cancer in immunocompetent mice. Another drug that has wider implications in blocking cancer activity is MDV3100; it blocks ligand receptor engagement and prevents nuclear shuttling of AR and coactivator recruitment of the ligand- receptor architecture (68) .

Targeting Chromatin-Modifying AR Co-Activators or AR Stability

Growing evidence has shown that co-regulators (factors recruited by transcription factors to either activate or repress transcription) are indispensable components of transcriptional regulation. AR, as a nuclear receptor, is no exception. AR co-regulators, such as FHL2 (four and a half LIM domains 2), have been reported previously (69). However recently, several chromatin modifying enzymes, namely histone demethylase proteins, were shown to complex with AR and facilitate its activation of gene targets (70).

Histone demethylases

Histone methylation was original thought to be a stable, irreversible mark as only histone methyl-transferases had been identified. However, the discovery of the first histone demethylase LSD1 (Lysine-specific demethylase 1), also known as AOF2 (Amine amine oxidase (flavin containing) domain 2) or KDM1 (Lysine (K) demethylase 1) in 2004 proved that histone methylation is reversible (71). Since then, a dozen more histone demethylases have been identified. Histone lysine methylation is a dynamic and versatile process, which has been observed on a number of lysine residues on histone proteins (72). The balance between repressive and active histone modifications (also known as the histone code) ultimately determines whether a gene will be actively transcribed or repressed, and AR target genes are no exception. The status of histone lysine methylation has been shown to be important for AR signaling, and several histone demethylase proteins are expressed or upregulated in prostate cancer. These include LSD1 and the Jumonji class of proteins, which include JMJD2C (Jumonji domain containing 2C, also known as GASC1 (Gene amplified in squamous cell carcinoma 1) and KDM4C (Lysine (K) demethylase 4C)), JMJD1A (Jumonji domain containing 1A, also known as JHDM2A (Jumonji domain containing histone demethylase 2A), and KDM3A (Lysine (K) demethylase 3A)), and JARID1B (Jumonji, AT rich interactive domain 1B, also known as PLU-1 and KDM5B (Lysine (K) demethylase 5B) (73). While the transcriptional targets of these proteins are largely unknown, all 4 complex with AR and facilitate its activation of downstream signaling

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pathways. Although the recruitment of JMJD1A to target genes was shown to occur solely in the presence of androgens, LSD1 and JMJD2C are bound to AR target genes even in the absence of androgen ligand or AR binding (74).

Interference with these histone demethylases with siRNA or pharmacological inhibitors leads to distinct changes in histone marks at AR target promoters. LSD1 is the best studied histone demethylase, and its activity and target specificity are largely dependent on interacting factors. In fact, it is a component of several transcriptional complexes (75). While LSD1 may demethylate the active di-methyl lysine 4 (2MK4) mark on histone H3, which leads to reduced gene expression, at AR target genes, several reports show that LSD1 binds to AR target AREs and facilitates demethylation of the repressive mono (1MK9) and di-methyl lysine 9 (2MK9) marks on histone H3 upon recruitment of ligand-bound AR, which results in transcriptional de -repression (76, 77). The demethylase activity of LSD1 is essential for this process, as deletion of the enzymatic domain of LSD1 or siRNA to LSD1 reduces androgen-induced transcriptional activation of AR targets, although direct biochemical evidence for LSD1 demethylation of 1MK9 and 2MK9 in cell -free systems is lacking. LSD1 may also mediate prostate cancer aggressiveness as high LSD1 mRNA levels or reduced levels of the 2MK4 mark on histone H3 that LSD1 demethylates, are significantly associated with prostate cancer recurrence, making these promising prognostic markers (78). Likewise, JMJD1A interference leads to increased levels of the repressive 2MK9 mark at AR target genes, which attenuates AR target gene transcriptional activation by ligand-bound AR. Unlike LSD1, 1MK9 and 2MK9 have been shown, in cell-free assays, to be direct substrates of JMJD1A. Like LSD1, however, knockdown of JMJD1A does not block AR recruitment, suggesting these two demethylases function after AR binding to facilitate AR's transcriptional program. Unlike LSD1 though, promoter occupancy of JMJD1A is dependent upon the presence of androgens or AR binding to its target genes, and RNA interference of JMJD1A results in more pronounced effects on blocking activation of AR targets than RNA interference of LSD1. Finally, JMJD1A expression is increased in response to androgens, although it is not clear whether JMJD1A is an AR target gene (79). The expression of another Jumonji class protein, JMJD2C, is also induced by androgens.

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JMJD2C may catalyze the removal of the repressive di and tri-methyl lysine 9 mark on histone H3(80). More importantly, JMJD2C interacts with LSD1 and co-localizes with LSD1 and AR at AR target gene AREs. Together, these two demethylases lead to the removal of repressive tri-, di-, and mono-methyl marks from lysine 9 on histone H3 to activate transcription of AR target genes. In addition, it was recently shown that JARID1B, whose only known substrate is the active methylated-H3K4 marks, interacts with AR. In reporter assays, JARID1B enhanced transcriptional activation by AR while a JARID1B mutant that was unable to bind AR did not. Finally, JARID1B is upregulated in prostate cancers samples compared to benign prostate samples. It can be concluded that, considering the importance of these enzymes in the activation of AR target genes, inhibition of these enzymes may be a rational, non-hormonal strategy to disrupt AR signaling. Efficient inhibitors of JmjC domain-containing histone demethylases have yet to be discovered, but two classes of LSD1 inhibitors have been identified. The first class is MAOIs (monoamine oxidase inhibitors) such as pargyline, which have been used clinically as antidepressants and which may also inhibit LSD1 activity. In addition, polyamine analogues, which have been previously shown to inhibit cancer cell growth and more recently which have been shown to inhibit LSD1 from demethylating the active 2MK4 mark, represent another class of agents to target LSD1, although the effect on AR target genes remains poorly characterized (81).

Conclusion

Spreading cancer before and after surgical, chemical or radiological treatment is one of the biggest challenge in the management of a cancer patient. Diagnosis of cancer metastasis could be a valuable approach while treating the cancer as prognostic factors helped in the survival of patients and reduce mortality rate. Prostate cancer, a major type of cancer in male, can be diagnosed by several molecular factors like PSA expression but diagnosis its metastatic stage is a challenging task. In this review, we have focused on the utilization of androgen receptors as a significant tool to diagnosis PCa metastasis state.

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