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Molecular mechanisms of castration-resistant prostate cancer progression

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Abstract

Hormone-refractory prostate cancer is the result of regrowth of prostate cancer cells that have adapted to the hormone-deprived environment of the prostate. The process by which castration-resistant prostate cancer (CRPC) cells are generated appears to be varied. The complex mechanism of hormone resistance has been the topic of research in most laboratories that have analyzed the process from different angles. This review compiles research findings that explain the methods of development of hormone resistance in prostate cancer. Research data show many different processes to be involved in the acquisition of hormone resistance. Interestingly, one observes interdependence between these processes, indicating a complex network at play in the development of hormone resistance. Cytokines such as IL-6 have been shown to initiate an alternative signaling pathway, compared with the androgen receptor signaling pathway, in CRPC. IL-6 has been proposed to be the effector of the intracrine signaling pathway by influencing the levels of metabolic enzymes. Neuroendocrine cells are present at low levels in normal prostate, and signify the transitory phase of normal hormone-sensitive cells to hormone-refractory cells. IL-6 induces growth of neuroendocrine cells or neuroendocrine-like features in cells in CRPC. The increased presence of neuroendocrine cells in CRPC signifies a change in the prostate cell microenvironment. The stromal microenvironment also influences the development of CRPC in the hormone-refractory stage. In addition, intracrine androgen metabolic enzymes play a significant role in the development of the hormone refractory process. Despite hormone ablation, there is a residual level of hormones in cells due to active intracrine metabolic pathways. It is acknowledged that the androgen receptor plays the most influential role in development of prostate cancer. In addition to mutation and amplification, the androgen receptor has been characterized and shown to differ in sequence in CRPC compared with the androgen-sensitive prostate cancer cells. These variants of the androgen receptor through sequence changes may preserve the basic function of the molecule, but have far-reaching consequences on the cell as a

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whole. A multicombinatorial drug treatment approach has been suggested to target these multiple pathways in an effort to reduce the possibility of recurrence of CRPC.

Keywords

androgen receptor; AR; AR variant; castration-resistant prostate cancer; CRPC; IL-6; interleukin-6; intracrine signaling

Castration-resistant prostate cancer

The growth of prostate epithelial cells requires physiological levels of androgen, both to stimulate proliferation and inhibit apoptotic death [1]. Androgen binds to the androgen receptor (AR), which causes AR to bind to androgen responsive elements (AREs) in the promoters of androgen-regulated genes. This interaction is affected by many other transcription coregulators. These complex interactions between AR, AREs and coregulators facilitate the activation or repression of genes regulating development, differentiation and proliferation of target cells. Several androgen-responsive genes have been identified, including prostate-specific antigen (PSA) and human glandular kallikrein 2 (hk2) [2].

Because the growth of prostate cancer cells depends on the presence of androgens, androgen deprivation therapy has been the primary treatment for patients with metastatic prostate cancer since the seminal recognition of the disease as androgen-sensitive by Huggins and Hodges in 1941 [3]. Almost all patients with advanced prostate cancer initially respond to androgen deprivation therapy. However, virtually every patient will relapse due to the growth of castration-resistant cancer cells. Castration-resistant prostate cancer (CRPC) cells often continue to express androgen-responsive genes such as PSA, and often express AR [4,5], suggesting that the AR becomes activated by a castration-resistant mechanism in AR-positive CRPC cells.

It is well-known that hormone ablation treatment of prostate cancer initially causes the cancer to regress. Regression often occurs within 12–18 months, although shorter periods of regression are also known to occur. The progression to metastatic disease is slow and can be monitored by the steady increase in PSA levels. Androgens maintain the ratio of cells within the prostate where there is a balance between dividing proliferating cells and the cells undergoing death. Upon hormone deprivation, the cells undergoing apoptosis increase, and the equal ratio of cells is affected, leading to cancer cell death and the regression of prostate cancer. Huggins and Hodges showed the effect of castration on acid phosphatase and alkaline phosphatase levels. Acid phosphatase levels decreased sharply on castration, while alkaline phosphatase levels decreased gradually. Alkaline phosphatase is an enzyme found in bone and cartilage and is indicative of activity of cells outside the prostate gland. Acid phosphatase is found in the cells of the prostate and is an effective indicator of the growth of prostate cells [6].

Theories of prostate cancer recurrence

Androgens are comprised of testosterone, dihydroepiandrosterone (DHEA), androstenediol and androstenedione. Testosterone makes up 90% of the androgens and freely diffuses into prostate cells. Testosterone that is synthesized in the testes is found in blood in the bound form where albumin or sex-hormone-binding-globulin binds the hormone. On entering prostate cells, the enzyme 5α-reductase (SRD5A2) converts testosterone to the active hormone, dihydrotestosterone (DHT), which binds to the AR. This causes a conformational change in the receptor, which then dissociates from heat shock proteins and is translocated into the nucleus where it binds to AREs in the promoter regions of genes to activate their

transcription [7–9]. So what causes the cells in the prostate to get recharged after castration and to proliferate, resulting in a more aggressive castration-resistant cancer? There have been many theories proposed on the causes of prostate cancer recurrence. Five main theories are:

- Hypersensitive pathway
- Outlaw pathway
- Promiscuous pathway
- Coactivators and corepressors
- Bypass pathway [7–9].

These theories deal with the AR and the role it plays in the development of castration-resistant cancer. In the hypersensitive pathway, the AR becomes extremely sensitive to very low amounts of androgens that may be present even after castration or medical blockade of androgens. The AR, through mutations or overamplification or increased 5α-reductase enzyme levels, may become sensitive to low levels of androgens. Many castration-resistant cancers show amplification of the AR in cells, which may be a result of selective outgrowth following death of cells during castration [8,10]. Increased 5α-reductase enzyme levels result from a polymorphism where there is a substitution of valine with leucine at codon 89. This is commonly observed in African men, indicating the genetic influence in prostate cancer [8,9]. CRPC, though independent of the supply of androgens, tends to express the AR at high levels in most cases. The outlaw pathway is functional when the AR pathway is activated by growth factors and receptor tyrosine kinases. In these cases, growth factor pathways such as IGF and KGF can bind and activate the AR in the castrated state, as they are overexpressed. Receptor tyrosine kinases such as HER-2/neu are overexpressed in castration-resistant cancers, which results in the activation of the AR. Interleukin-6 and interleukin-4 are also activators of the AR pathway in the castration-resistant state [7,8,11,12]. The cross-talk between NF-κB and AR has indicated the role of the NF-κB signaling pathway in the development of castration resistant cancer. The promiscuous pathway results in the AR being receptive to ligands other than DHT. Nonandrogenic steroids, antiandrogens, can activate the AR due to missense and other mutations, which may expand the ligand binding specificity of the AR. LNCaP cells possess an AR where threonine is substituted with alanine at codon 877, which is sensitive to a wide range of steroid ligands. CWR22 cells also show a substitution of histidine with tyrosine at codon 874 in the sequence that encodes AR. Mutations in AR are observed in TRAMP mice in three regions – that is, viz. the signature loop of the receptor, the flanking region where p160 coactivators bind and the ligand-binding domain [8]. Coactivator levels are increased in castration-resistant cancers, which enhances the sensitivity of the AR to various ligands other than androgens. Coactivators such as ARA70, ARA55, SRC-1, P/CAF and GRIP1/TIF2 are overexpressed in prostate cancer [7,8,13]. The bypass pathway, as the name suggests, circumvents the AR pathway and utilizes other pathways in stimulating prostate cells to proliferate in a castration-resistant environment. The activation of *bcl2* in prostatic intraepithelial neoplasia (PIN) lesions can cause cells to avoid utilizing the AR pathway. Similarly, the activation of oncogenes or inactivation of tumor suppressor genes may result in other bypass pathways. Neuroendocrine (NE) cells secrete neuropeptides such as bombesin, which enhance the proliferative rate of cells in a cancerous environment in the absence of hormones. Bombesin activation of AR is Src-dependent. This has been shown through analyses of LNCaP and PC-3. Myc upregulation is a striking feature of bombesin activation of AR. This study shows an alternate route of AR activation that is independent of androgen and dependent on Src kinase [7,8,13]. Shi *et al.* have examined the alterations involved in the transition of LNCaP cells into castration-resistant cells, and have observed upregulation of growth factors, the Bcl-2 protein and the Akt pathway. The three sublines

generated were representative of the theories proposed for development of castration resistance [14]. Chen *et al.* also performed similar analyses using LNCaP and castration-resistant C4-2 cells and analyzed genes such as *TMEFF2*, *NKX3.1* and *AMACR* to compare the gene-expression differences. In addition, these genes were further analyzed in xenografts of these cells to observe consistency in expression. The study analyzed 51 candidate genes and compared the results to human prostate cancer tissues and showed a correlation. The study by Shi *et al.*, as well as Chen *et al.*, showed the difference in gene expression in androgen-dependent and castration resistant prostate cancer [15].

Influence of cytokine IL-6

Interleukin-6 (IL-6) is a glycoprotein and has been implicated in the modulation of growth and differentiation in many cancers, including prostate [16–18]. The expression of IL-6 and its receptor has been consistently demonstrated in human prostate cancer cell lines, and clinical specimens of prostate cancer and benign prostate hyperplasia [19–21]. Multiple studies have demonstrated that IL-6 is elevated in the sera of patients with metastatic prostate cancer, and that the levels of IL-6 correlate with tumor burden, serum PSA, clinically evident metastases and CRPC [22,23]. In addition to the clinical data that IL-6 is associated with CRPC, experimental studies demonstrate that IL-6 plays a critical role in prostate cancer cell growth and differentiation. IL-6 functions as a paracrine growth factor for the human LNCaP androgen-sensitive prostate cancer cells, and as an autocrine growth factor for the human DU145 and PC3 androgen-insensitive prostate cancer cells [24]. IL-6 activates AR-mediated gene expression by activation of the AR through a Stat3 pathway in LNCaP cells [25–27]. Further studies demonstrated that overexpression of IL-6 enhanced PSA mRNA expression in LNCaP cells and can partially rescue LNCaP cells from growth arrest induced by androgen deprivation therapy [12,28]. In addition, overexpression of IL-6 protects LNCaP cells from undergoing apoptosis induced by androgen deprivation therapy. The antiapoptotic effect of IL-6 is mediated by Stat3. IL-6 over-expressing LNCaP clones show increased Stat3 levels. LNCaP cells grown in charcoal-stripped medium, when transfected with constitutively active Stat3, show a reduced rate of apoptosis in the presence of Stat3 [28]. Collectively, these findings suggest that IL-6 can regulate the expression of androgen-responsive genes in a castration-resistant manner, and induces castration-resistant growth of androgen-dependent human prostate cancer cells.

Influence of cytokine IL-8

IL-8 is a chemokine that was first discovered as a chemoattractant in leukocytes [29]. It is a mitogen and shows chemotactic activity for endothelial cells, thus promoting angiogenesis [29–31]. It is also secreted in tumor cells such as prostate cancer. IL-8 is specifically found in metastatic prostate cancer compared with benign prostatic hyperplasia or normal cells [29,31].

In the study by Inoue *et al.*, it was observed that IL-8 overexpression in the prostate cancer cell line, PC-3P, transformed the cell line to a more metastatic phenotype. This transformation resulted in the cell line developing angiogenic factors such as MMP-9, which caused increased tumorigenicity as observed *in vivo* after orthotopic implantation in mice [29]. Araki *et al.* have shown that overexpression of IL-8 in androgen-dependent prostate cancer cells transforms them into cells with a castration-resistant phenotype. Such a transformation results in the cells acquiring greater invasive capacity, motility and increased angiogenesis [30]. IL-8 has also been shown to mediate the transition of androgen dependence in prostate cancer cells to castration resistance using the tyrosine kinases such as Src and FAK [32]. Parathyroid hormone-related protein is responsible for IL-8 induction in prostate cancer cells [33]. IL-8 has been shown to be highly expressed in men with advanced

prostate cancer who also have bone metastases [34]. Further evidence for the transitory role of IL-8 to castration resistance is provided by the results of Schauer *et al.* which state that IL-8 upregulation correlated with the production of reactive stroma in benign prostatic hyperplasia [35].

Neuroendocrine cells

Neuroendocrine cells represent a minor cell population in the epithelial compartment of normal prostate glands and may play a role in regulating the growth and differentiation of normal prostate epithelia. In prostatic adenocarcinoma cells, the NE phenotype acquisition is associated with tumor progression and castration resistance. Although a small percentage of prostrate cancer cases are classified as NE cancers, there is growing appreciation for the role prostatic NE cells may play in the ability of prostrate cancer to survive castration. Prostatic NE cells are typically post-mitotic cells that resist androgen deprivation therapy due to their lack of AR expression. NE cells secrete many factors that induce paracrine mitogenic effects on prostrate cancer tumor cells. IL-6 is among the most well-documented factor induced by castration. IL-6 can further enhance the NE phenotypes, thereby amplifying the wave of survival factor release [36–38]. IL-6 treatment induces neuron-like morphology and elevated NE marker expression in LNCaP cells [36,39,40]. Although the NE-transdifferentiated cells are growth arrested, the factors released by them contribute to the survival and eventually the growth of the CRPC in androgen-deprived conditions. Our team has extensively characterized the soluble factors released by either IL-6 or androgen deprivation-induced NE differentiation. We performed an extensive survey based on microarray, real-time reverse transcription (RT)-PCR and found neurokines such as GRP, neurotensin, chromogranin A and parathyroid hormone related-peptide, chemokines such as IL-8, endothelin-1 and H2 relaxin, TGFβ family peptides such as BMP and PDF, as well as angiogenic factors such as VEGF and VEGF-C. We previously demonstrated that IL-6 can induce CRPC, when supplied to LNCaP either in an autocrine or paracrine fashion [37,41,42], as IL-6 is also one factor upregulated in NE cells. Jin *et al.* showed that NE cells could facilitate the growth of LNCaP tumors in castrated nude mice even when injected into the opposing flank [43], suggesting a paracrine and endocrine mechanism. Components of these pathways are potential targets for intervention. IL-6 is released upon androgen withdrawal, and has the capacity to ‘reactivate’ AR in the presence of a low level of androgen.

Influence of microenvironment

Besides the AR, the microenvironment plays a role in the development of castration-resistant cells. Studies by Cunha *et al.* have shown the effect of the stroma on the epithelial cells of the prostate. Recombination of mice epithelial cells possessing inactive AR with stromal cells of mice containing functional AR showed that the mice epithelial cells developed normal prostatic cells, yet the reverse combination failed to yield functional epithelial cells despite the addition of androgens. This indicated the importance of the stroma in prostate cell development [10]. The stromal cells can activate the epithelial cells through paracrine signals, while the growth of prostate cells in a castration-resistant environment can also occur in an autocrine manner. This was observed in experiments where prostate cancer cells could proliferate in nude mice that possessed either wild-type AR or where the AR was inactive. The proliferation of prostate cells in an AR-null environment indicated autocrine signaling of cells to activate pathways besides the AR signaling pathway to survive in a hormone-refractory environment [10]. Akakura *et al.* have demonstrated the effect of intermittent androgen therapy on androgen-dependent tumors, where it was observed that androgen-dependent tumors transplanted in castrated mice showed a regression. When the tumors had regressed 30%, they were transferred to intact mice. The tumors started to develop only to be transplanted back into castrated mice. This

intermittent procedure was conducted to observe the rate at which the tumors became castration-resistant. The time taken for the tumors to develop into castration resistant-tumors was threefold. Such intermittent therapy could delay the development of the aggressive tumor that is hormone refractory [44]. Reactive stroma is a term used to describe the changes that stroma undergoes during the transition from normal cells to cancer. In the prostate, the reactive stroma is characterized by a myofibroblast/fibroblast mix of cells and a decrease in smooth-muscle content. Other distinguishing features of reactive stroma are an increase in levels of tenascin and collagen type I proteins [45–47]. It has also been observed that an increase in IL-8 correlates with the development of reactive stroma in benign prostatic hyperplasia [35].

Research has been carried out on androgen-dependent prostate cancer cells to observe their growth into castration-resistant cells and to analyze the changes that occur with this transition. Hendriksen *et al.* have analyzed the AR pathway in LNCaP cells, as well as xenografts from intact and castrated mice to observe the genes that are upregulated or downregulated in the transition to androgen independence. It was observed that AR genes that were not stroma associated were upregulated in primary prostate cancer that shows low levels of stromal cells. In low-grade carcinoma, genes that were involved in cell growth, proliferation and apoptosis were expressed to higher levels compared with high-grade carcinoma, where differentiation is lowered. The genes that are upregulated in low- and high-grade carcinomas were involved in metabolism, exocytosis and protein folding. *SIM2* and *AMACR* were upregulated in prostate carcinoma. The study showed that the stress response genes, *HERPUD1* and *STK39*, were downregulated and were good indicators of metastasis [48].

Intracrine androgen metabolism

One highly significant recent development concerning CRPC is the discovery that levels of intracellular androgens and the expression of enzymes involved in androgen biosynthesis such as HSD3B1/2 and AKR1C1-3 are upregulated in CRPC [49–55]. Although serum testosterone concentrations were significantly reduced after androgen deprivation therapy, levels of intraprostatic androgens are reproducibly measured at concentrations sufficient to activate the AR and stimulate tumor growth [49,51–57]. Accumulating evidence demonstrated that prostate cancer cells may survive androgen deprivation therapies by regulating intracrine androgen synthesis within the prostate. Intraprostatic androgens can be *de novo* synthesized from cholesterol or other ubiquitous molecular precursors such as DHEA, mediated by genes encoding many steroidogenic enzymes, including AKR1C3, HSD3B2, SRD5A1, CYP17A1, CYP19A1 and UBT2B15 (Figure 1). Specifically, AKR1C3 (also known as 17βHSD5) converts androstenedione to testosterone in the prostate. HSD3B2 converts DHEA to androstenedione, a substrate for conversion to testosterone. SRD5A1 converts testosterone to the higher affinity dihydrotestosterone (DHT). The expression of these genes involved in regulating androgen metabolism is increased in castration-resistant cancers versus prostate cancers analyzed from untreated patients [49–55]. These data clearly suggest that testosterone and DHT can be biosynthesized within prostate tumors through the utilization of adrenal androgen precursors or through the metabolism of precursors incorporated earlier in the androgen biosynthetic pathway. Recent data has shown the regulation of enzymes such as AKR1C3, HSD3B2 and SRD5A1 by the cytokine IL-6 [58]. The study shows the regulation of AKR1C3 at the transcriptional and translational level by IL-6. The specificity of IL-6 on AKR1C3 is confirmed by downregulating the IL-6 receptor, gp130 receptor or a combination of both with siRNA treatment, and observing the decrease in AKR1C3 levels. Testosterone levels are increased in the presence of IL-6 in castrated mice, indicating a functional androgen signaling pathway despite the absence of testicular androgen. The study shows for the first time a source for the rise in intraprostatic androgen

levels despite castration [58]. The study by Arnold *et al.* have shown the effect of the stroma of a cancerous cell on the induction of androgen-responsive genes by DHEA. The effect of DHEA on a potential cancerous environment was analyzed, and it was observed that in the presence of a coculture of prostate cancer-associated stromal cells and LAPC-4 cells, DHEA induced PSA mRNA by 15-fold and an increase in testosterone levels [59]. The induction of androgen-responsive genes and androgen signaling appears to be a result of complex signals from cytokines and the microenvironment.

Androgen receptor variants

Research on CRPC focuses on the AR, the key molecule involved in processing the androgen hormone. With the depletion of androgen levels, it has been observed that the AR undergoes structural and functional changes to be able to survive the hormone-deprived environment. Gene amplification and AR mutations in the ligand-binding domain (LBD) are some of the changes observed in the AR which continues to perform its genomic function [8,9,60].

Recent analysis of the AR has identified an isoform of the AR developed from a CWR22 relapsed xenograft [61,62]. The AR mutation was observed in hormone-refractory CWR22 as compared with the original hormone-dependent cell line. The AR mutation is characterized by a duplication of exon 3 which is in-frame with the rest of the AR protein sequence with a molecular mass of 114 kDa. There is a simultaneous generation of a second protein fragment of lower molecular mass (75–80 kDa) with a truncated ligand-binding domain [62]. The 75–80 kDa AR protein (ARΔLBD) possesses the transactivation and DNA-binding domains, but lacks the ligand-binding domain as the carboxyl terminal is truncated. The AR-specific mutation observed in the CWR22R*v1* cell line promotes growth in androgen-deprived conditions independent of hormone concentrations. The doublet ARΔLBD becomes more pronounced in hormone-deprived conditions, and is reduced with the addition of exogenous androgen. However, androgen reduced the transcriptional activity of AR in CWR22R*v1* cell lines. PSA levels were also found to be low in this cell line.

The ARΔLBD isoform was further characterized in 2008 by Dehm *et al.* [61]. This manuscript went on to show that ARΔLBD was not excised from the full-length 114 kDa AR protein found in CWR22R*v1* cells, but was independently generated. Further verification of the roles of the two AR species were obtained from cell growth and cell proliferation assays that showed that AR^{Ex3dup} inhibition affected growth of cells in the presence of hormones, while ARΔLBD inhibition affected cell growth in the absence of hormones. These were clear indications of the distinct pathways of the two AR species. The siRNA results confirmed that ARΔLBD species was a result of transcription from a distinct set of mRNA distinct from those transcribing AR^{Ex3dup}. Sequence analysis of the region surrounding exon 2 revealed a 90–120 bp sequence inserted between exon 2 and exon 3. This sequence was termed exon 2b and was found to be spliced to either exons 1 and 2 or a combination of exons 1, 2 and 3. The result of this splicing is the generation of two types of AR. The two short isoforms are referred to as $AR^{1/2/2b}$ and $AR^{1/2/3/2b}$. It has been observed that $AR^{1/2/3/2b}$ is present in cells that contain a-full length AR^{Ex3dup}, whereas $AR^{1/2/2b}$ can be found in cells that contain the 110-kDa full-length AR. There is a significant increase in expression of full-length AR coupled to the short isoforms both at the transcriptional and translational level. Xenografts in castrated mice show an increase in $AR^{1/2/2b}$ at the mRNA and protein level, indicating ligand independence of prostate cancer cells in hormone-deprived conditions [61].

Guo *et al.* have characterized three other variants of AR in hormone-refractory cell lines, which lack the ligand-binding domain [63]. The three variants, AR3, AR4, AR5, were

detected by amplifying the AR sequence following the DNA-binding domain. AR3 was found to be the most abundant of the variant transcripts in the hormone-refractory cell lines C-821, CWR-R1 and CWR22R*v1*. AR3 activity and expression is unaffected by the presence of androgens or anti-androgens indicating the correlation to androgen independence in prostate cancer cells. AR3 was detected both in the cytoplasm, as well as nuclei of hormone-refractory prostate cancer cells. This was further confirmed through tissue staining, which detected increased AR3 staining in malignant hormone-resistant prostate cells compared with the benign and androgen-sensitive prostate cells. An interesting observation is that increased cytoplasmic staining of AR3 is an indicator of prostate cancer recurrence. Microarray analysis has revealed the set of genes that are differentially regulated by AR3 compared with AR. Akt1 is a target protein of AR3 that appears to be a mechanism by which AR3 regulates hormone-refractory growth of prostate cancer cells. This study established an independent pathway of AR3 in regulating growth of prostate cancer cells compared with wild-type AR [63].

A second study by Hu *et al.* discusses additional variants or isoforms observed in the region downstream of the DNA-binding exons of AR, resulting in the generation of proteins with truncated ligand-binding domains [64]. These variants were found to be overexpressed in hormone-refractory prostate cancer. Two variants, AR-V1 and AR-V7, were studied at length. The variants were detected after sequencing the intron region that followed exon 3. Three cryptic exons were detected that give rise to seven variants of the AR based on the combination with the remaining exons of the AR sequence. These variants possess premature termination codons that result in truncated AR species. The expression levels of the variant mRNA were higher in clinical specimens with hormone-refractory prostate cancer, although the expression is lower relative to the full-length AR. PSA expression cannot be correlated to AR-V7 and AR-V1 expression levels. Increased expression of the variant species in hormone-dependent prostate cancer correlated with poor prognosis [64].

An AR splicing variant, AR23, which impairs the nuclear localization function has been described by Jagla *et al.*, where a 23-aminoacid insertion between exon 2 and exon 3 of the AR results in a variant of the AR that fails to enter the nucleus upon androgen stimulation [65]. AR23 was detected in a patient with hormone-refractory metastatic prostate cancer. Unlike wild-type AR, AR23 is retained in the cytoplasm as aggregates, despite hormone treatment. AR23 is found to enhance the functions of endogenous wild-type AR in the cell. Thus, the androgen signaling pathway is induced by AR23 only in the presence of endogenous wild-type AR. The expression of endogenous AR is enhanced in the presence of AR23. NF-κB transcriptional activity is increased in prostate cancer cells in the presence of AR23, while AP-1 activity is reduced. AR23 appears to regulate the effects of cell growth, differentiation and migration through these signaling pathways [65].

In summary, one of the greatest challenges facing prostrate cancer is its evolution to castration resistance. Accumulated data emphasize the presence of residual androgens and persistent activation of the AR signaling axis in castration-resistant prostate tumors despite castration (Figure 2). Data regarding the molecular response of prostate cancer to hormone therapy continues to emerge, providing critical insight into growth and signaling pathways that may be exploited as therapeutic targets.

Future perspective

Research in the field of CRPC is advancing. The traditional idea of androgen blockade as the means of eradicating the spread of prostate cancer is now being closely examined. This review provides different aspects of development of castration resistance. The elucidation of these different pathways is an opportunity for drug development, which can be designed to

target these pathways specifically. Further research based on the current findings in CRPC will result in detailed characterization of the castration-resistant pathway. Patients can be specifically treated based on the kind of castration resistance development occurring in the body. Prostate cancer research has reached a stage where fine characterization of elusive pathways is possible, and this promises to be a very exciting and crucial phase for treatment benefits.

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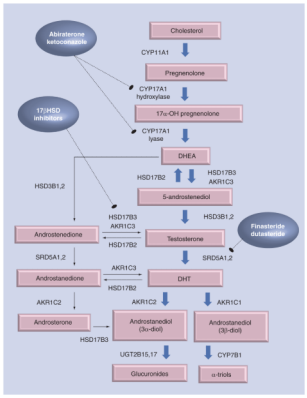

Figure 1. Pathways of androgen biosynthesis from cholesterol to DHT

Broken lines show enzymes targeted by inhibitors.

DHEA: Dihydroepiandrosterone; DHT: Dihydrotestosterone.

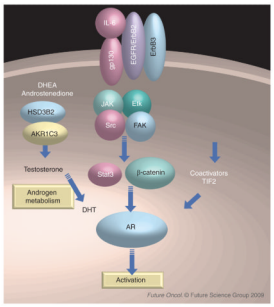

Figure 2. Androgen receptor activation by growth factors and cytokines, intraprostatic androgens and coactivators

Growth factors such as IL-6, ErbB3 and EGFR activate AR through tyrosine kinases such as JAK, Src and FAK, which in turn mediate their activity through Stat3 or β-catenin. In the absence of androgen ligand, intracrine metabolism with the help of enzymes such as AKR1C3 or HSD3B2 occurs where precursors such as DHEA and androstenedione are converted to testosterone and, finally, DHT. Coactivators such as TIF2 provide an alternate mechanism of AR activation in the absence of androgen ligand in a castration-resistant environment.

AR: Androgen receptor; DHEA: Dihydroepiandrosterone;
DHT: Dihydrotestosterone; EGFR: Epidermal growth factor receptor.