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Delineation of the androgen-regulated signaling pathways in prostate cancer facilitates the development of novel therapeutic approaches

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Abstract

While androgen deprivation therapy (ADT) is initially effective for the treatment of progressive prostate cancer, it inevitably fails due to the onset of diverse resistance mechanisms that restore androgen receptor (AR) signaling. Thus, AR remains a desired therapeutic target even in the relapsed stages of the disease. Given the difficulties in stopping all AR reactivation mechanisms, we propose that the identification of the driver signaling events downstream of the receptor offer viable, alternative therapeutic targets. Here, we summarize recently described, AR-regulated processes that have been demonstrated to promote prostate cancer. By highlighting these signaling events and describing some of the ongoing efforts to pharmacologically modulate these pathways, our goal is to advocate potential new therapeutic targets that would represent an alternative approach for blocking AR actions.

Introduction

Prostate cancer is the most commonly diagnosed non-skin cancer in men and ranked third in cancer-related deaths [1]. One of the main drivers of prostate cancer is the androgen receptor

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(AR), a ligand inducible transcription factor that is also needed for prostate development and homeostasis. Upon binding to androgens, AR translocates from the cytoplasm to the nucleus where the receptor homodimerizes and binds specific sequences of DNA termed androgen response elements (AREs). This leads to the transcriptional regulation of a variety of genes [2]. Due to the importance of AR in cancer progression, prostate cancer patients with metastatic or intermediate to high-risk, localized prostate cancer are treated with androgen deprivation therapy (ADT) [3]. However, most patients relapse within 2-3 years due to the emergence of resistance mechanisms that allow AR to remain active [4]. These mechanisms of resistance underline the importance of understanding the androgen-regulated pathways that contribute to the progression of prostate cancer (Figure 1). This review highlights some of the recent insights into androgen-regulated pathways in prostate cancer and their role in the progression of the disease with an eye towards those that may present new therapeutic avenues.

Proliferation

One of the key processes by which AR drives prostate cancer progression is through the regulation of genes involved in proliferation. It is known that knockdown of AR can significantly decrease proliferation in both hormone-sensitive prostate cancer (HSPC) and metastatic castration-resistant prostate cancer (mCRPC) [5,6]. This lends credence to the concept that AR is still the main driver of mCRPC even after hormone ablation therapy fails.

Canonical AR-mediated regulation of proliferation

In HSPC, androgen signaling primarily promotes the G1 to S phase transition. Accordingly, androgen depletion leads to a G1 cell cycle arrest [7]. This arrest is thought to be mainly due to the loss of AR-mediated cyclin D1/D3 expression via mTOR, attenuated CDK4 activity, and hyper-activation of the cell cycle regulator Rb [8,9]. In early G1, AR indirectly activates cyclin D1/3 and CDK4/6 complexes through expression of p21^{cip1} (which promotes formation of cyclin D/CDK4/6 complexes early in G1, but later inhibits CDK2 activity in late G1 and S phases in cancer) [7]. These complexes perform the first round of Rb phosphorylation/deactivation [7]. In late G1, AR-mediated degradation of the cell cycle inhibitor p27^{kip} activates the cyclin E- and CDK2-mediated phosphorylation/deactivation of Rb [8]. Once Rb is deactivated, prostate cancer cells move into S phase unimpeded.

Emerging cell cycle genes regulated by AR

CLDN8 was identified as a potential AR-regulated gene in a search for global AR target genes in prostate cancer [10]. *CLDN8* is expressed at higher levels in clinical samples of prostate cancer compared to benign prostate tissue. *CLDN8* is directly regulated by AR and has been found to promote prostate cancer cell proliferation and migration. Mechanistically, *CLDN8* provides the cytoskeletal remodeling and signaling necessary for proliferation by binding to peripheral membrane proteins to manipulate cytoskeletal structure at tight junctions and as an upstream activator of Akt and ERK. These recent data suggest that *CLDN* family inhibitors or novel *CLDN8* inhibitors may have clinical utility in the treatment of prostate cancer, an area that has not been fully investigated.

Endoplasmic Reticulum (ER)-associated protein degradation (ERAD) is a pathway involved in the degradation of misfolded proteins through the unfolded protein response and regulation of normal ER proteins. ERAD consists of several genes that regulate protein flux through degradation. These genes have recently been demonstrated to be under AR control (either directly by AR or indirectly through AR-mediated UPR-IRE1 α activation) [11]. In response to androgens, the expression of several ERAD genes are increased while the expression of the ERAD inhibitor SVIP is decreased. Further, there was an increase in the degradation of ERAD target substrates in response to androgens which, in turn, promoted prostate cancer cell proliferation. The knockdown of one such ERAD gene, ER membrane-bound transcription factor *CREB3L4*, led to cell cycle arrest in the G2/M phase, elevated expression of cyclin B1 (must be degraded for mitotic exit), reduction of cyclin D1, and upregulation of the CDK inhibitors p21^{cip} and INCA1 [12]. Knockdown also caused a decrease in the expression of canonical AR-regulated genes. Conversely, overexpression of *CREB3L4* increased cell proliferation [12]. Taken together, these data suggest a positive feedback loop in which CREB3L4 can bind AR and enhance AR gene activation which further increases CREB3L4 expression and activity [12]. Correspondingly, CREB3L4 is expressed higher in PIN and cancer compared to benign prostate. Taken together, these recent discoveries indicate that ERAD may represent a new source for therapeutic targets and clinical biomarkers.

TMPRSS2:ERG is a fusion gene found in ~50% of prostate cancers. Due to the translocation of the AR-regulated portion of the *TMPRSS2* gene, AR activity increases expression of the oncogenic transcription factor *ERG* [13]. While *TMPRSS2:ERG* actions in prostate cancer have been well documented, recent work indicates *TMPRSS2:ERG* could be driving proliferation through co-opting the transcription factors HOXB13 and FOXA1 to activate NOTCH signaling (a known regulator of proliferation) [14]. These data suggest that NOTCH inhibitors may have enhanced efficacy in *TMPRSS2:ERG*-positive patients.

Recently, there has been a boom in the identification of non-coding (nc) RNAs that play a role in prostate cancer signaling. Not surprisingly, several ncRNAs have been shown to 1) be regulated by AR and 2) control proliferation [15]. miR-21, widely considered an oncomir, is upregulated in both HSPC and mCRPC [16]. miR-21 increases proliferation by targeting several genes that are involved in cell cycle maintenance including *SPRY1/2*, *SNARCA4*, *TGFBR2*, *PTEN*, and *HNRPK* [17]. Another miRNA, miR-221, has been shown to be suppressed by AR and is implicated in cell cycle aberrations [15]. A recent study found that two miR-221 targets, *HECTD2* and *RAB1A*, inhibit AR-cyclin signaling. As a result, new cyclins are generated after downregulation of these miR-221 targets [15]. Conversely, miR-135A, an inhibitor of proliferation, was recently found to be a direct target of AR. miR-135A-mediated inhibition of prostate cancer cell proliferation was accomplished through the inhibition of RBAK and MMP11 [18]. Hence, ADT may promote disease progression through downregulating this potential tumor suppressor. PCA3 is an AR-regulated, long ncRNA (lncRNA) [19]. Inhibition of PCA3 leads to a reduction in cell growth and increase in apoptotic cell death [19]. It has recently been shown that this reduction in cell growth is due to its ability to inhibit PRUNE2, a novel tumor suppressor that inhibits proliferation [20]. Due to the prevalence of *PCA3* expression, it has been developed as a biomarker for prostate cancer detection and may have added prognostic

power in combination with TMPRSS2:ERG [21]. PCAT18 is another AR-regulated lncRNA that promotes cell proliferation, invasion and migration [22]. Like PCA3, PCAT18 is also a promising biomarker due to its correlation with disease progression [15]. Collectively, while possessing important functional roles, the most near-term promise of ncRNAs may be in their prognostic power.

AR-regulated proliferation in CRPC: A shift to transcription of M phase genes

In mCRPC, there is a shift in transcriptional programming that promotes the activation of M phase genes such as *UBE2C*, *CDC20*, *CDKI*, and *ANPC10* [23]. *UBE2C* in particular is a ubiquitin ligase that inactivates a critical M phase checkpoint [23]. AR binding to the *UBE2C* promoter relies on elevated levels of the coregulator FOXA1 and methylation of H3K4. FOXA1 binding and H3K4me2 are also found at the enhancers of *CDC20*, *CDKI*, and *ANPC10*, demonstrating that changes in transcription factor expression and epigenetic regulation can alter the genes activated by AR to drive mCRPC [23]. Further studies have shown that AR splice variants induce the transcription of cell cycle genes. Accordingly, AR-V7 splice variant is associated with expression of *UBE2C* in clinical samples [24]. Together, these data indicate that AR-regulated transcription can evolve as the disease progresses, but AR still promotes progression through the cell cycle (Figure 2).

Cell death and survival

CRISPR knockout of AR in androgen-dependent cells decreases cell growth in part by inducing apoptosis [25]. Classical AR-regulated transcription can promote cancer cell survival, for example by upregulating expression of G3BP2, a protein that can SUMOylate p53, causing it to translocate from the nucleus to the cytoplasm [26,27]. The androgen-regulated gene *NFKBIA*, which encodes the NF κ B pathway inhibitor I κ B α , was also recently demonstrated to promote resistance to apoptosis [28].

In addition to regulating transcription, AR can interact with proteins in the cytoplasm to activate or repress cell death and survival pathways. In this regard, AR can protect prostate cancer cells from apoptosis through various signaling pathways modulating the BCL-2 family of anti-apoptotic proteins. BCL-2 is overexpressed in CRPC [29]. Hence, its associated pathways may represent potential therapeutic targets for the treatment of CRPC. Non-transcriptional AR signaling can inhibit apoptosis in prostate cancer via BCL-2 proteins activating pathways including PI3K/AKT and MEK/ERK [30]. Interestingly, AR signaling can also promote apoptosis under certain context. For example, AR can interact with PIRH2 to downregulate the cell cycle inhibitor p21 by degradation of its transcriptional activator, p53 [31]. This sensitizes cells in particular to UV-mediated apoptosis. In this way, AR regulates cell death by activating the PIRH2-p53-p21 pathway. Given that AR has dual roles in cell death, promoting both survival and apoptosis, targeting specific downstream effectors could be more efficacious than broadly targeting AR. Of note, the FDA recently approved the anti-BCL-2 drug venetoclax [32] for the treatment of chronic lymphocytic leukemia (CLL), a disease in which BCL-2 is consistently upregulated. The success of this drug in promoting regulated apoptotic events suggests that BCL-2 inhibitors may also have promise in prostate cancer therapy.

Migration and Invasion

Androgens can promote the migration and invasion of prostate cancer cells and thus may contribute to this biological aspect of metastasis [33]. While the exact mechanisms remain poorly understood, emerging evidence indicate that migration and invasion may result in part from changes in metabolism. Recent work has demonstrated that knockdown of carnitine palmitoyltransferase-1 (CPT1), a rate-limiting step of β -oxidation, was required for prostate cancer cell invasion [34], possibly through the modulation of cell adhesion. Given that androgens increase β -oxidation [35], this suggests that AR promotes invasion through altering lipid metabolism. This is consistent with work demonstrating AR increased prostate cancer cell migration and invasion through directly increasing the expression of *CAMKK2* and subsequent AMP-activated protein kinase (AMPK) signaling [36,37], whose activity can increase β -oxidation [35]. However, like AR's dual roles in cell survival, androgens can also suppress prostate cancer cell migration and invasion through the upregulation of miR-135a [38]. AR-mediated expression of miR-135a inhibits migration and invasion through the downregulation of rho-associated, coiled-coil-containing protein kinase 1 (ROCK1), ROCK2 and matrix metalloproteinase 11 (MMP11) [18,38]. Hence, broadly targeting AR could be suboptimal since inhibition of the receptor may block tumor suppressive processes as well as oncogenic effects. Certainly, additional studies are warranted to determine specifically when AR signaling is promoting versus inhibiting migration and/or invasion and whether targeting the specific AR-regulated pathways that govern these processes could prevent the spread of the disease.

Metabolism

Glucose metabolism

Luminal prostate epithelial cells secrete large amounts of citrate as part of the prostatic fluid. Citrate is generated because of a truncated TCA cycle due to inhibition of aconitase by high zinc levels present in these cells (Figure 3) [39]. As part of the initial transformation to malignancy, zinc uptake drops, causing a de-repression of aconitase. This results in a shift from a truncated TCA cycle to increased oxidative phosphorylation (OXPHOS) [40]. AR can increase glucose uptake through directly and indirectly upregulating the glucose transporters GLUT1 and GLUT12 [37,41,42]. Androgen can further increase flux through glycolysis by the transcription of key enzymes such as hexokinase 2 and 6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (PFKFB2), a rate-limiting step of glycolysis [37,43]. Alternatively, androgens can also promote flux through the pentose phosphate pathway via an mTOR-mediated mechanism [44]. This alternative metabolic route 1) increased the pool of NADPH that can, amongst other anabolic roles, be used to modulate reactive oxygen species (ROS) and 2) lead to the production of nucleotide precursors. The hexosamine biosynthetic pathway (HBP) is another pathway for glucose metabolism that is upregulated by AR in HSPC [45–47]. However, recent work has suggested that this same pathway may paradoxically be downregulated in CRPC [48]. More work is needed to understand why this shift occurs, how prevalent it is, and what are the downstream targets ultimately affected by this change.

Despite the known role of glucose metabolism in prostate cancer, ^{18}F -fluorodeoxyglucose positron emission tomography (FDG-PET) imaging, a marker of glucose uptake commonly used to detect other cancer types, is rarely used in prostate cancer [49]. This is because glucose uptake in most prostate cancers relative to benign tissue is not dramatically increased. Conversely, FDG-PET can be readily used to detect advanced prostate cancer. These imaging observations indicate that any attempts to target glucose metabolism in prostate cancer may be limited to the most aggressive stages of the disease.

Lipid metabolism

One of the hallmarks of prostate cancer is the alteration of lipid metabolism. The synthesis, uptake and storage of fatty acids in prostate cancer have all been shown to be regulated by AR [50]. While it has been reported that prostate cancers can take up fatty acids through increasing fatty acid binding protein levels, an AR-regulated event, prostate cancer appears to largely rely on *de novo* lipogenesis [51]. Lipid synthesis is controlled indirectly via the SREBP cleavage-activating protein (*SCAP*), a direct target of AR and activator of sterol regulatory element binding proteins (SREBPs) [50,52]. SREBPs are master transcriptional regulators of *de novo* lipogenesis, increasing the expression of genes such as *ACLY* (ATP citrate lyase), *ACACA* (acetyl-CoA carboxylase alpha) and *FASN* (fatty acid synthase) [50]. Resulting palmitate can then be used for the storage of lipids (lipid droplets), fatty acid oxidation or the building of cellular membranes (ex. phospholipids). It was shown recently that AR can also upregulate lipid synthesis via nuclear mTOR-mediated rearrangement of the chromatin [42]. Importantly, *de novo* lipid synthesis may determine the sensitivity to chemotherapeutics due the change of membrane composition. To that end, aggressive prostate cancers contain more saturated fatty acids in their membranes, perhaps making them more rigid and resistant to oxidative stress [53]. Because of the prevalence of lipogenesis in prostate cancer, several groups have targeted this process in preclinical models of prostate cancer (reviewed in [54]). Recently, triclosan, an anti-microbial compound found in toothpaste and other household items, was shown to impair prostate cancer growth and had less toxicity compared to previously used inhibitors of lipogenesis such as C75 and orlistat [55].

Paradoxically, androgens also promote fatty acid oxidation [34,35]. One study found that knockout of CPT1 resulted in decreased migration and growth [34]. Our group demonstrated that androgens could promote fatty acid oxidation through increasing mitochondrial biogenesis in an AMPK-dependent manner [35]. Taken together, AR fuels both lipid accumulation and the catabolism of fats to augment prostate cancer progression. AR-mediated lipid metabolism likely promotes cancer through a variety of processes (ex. membrane synthesis, ATP generation, lipid signaling, steroidogenesis, etc). As such, targeting lipid metabolism for the treatment of prostate cancer is an exciting concept as it could affect multiple oncogenic processes and thus exhibit robust efficacy. A major issue that needs to be addressed is whether such an approach would have severe toxicity on other tissues/organs that rely heavily on lipid metabolism (ex. heart).

Amino acid metabolism/protein synthesis

AR increases protein synthesis via mTOR [9]. Additionally, androgens increase the uptake of amino acids such as glutamine by upregulating the expression of their transporters (ex. SLC1A5/ASCT2) [56–58]. Glutamine can then function as a nitrogen and carbon source for the cell and serves to replenish intermediates of the TCA cycle, a process called anaplerosis. Interestingly, AR can also serve to provide building blocks to the cell by augmenting the breakdown of proteins and recycling of amino acids via autophagy [59,60]. In a 2017 study, four autophagy genes (*ATG4B*, *ATG4D*, *ULK1* and *ULK2*) and *TFEB*, a master regulator of lysosomal biogenesis and function, were shown to be upregulated by AR in prostate cancer and required for androgen-mediated proliferation [59]. Clinically, the importance of amino acid metabolism in prostate cancer has been supported by the recent (May 2016) FDA approval of fluciclovine for the detection of prostate cancer recurrence. Fluciclovine is a PET imaging agent that is an amino acid analog reported to be taken up by SLC1A5. Therapeutically, preclinical studies suggest that SLC1A5 may also represent a promising drug target in prostate cancer [56,57,61]. To that end, inhibitors of SLC1A5 have recently been developed [62]. While single agent mTOR inhibitor approaches have not yet worked for the treatment of prostate cancer, there are considerable ongoing efforts to test the effectiveness of mTOR inhibitors in combination with other therapies (summarized in [63]). In addition, inhibitors of glutaminase, the rate-limiting step of glutamine metabolism, have now entered early phase clinical trials (NCT02071927, NCT02944435, NCT02071888, NCT02861300, NCT02771626, NCT02071862). Finally, several clinical trials have tested the efficacy of hydroxychloroquine, a nonspecific inhibitor of autophagy, in prostate cancer (NCT01828476, NCT00786682, NCT02421575). To date, none of these trials have demonstrated a clinical benefit of this approach. This may be because it is difficult to achieve the levels of hydroxychloroquine needed to block autophagy in the tumor prior to the onset of toxicity (reviewed in [64]). Whether this problem can be overcome with more selective inhibitors of autophagy, an ongoing effort in the field, better patient selection or different combination therapies remains to be determined.

Polyamine synthesis

Prostate cells secrete polyamines together with citrate as part of the prostate fluid. The upregulation of polyamine synthesis correlates with increased proliferation in different cancer types [65]. Polyamines can accumulate in the cell either by the uptake of putrescine, spermidine or spermine through the diet or *de novo* synthesis [65]. One key step in polyamine biogenesis is the conversion of ornithine to putrescine, catalyzed by the enzyme ornithine decarboxylase (ODC), which was shown to be directly regulated by AR [66]. Recently, it was reported that overexpression of *ODC* was sufficient to transform normal prostate epithelial cells to malignant cells *in vivo* [67]. In the same report, a crosstalk between ODC and AR was reported. The knockdown of ODC *in vitro* led to a reduction of AR at the RNA and protein levels and a subsequent decrease in AR-mediated transcription [67]. Inhibition of polyamine uptake using the compound OR1202 (N¹-spermine-L-lysine amide), as well as the inhibition of ODC by α -difluoromethylornithine (DFMO), can inhibit the growth of prostate cancer cells in cell culture and human prostate cancer growth in xenograft mouse models [68]. Importantly, a recent chemoprevention study targeting ODC concluded promising results with the potential to prevent prostate cancer progression [69].

DNA repair

The DNA damage response (DDR) involves the joint effect of several sophisticated mechanisms including base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR) and classical non-homologous end-joining (NHEJ) to maintain genome integrity [70]. Growing evidence indicates that genome instability and alterations in the DDR play a critical role in the progression of prostate cancer. A recent Stand Up To Cancer study revealed that ~90% of mCRPC patients harbored clinically actionable molecular alterations and 23% of them harbored DNA repair pathway aberrations [71]. Moreover, mutations in some important DDR genes, including *ATM*, *XRCC1* and *CHEK1* correlated with an increased prostate cancer risk [72]. Importantly, some of these findings have begun to translate to clinical success by establishing a synthetic lethality strategy in advanced prostate cancer by exploiting Poly [ADP-ribose] polymerase 1 (PARP1) inhibitors when *BRCA1/2* or *ATM* aberrations are present [73]. In a landmark study, a phase 2 trial reported that mCRPC patients with DDR defects including *BRCA2* loss or *ATM* aberration responded well (88%) to the PARP1 inhibitor olaparib [74]. While these studies highlight the potential for targeting DDR with a precision medicine approach, the complete list of actionable DDR alterations has not been fully defined.

Besides canonical DDR signaling, emerging evidence support the concept that cross-talk between AR and DDR signaling is critical for DDR-linked prostate cancer development and therapeutic resistance. Many DDR components such as human G2 checkpoint control protein (hRad9), MAGE family member A11 (MAGE-11), breast cancer 1/2 (*BRCA1/2*), PARP1, and mediator of DNA damage checkpoint protein 1 (*MDC1*) function as AR coactivators to alter AR transcriptional activity. The detailed mechanisms have been previously discussed [70,72,75]. In addition, AR signaling augments the expression and activity of important factors involved in DDR. Transcriptome analysis of mCRPC has uncovered a network of AR-regulated DNA repair genes that play key roles in DNA damage-sensing (*ATR*, *NBN*, *MRE11*), non-homologous end joining (NHEJ) (*XRCC4*, *XRCC5*), homologous recombination (HR) (*RAD54B*, *RAD51C*), DNA mismatch repair (MMR) (*MSH2*, *MSH6*), base excision repair (BER) (*LIG3*, *PARP1*) and the Fanconi pathway (*FANCI*, *FANCC*, *USP1*) [76]. Furthermore, a DNA damage-gene expression profile combined with the AR chromatin-binding data suggested three DDR genes, *XRCC2*, *XRCC3* and *PRKDC* (gene encoding DNA-PKcs) harbored AR-occupied regions and were regulated by androgen after radiation [77]. Importantly, DNA-PK (DNA-dependent protein kinase), composed of the Ku70-Ku80 heterodimer and DNA-PKcs, acts as a crucial connection between AR signaling and DSB repair. DNA-PK is highly activated in advanced prostate cancer and its levels track tightly with tumor metastases, recurrence, and poor prognosis [78]. Interestingly, DNA-PK has been demonstrated to act as a part of the AR transcriptional machinery, stimulating AR gene transcription. The binding between Ku70 and AR has been described in prostate cancer tissues [79]. Here, castration resulted in a decrease of Ku70 which correlated with PSA decreases and γ H2AX foci formation. In addition, AR was observed to directly increase DNA-PKcs expression by binding to the *PRKDC* locus and promote its activity [77]. This indicated the existence of a positive

feedback loop between AR and DNA-PK and provided one possible mechanistic basis for clinical observations that ADT therapy boosts the response to radiotherapy. Recently, *CDC6*, a regulator of DDR, has been identified as a novel AR target gene [80]. The expression of *CDC6* was upregulated during prostate cancer progression and strongly correlated with AR in patient samples. *AR* or *CDC6* knockdown, combined with the Chk1/2 inhibitor AZD7762, decreased TopBP1-ATR-Chk1 signaling and increased ATM phosphorylation, leading to synergistic DNA damage and apoptosis in advanced prostate cancer cells. This finding suggested the promising therapeutic strategy to target DDR through a combination of AR signaling inhibitors and DDR-targeted agents in mCRPC. Of interest, this same group provided another potential treatment strategy using ADT with a PARP1 inhibitor [81]. In this study, they found enzalutamide decreased some HR-associated genes (*BRCA1*, *RAD54L* and *RMI2*), which led to the development of BRCA- and HR-deficient cells. These cells were analogous to the BRCA deleted/mutated state and, importantly, more sensitive to PARP1 inhibition. This treatment strategy is of particular interest because it expands the application of PARP1 inhibitors to patients without BRCA mutations [81].

Angiogenesis

Matrix metalloproteases (MMPs), vascular endothelial growth factor (VEGF), transforming growth factor β (TGF β), and cyclooxygenase 2 (COX-2) are known promoters of angiogenesis, but VEGF is the only angiogenic factor shown to be under the regulation of AR [82]. Angiogenesis is required for growth of tumors beyond 1–3 mm³ in volume which indicates that it plays a critical role in tumorigenesis [83]. It is not precisely known how AR regulates VEGF expression, but it is hypothesized that it could be by tethering to known AR coregulators (HIF1 α and the zinc finger transcription factors Sp1, Egr1, and WT1 [83]. This is evidenced by the presence of Sp1/Egr1 binding sites just upstream of the *VEGFA* gene [83]. To date, angiogenesis inhibitors have not been demonstrated to be efficacious for the treatment of prostate cancer [84,85]. While they are initially effective, tumors quickly develop resistance. This could be due to the fact that administration of these drugs is long term, allowing tumors to gradually adapt [84,85]. Also, surrounding vessels are not affected, so tumors can grow back rapidly once they develop resistance [84,85]. Given these setbacks, future studies will have to address why previous efforts failed in the clinic [86]. In this regard, angiogenesis inhibitors will likely need to be combined with other treatments to provide sustained efficacy.

Conclusions

Androgens control many physiological cellular processes in the prostate and, as highlighted in this review, pathological processes in prostate cancer (Table 1). So while important for the development and upkeep of a healthy prostate, AR also represents a key driver of prostate cancer at multiple stages of the disease. Unfortunately, while current AR-targeted therapies are effective in slowing disease progression, they invariably fail within a few years. Despite the failure of existing AR-targeted drugs, AR activity often continues to drive disease progression. This reactivation of AR signaling occurs through a variety of mechanisms including, but not limited to, *AR* gene amplifications, *AR* splicing, intratumoral androgen synthesis, shifts in AR co-regulators, AR ligand-binding domain mutations and potentially

signal transduction pathways that increase ligand-independent AR activity. Interestingly, other nuclear receptors (NRs) such as the related glucocorticoid receptor (GR) have been demonstrated to essentially substitute for AR in CRPC by regulating many of the same pathways [87–93]. Collectively, these data indicate that prostate cancer cells will use a variety of mechanisms to maintain certain signaling events and downstream biological processes that were initially under the control of wild-type AR. To circumvent these existing hormone-mediated resistance mechanisms, we propose that the identification and targeting of the signaling events downstream of the receptor that functionally drive the disease offer an alternative therapeutic approach. In doing so, we can bypass the known reactivation of NR activity. Further, by parsing out the true drivers of the disease versus bystanders that were, for example, androgen-mediated processes involved only in physiological prostate development and homeostasis, we may be able to develop new treatments that avoid some of the side effects of ADT. In addition, identification of these downstream pathways could provide more faithful biomarkers of meaningful AR activity in prostate cancer. Finally, we postulate that many hormone-mediated signaling processes may be regulated by additional, oncogenic pathways at different points throughout the disease progression. Thus, targeting these downstream processes may have the added benefit of blocking a common conduit required for multiple signals to promote cancer. In this regard, blocking such a shared target may help to limit resistance. Taken together, we encourage future research to look beyond the receptor in their search for the next innovative treatment.

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Highlights

- Androgens promote prostate cancer by regulating diverse cellular processes.
- Pathways downstream of AR represent alternative therapeutic targets.
- AR-regulated transcription can evolve as the disease progresses.

Figure 1. The androgen receptor (AR) regulates a broad range of cellular functions

AR regulates a complex network of cellular functions that include proliferation, metabolism, DNA repair, angiogenesis, cell death/survival and metastasis. **AR** androgen receptor; **ERAD** endoplasmic reticulum-associated degradation; **HR** homologous recombination; **NHEJ** non-homologous end-joining; **MMR** mismatch repair; **BER** base excision repair;

Figure 2. Transcriptional reprogramming by the androgen receptor causes a shift from S phase in HSPC to M phase gene expression in mCRPC

Distinct transcriptional programming of M phase genes in mCRPC is facilitated by the binding of the transcription factor FOXA1 and histone H3K4 methylation. This causes the AR transcriptome to potentially evolve during disease progression. **AR** androgen receptor; **HSPC** hormone-sensitive prostate cancer; **mCRPC** metastatic castration-resistant prostate cancer

Figure 3. AR promotes diverse metabolic processes that regulate carbohydrate, lipid, amino acid and polyamine levels

Several metabolic pathways are influenced by AR, leading to a metabolic switch observed in prostate cancer. AR can increase the uptake of glucose and glutamine. Further, AR can modulate the subsequent metabolism of nutrients. For example, AR regulates glucose flux through glycolysis, the TCA cycle, the pentose phosphate pathway and the hexosamine biosynthetic pathway. AR also increases *de novo* lipogenesis (through controlling expression of SCAP (SREBP cleavage protein)), fatty acid uptake and lipid storage. Elevated polyamines have also been demonstrated to promote cancer. Accordingly, AR directly regulates ornithine decarboxylase (ODC), a key step in polyamine synthesis. **AR** androgen receptor; **ACLY** ATP citrate lyase; **ACACA** acetyl-CoA carboxylase alpha; **FASN** fatty acid synthase; **ODC** ornithine decarboxylase; **TCA** tricarboxylic acid cycle

Table 1

Recently described AR downstream targets in prostate cancer.

<i>Gene(s) or Protein(s)</i>	<i>Function(s)</i>	<i>AR regulation</i>	<i>References</i>
Proliferation			
<i>CLDN8</i>	Regulates cytoskeletal rearrangements at tight junctions and cytoplasm. Activation of Akt and ERK signaling	Direct	[10]
<i>CREB3L4 (and other ERAD genes)</i>	Involved in endoplasmic reticulum-associated degradation (ERAD). CREB3L4 also promotes transcription of AR target genes in a positive feedback loop	Direct and indirect	[11,12]
<i>NOTCH</i>	Transmembrane receptor that promotes proliferative signaling	Indirect	[14]
<i>M Phase genes (UBE2C, CDC20, CDKI, ANPC10)</i>	Promote advancement of the cell cycle through M phase	Direct	[23]
Cell death and survival			
p53	Tumor suppressor	Indirect	[26,27]
<i>NFKBIA</i>	Inhibitor of NFκB	Direct	[28]
<i>BCL-2</i>	Pro-survival	Direct and indirect	[29]
<i>PIRH2</i>	Regulator of p53 levels	Protein interaction	[31]
Migration and Invasion			
<i>CAMKK2</i>	Serine/threonine kinase, major upstream kinase of AMPK in the prostate	Direct	[36,37]
miR-135a	Decreases migration and invasion through downregulation ROCK1 and ROCK2	Direct	[38]
Metabolism			
<i>SLC2A1</i>	Glucose transporters	Direct	[37]
<i>SLC2A12</i>	Glucose transporters	Indirect	[41]
<i>PFKFB</i>	rate-limiting step of glycolysis	Direct and indirect	[37,43]
<i>ODC</i>	polyamine biosynthesis, conversion of ornithine to putrescine	Direct	[66]
<i>CAMKK2</i>	Serine/threonine kinase, major upstream kinase of AMPK in the prostate	Direct	[36,37]
<i>SCAP</i>	Lipid synthesis, activates SREBP-mediated transcription	Direct	[94]
<i>ATG4B, ATG4D, ULK1, ULK2</i>	Autophagy core proteins	Direct and indirect	[59]
<i>TFEB</i>	Master regulator of lysosomal biogenesis and function, autophagy	Direct	[59]
<i>SLC1A4, SLC1A5</i>	Amino acid transporters	Indirect	[56,57]
DNA repair			
<i>DNA-PKcs</i>	Catalytic subunit of DNA-PK complex. Involved in double- strand break repair	Direct	[77]
<i>Ku70</i>	Regulatory subunit of DNA-PK complex. Involved in double-strand break repair	Unknown	[79]
<i>CDC6</i>	Required for the initiation of DNA replication and checkpoint controls	Direct	[80]
<i>XRCC2</i>	Involved in the homologous recombination repair	Direct	[77]
<i>XRCC3</i>	Involved in the homologous recombination repair	Direct	[77]
Angiogenesis			
<i>VEGFA</i>	Promotes vascularization	Direct	[83]