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Review Article

Looking beyond Androgen Receptor Signaling in the Treatment of Advanced Prostate Cancer

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This review will provide a description of recent efforts in our laboratory contributing to a general goal of identifying critical determinants of prostate cancer growth in both androgen-dependent and -independent contexts. Important outcomes to date have indicated that the sustained activation of AR transcriptional activity in castration-resistant prostate cancer (CRPC) cells results in a gene expression profile separate from the androgen-responsive profile of androgen-dependent prostate cancer (ADPC) cells. Contributing to this reprogramming is enhanced FoxA1 recruitment of AR to G2/M phase target gene loci and the enhanced chromatin looping of CRPC-specific gene regulatory elements facilitated by PI3K/Akt-phosphorylated MED1. We have also observed a role for FoxA1 beyond AR signaling in driving G1/S phase cell cycle progression that relies on interactions with novel collaborators MYBL2 and CREB1. Finally, we describe an in-depth mechanism of GATA2-mediated androgen-responsive gene expression in both ADPC and CRPC cells. Altogether these efforts provide evidence to support the development of novel prostate cancer therapeutics that address downstream targets of AR activity as well as AR-independent drivers of disease-relevant transcription programs.

1. Introduction

The androgen receptor (AR), a member of the steroid receptor superfamily [1], is a classic example of a ligand-inducible transcription factor whose activity is tightly linked to numerous physiological processes and disease states. Within the various mammalian tissues expressing AR, its essential role in organ development and function has been demonstrated, which ranges from contributions to spermatogenesis, sexual behavior, and skeletal maintenance [2]. Mechanistically, induction of AR activity relies upon binding to male hormones, androgens (e.g., testosterone or the more potent 5α-dihydrotestosterone [DHT]), resulting in release of AR from stabilizing interactions with cytoplasmic heat shock proteins (HSPs) [3]. Homodimerization, activation by posttranslational modification, and nuclear translocation of ligand-bound AR is then essential for its established role in determining tissue-, cell type-, and disease stage-specific gene expression patterns [3]. Within the nucleus, AR binds genomic regions enriched with its cognate DNA-binding motif, or androgen response element (ARE), consisting classically of the 15-base pair sequence 5′-AGAACAnnnTGTTCT-3′ and providing a degree of specificity to global AR distribution within regulatory elements of androgen responsive genes [4]. These regulatory regions further serve as a substrate for the assembly of AR coactivator and transcription complexes that initiate gene expression.

Advances in the understanding of this AR activity have had no greater impact than in the human prostate cancer field. Development and normal function of the prostate secretory epithelium are understood to rely on hormone-inducible gene expression mediated by AR [2, 5], and knowledge of the subsequent dependence on androgens for the malignant proliferation of prostate adenocarcinoma dates back to the Nobel Prize-winning insights of Dr. Charles Huggins, who demonstrated that surgical castration or application of endogenous estrogens resulted in decreased cellular proliferation in benign prostatic hyperplasia (BPH) and regression of local and metastatic prostate cancer [6–9]. Leveraging the sensitivity of prostate cancer to castrate levels

of androgens, therapeutics targeting testicular and adrenal androgen synthesis (luteinizing hormone-releasing hormone agonists/antagonists [e.g., leuprorelide and cetrorelix, respectively] and steroidogenic cytochrome P450 [CYP] enzyme inhibitors [e.g., ketoconazole]) would later underlie the medical treatment of advanced disease [10, 11]. Discovery and characterization of AR [12–14] also allowed for the development of nonsteroidal antiandrogens (e.g., flutamide) that could compete with DHT for AR binding, thus inhibiting its transcriptional activity [15].

Today, combination therapy involving androgen ablation and the application of antiandrogens is the mainstay of treatment for metastatic disease and is initially effective against androgen-dependent prostate cancer (ADPC) cases naïve to hormone therapy [16–18]. Despite the early response, the heterogeneous nature of prostate cancer cell populations, characterized by both androgen-dependent and -independent sub-populations, provides an initial rationale for the nearly inevitable progression of the disease to a treatment-insensitive, castration-resistant prostate cancer (CRPC) phenotype. However, recent insights suggest that it is overly simplistic to regard such lethal cases as entirely independent of the activities of AR. As previously reviewed [19–21], a number of mechanisms have been explored that support reestablished AR activity as a prominent driver of CRPC growth even in the presence of the modern, more potent second generation antiandrogen (enzalutamide), and CYP inhibitor (abiraterone) [22–28]. Briefly, through amplification, overexpression, or perhaps stabilization of AR, cancer cells may exhibit enhanced sensitivity to low levels of circulating androgens [29, 30]. Mutations within the AR ligand binding domain (LBD) or alternative splicing that excludes the LBD altogether can allow cells to utilize antiandrogens as ligands or deviate completely from the need for ligand induction for AR transactivation, respectively [31–36]. Alternately, intratumoral androgen synthesis, via upregulation of steroidogenic enzymes [37–39] or aberrant activation of AR by upstream kinases responding to growth factor stimulation, such as epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF1), may stimulate AR activity following androgen ablation [40, 41]. Finally, and most relevant to the current review, overexpression or activation of AR coactivators/collaborating transcription factors and lost activity of AR corepressors may contribute to the redistribution of AR, promoting a disease-stage specific transcription profile, or to the activation of AR by antiandrogens [42–44].

It has been demonstrated that the selective pressure applied by the therapeutic strategies described above can result in the outgrowth of treatment-insensitive cancer cell populations utilizing the aforementioned resistance mechanisms [45]. Still other CRPC cells lose expression of AR and are thus entirely refractory to compounds targeting the androgen signaling axis. Our current inability to address the molecular determinants of CRPC growth contributes to the role of prostate cancer as the second leading cause of cancer-related deaths in American men and encourages continued investigation into the role of AR as well as additional factors throughout disease progression [46]. The primary focus of our recent investigations, outlined in this review, has been to evaluate alterations to AR-mediated gene expression profiles in the progression from ADPC to CRPC. The elucidation of factors contributing to disease stage-specific redistribution of AR binding has been central to this aim. Emerging from the earliest efforts of our lab, this review will highlight the central importance of the pioneer transcription factors GATA2 and FoxA1 in models of early and late stage disease by describing the manner in which FoxA1 directs CRPC-specific AR binding and drives androgen-independent cell cycle progression and the in-depth mechanism by which GATA2 contributes to androgen/AR-mediated gene expression in both ADPC and CRPC. Finally, in addition to highlighting how the coordinated activities of these factors with the Mediator coregulatory complex subunit MED1 has been shown to facilitate chromatin loop formation/maintenance allowing for enhancer-driven expression of AR target genes, this review will discuss our work in revealing the relative importance of MED1 in various cell line models of prostate cancer.

2. Distinct Transcriptional Outcomes of Sustained AR Activity in CRPC

A prominent mechanism by which androgen/AR contributes to ADPC growth and proliferation is through regulation of G1/S-phase cell cycle transit. Reviewed previously, androgen starvation of prostate cancer cells results in G1 arrest as expression levels of cyclin D1 (CCND1) and p21 are diminished, active cyclin/cyclin-dependent kinase (CDK) complex formation is inhibited, and retinoblastoma (RB) tumor suppressor activity is enhanced [47]. We demonstrated in an early study from our lab that AR supports the androgen-independent progression of CRPC cells through G2/M phase of the cell cycle as a primary means of enhancing CRPC proliferation in the absence of hormone [48]. We characterized the gene expression profiles of the ADPC cell line model LNCaP and its derivative CRPC model, abl, over a time course of androgen stimulation and after silencing of AR to find that the basal AR-mediated gene expression profile of CRPC cells differs remarkably from the hormone-stimulated gene expression profile of ADPC cells. This result suggested that continued AR function in CRPC is directed towards the definition of a unique gene expression profile. In fact, ADPC-specific DHT-upregulated genes were enriched within the biological process, "cellular lipid metabolism," while CRPC-specific basal AR-upregulated genes were enriched in "mitotic cell cycle." A more recent comprehensive characterization of a core set of androgen-stimulated, direct AR target genes also revealed metabolic pathways under the control of this signaling axis in two separate cell line models of ADPC: LNCaP (characterized by AR LBD mutation) and VCaP (characterized by AR overexpression) [49]. That metabolic process deregulation, intuitively required to support rapid proliferation of cancer cell populations, appears in independent studies to be the result of AR activity across distinct molecular subtypes of ADPC suggests a therapeutic focus on these downstream products of androgen signaling may be broadly applicable to the initial treatment of prostate cancer.

AR chromatin immunoprecipitation combined with tiled oligonucleotide microarray (ChIP-on-chip) analysis of

LNCaP and abl cells provided information regarding the genome-wide redistribution of AR binding sites from ADPC to CRPC [48]. Increased enrichment of AR binding within enhancers of cell cycle, and specifically M phase, genes (*CDK1*, *CDC20*, and *UBE2C*) in abl cells indicated a direct mechanism of AR-mediated CRPC cell cycle progression distinct from the androgen-stimulated G1/S phase progression of ADPC cells. It was further demonstrated that epigenetic definition of abl-specific enhancers, marked by histone H3 lysine 4 mono- and dimethylation (H3K4me1/2), provided a cell type-specific chromatin environment amenable to androgen-independent, pioneer factor (FoxA1)-mediated AR recruitment to the *UBE2C* locus. Expression of UBE2C, a ubiquitin-conjugating enzyme involved in mitotic cell cycle progression by promoting the accumulation and activation of the anaphase-promoting complex/cyclosome (APC/C) [50, 51], was finally shown to be strongly correlated with clinical cases of CRPC versus ADPC and necessary for androgen-independent CRPC but not ADPC cell proliferation. A 2012 study further clarified this point by demonstrating that functional-LBD-deficient AR splice variants (e.g., AR-V7), which accumulate in CRPC cells following enzalutamide treatment, promote a gene expression profile that includes *UBE2C* and is largely unique from the full-length AR-mediated gene profile [52]. Importantly, in CRPC cells treated with enzalutamide and in CRPC xenografts treated with abiraterone, expression of AR splice variants, but not full-length AR, was correlated with UBE2C expression, and this relationship was further reflected in the analysis of AR, AR-V7, and UBE2C expression in CRPC tissue microarrays.

In light of the high prevalence of prostate cancer progression following therapy targeting the androgen/AR signaling axis and the overwhelming evidence supporting the sustained, disease-driving activity of AR in the presence of such treatments, it is desirable to continue to identify direct targets of this transcription factor that contribute to AR-mediated cancer cell proliferation. Our study suggests that these targets may be disease state specific and encourages further characterization of unique AR-transcriptional targets across a broad spectrum of prostate cancer severity, which may offer opportunities to address unique drivers of late stage disease. A recent investigation from the Brown lab, describing a role of enhancer of zeste homolog 2 (EZH2) as a coactivator of AR-driven gene expression in CRPC cells, revealed that enhancer cooccupancy of AR and EZH2 facilitates the expression of DNA-repair proteins KIAA0101 and RAD51C [53, 54]. As FoxA1 pioneer factor activity was found to support AR-driven G2/M phase gene expression, these combined results indicate that interactions with specific factors may allow AR to drive the expression of genes within nonoverlapping biological processes, adding complexity to its role in supporting CRPC growth. In light of this finding and the recent analysis of AR genomic distribution in CRPC clinical samples, the results of which suggest that CRPC-specific AR binding sites may occur alongside context-specific collaborating transcription factors (e.g., MYC, E2F, and STATs) [55], targeting CRPC-specific AR collaborators/coactivators could prove a viable means of inhibiting significant portions of AR-mediated transcription profiles in treatment-resistant contexts.

We have explored the therapeutic approach of targeting downstream products of AR transcriptional activation that contribute specifically to the growth of CRPC by investigating the use of cell-cycle inhibitor 779 (CCI-779), targeting mTOR activity [56]. We found that by downregulating the expressions of CCND1 and UBE2C, CCI-779 treatment resulted in G1 and G2/M phase accumulation, respectively, of two distinct CRPC cells lines (abl and C4-2B), as well as significantly reducing CRPC cell proliferation. These effects were reproducible in an abl xenograft model, where CCI-779 treatment resulted in a 75% reduction in average tumor volume relative to control and dramatically reduced UBE2C and CCND1 protein expression compared to control tumor tissue. Importantly, as UBE2C expression has been correlated with metastasis in nonprostatic cancers [57–59], CCI-779 treatment or UBE2C knockdown were both found to inhibit CRPC cell invasion potential. Additionally, CCI-779 treatment was able to inhibit the enhanced invasiveness of CRPC cells transiently overexpressing UBE2C. Mechanistically, decreased UBE2C expression was determined to be the result of UBE2C mRNA destabilization and failed coactivator/transcription complex assembly at *UBE2C* enhancers (involving SRC1/3, p300, MED1 [discussed below], and Pol II), though AR binding was unaffected.

Other lines of evidence suggest that a therapeutic focus on mTOR signaling may be effective in inhibiting AR target-mediated prostate cancer cell proliferation before and after the development of therapeutic resistance. A 2011 study found that expression of the L-type amino acid transporter LAT3 is controlled directly by AR in the presence of androgen while LAT1 expression, controlled by ATF4, was induced upon androgen deprivation [60]. Concordantly, LAT3 overexpression was observed in clinical samples of ADPC compared to benign tissue and samples of CRPC, while LAT1 expression correlated with progression to CRPC. Both transporters maintain intracellular amino acid homeostasis required for mTORC1 activity [61]. A subsequent investigation from the same group found that silencing of LAT family members or inhibiting their activity with the leucine analog BCH resulted in lost expression of not only *UBE2C* but also *CDK1* and *CDC20 in vitro* and in xenograft models of CRPC [62]. Thus, addressing critical downstream targets of AR activity may be accomplished via therapeutic strategies focused beyond the canonical androgen/AR signaling axis resulting in potent cell cycle inhibition.

3. FoxA1: Master Cell Cycle Regulator

Considerable interest in collaborating factors that contribute to AR transcriptional activity has evolved from analyses of genome-wide AR binding sites in prostate cancer cells, which revealed a number of cooccurring DNA motifs within AR-occupied regions. Chief among the activities that recognize these motifs are the pioneer factors, FoxA1, and GATA2 (discussed below), whose role in determining nuclear receptor (NR, e.g., AR and estrogen receptor [ER]) binding patterns has long been appreciated, as reviewed previously [63]. In short, pioneer factors are understood to engage regions of compact chromatin, facilitating their decondensation in

advance of ligand-stimulated NR binding. FoxA1 accomplishes this by displacing the linker histone H1, owing to the structural similarity of its DNA-binding domain to H1 [64–66]. As briefly mentioned above, enrichment of the epigenetic marks H3K4me1/2, known to partially determine FoxA1 chromatin occupancy [67], within CRPC cell-specific *UBE2C* enhancers facilitates androgen-independent pioneer factor binding within these gene regulatory elements. The net result is androgen-independent, FoxA1-mediated AR binding at the *UBE2C* locus and enhanced expression of this clinically relevant oncogene, suggesting that an important role of FoxA1 in CRPC is the reprogramming of AR activity to drive G2/M transit, rather than G1/S cell cycle progression as in ADPC [48].

In a subsequent study, our lab further investigated the observation that, in contrast to FoxA1 silencing in G2/M phase-synchronized CRPC cells resulting in G2/M accumulation, unsynchronized cells exhibited significant G1 arrest following FoxA1 knockdown, suggesting that this pioneer factor may have functional significance throughout the cell cycle [68]. mRNA and protein expression analysis of several G1 phase genes identified *CCNE2* and *CCNA2* as being robustly overexpressed in CRPC compared to ADPC cells in an androgen-independent yet FoxA1-dependent manner. Silencing of *CCNE2* and *CCNA2* significantly decreased CRPC cell proliferation in the absence of androgen, and, remarkably, overexpression of either factor in LNCaP cells enhanced their sensitivity and ability to grow in the presence of subphysiological androgen concentrations.

As in our AR study [48], FoxA1 ChIP-on-chip analyses in LNCaP and abl cells were utilized to provide a picture of genome-wide FoxA1 binding patterns and evidence of its transcriptional control over these G1 phase genes. In general, CRPC cell-specific FoxA1 binding was observed within the vicinity of genes differentially regulated (upregulated and, to a lesser extent, downregulated) in clinical samples of CRPC versus ADPC [69–71]. Increased FoxA1 binding in abl cells was observed at enhancer elements within the *CCNE2* locus, corresponding to enhanced coactivator/transcription complex assembly (Pol II, CREB binding protein [CBP], and MED1) and chromatin accessibility in CRPC cells, suggesting direct regulation of *CCNE2* by FoxA1. On the other hand, our work determined that *CCNA2* upregulation was the result of enhanced E2F1 binding to the *CCNA2* promoter subsequent to direct regulation of *E2F1* in CRPC cells by enhanced FoxA1 occupancy of *E2F1* regulatory elements. Importantly, motif analysis of CRPC-specific FoxA1 binding sites revealed a significant cooccurrence with CREB and MYB recognition motifs in this cellular context. We then demonstrated that MYBL2/FoxA1 and CREB1/FoxA1 cooccupancy of *CCNE2* and *E2F1* regulatory elements occurs in a codependent fashion and that silencing of either CREB1 or MYBL2 resulted in reduced enhancer chromatin accessibility, androgen-independent expression of *CCNE2*, *CCNA2*, and *E2F1* in CRPC cells, and CRPC cell proliferation. We finally showed that enhanced CREB1/MYBL2 occupancy of FoxA1-bound *UBE2C* and *CDK1* regulatory elements was necessary for the androgen-independent overexpression of these G2/M phase genes in abl versus LNCaP cells.

Together these results identified CREB1/MYBL2 as potential FoxA1 collaborators facilitating diverse, disease-relevant transcriptional outcomes in a CRPC context. Positive correlation between MYBL2/CREB1 expression and disease progression from ADPC to CRPC has been reported and may provide some basis for the observed redistribution of FoxA1 to regions cooccupied by these factors in a model of late stage disease [72, 73]. CREB1 activity has been the focus of several studies in prostate cancer, and ongoing research should focus on elucidating its global impact on FoxA1 binding patterns and gene expression profiles. Looking back to our AR study, these results also suggest that FoxA1 engages in distinct collaborative relationships as it determines the expression of genes responsible for progression through multiple phases of the cell cycle. Previous studies have shown that AR expression and transactivation potential are significantly reduced in cells immediately following mitosis, providing some rationale for its selective involvement in FoxA1-mediated cell cycle transit during G2/M phase alone [74, 75]. It will therefore be important to comprehensively identify collaborators common to FoxA1 transcriptional complexes throughout the cell cycle (e.g., CREB1 and MYBL2) that may serve as therapeutic targets in the inhibition of this master cell cycle regulator.

4. GATA2 Promotes AR Target Gene Expression

Though the nature of FoxA1 pioneer factor function has been the subject of numerous studies, an in-depth mechanism by which GATA2 contributes to NR chromatin loading remained somewhat elusive prior to a very recent investigation from our lab [76]. Analysis of AR, GATA2, and FoxA1 chromatin immunoprecipitation combined with high-throughput sequencing (ChIP-seq) datasets revealed that approximately 55% of all androgen-stimulated AR binding sites in LNCaP cells overlapped with basal pioneer factor-occupied regions, and so we asked how GATA2 contributes to AR genome-wide binding and androgen-responsive gene expression in both ADPC and CRPC cells. We first characterized two novel GATA2 binding sites in the *AR* gene locus, finding that pioneer factor occupancy at these locations positively regulated the expression of *AR* itself. We next showed that androgen-independent GATA2 and FoxA1 occupancy of AR target gene loci (*ABCC4* and *ADPGK*) occurs in advance of hormone-induced AR binding. AR loading and target gene expression were inhibited by silencing of either pioneer factor, in some cases to levels achieved by silencing of AR. Importantly, we showed that in occupying gene regulatory elements as a prerequisite to AR binding, GATA2 and FoxA1 exhibit site-specific cobinding characteristics (i.e., codependent and independent).

In regions where GATA2 occupancy was independent of FoxA1, silencing of GATA2 but not FoxA1 was sufficient to inhibit androgen-independent recruitment of the histone acetyltransferase (HAT) p300 [77], accumulation of the active epigenetic mark histone H3 lysine 27 acetylation (H3K27ac) [78], and chromatin accessibility. This result provided a second layer to the role of GATA2 as a pioneer factor in activating chromatin within androgen-responsive gene

loci, priming them for AR binding and hormone-inducible expression. The central importance of these two GATA2 activities to global AR-DNA binding was demonstrated in the results of AR ChIP-seq assays performed following GATA2 silencing. Here we showed that nearly 80% of AR binding sites were lost following GATA2 knockdown, and of the 12,529 sites that remained, dramatically reduced ChIP-seq signal intensity was observed in GATA2-silenced conditions. Finally, as it is understood that AR primarily occupies distal enhancer elements within target gene loci, a critical component to AR-mediated gene expression involves chromatin looping from distal regulatory regions to target gene promoters via protein scaffolding facilitated in part by the Mediator coregulatory complex subunit MED1 (discussed in-depth below). We showed that enhanced locus looping between the AR/GATA2/FoxA1-bound *ABCC4* enhancer and promoter follows androgen stimulation and that silencing of either GATA2 or FoxA1 resulted in lost MED1 recruitment and inhibited basal chromatin loop formation. Thus, GATA2 plays a third role in supporting AR activity by preforming functional chromatin loops at androgen-responsive gene loci in advance of AR binding.

These results were largely consistent with previous studies of related GATA family pioneer factors in relation to NR activity and clarifying to previous correlative studies suggesting such roles for GATA2 [79–81]. However, to observe an almost exclusively positive relationship of GATA2 with AR in determining its global binding is in contrast to similar studies of FoxA1 in relation to AR [82, 83] and of GATA3 in relation to ER [84]. In these investigations, large numbers of new AR and ER binding sites were observed following FoxA1 and GATA3 silencing, respectively, while only 131 new, low-affinity AR binding sites were found in our analysis [76]. Due to the unique and highly dependent nature of AR activity on GATA2 function, it is of interest to further characterize the impact of GATA2 inhibition in both treatment sensitive and resistant contexts, as expression of this pioneer factor has been positively correlated with more advanced disease [85], and on global gene expression profiles as a foundation for developing novel GATA2-targeted therapeutics for prostate cancer. The Knudsen group demonstrated the feasibility of such a treatment strategy, using the natural compound curcumin to inhibit accumulation of GATA2/p300/histone acetylation at AR target gene loci in cell line models of ADPC and CRPC, leading to reduced target gene expression, *in vitro* cell proliferation, and xenograft tumorigenesis [79]. This promising approach may offer the added benefit of sparing patients the unwanted side effects of androgen ablation by focusing on a separate determinant of AR activity. As we have demonstrated for FoxA1 [68], the large number of GATA2 binding sites outside AR-bound regions suggests a potential role for GATA2 beyond the androgen/AR signaling axis, and thus additional GATA2 collaborators and downstream targets must be identified and explored.

5. CRPC-Specific MED1 Activation Drives Chromatin Looping

We have demonstrated the importance of chromatin looping between AR-bound enhancers and the promoters of AR target genes in both ADPC and CRPC cells in driving gene expression contributing to disease progression [48, 76]. Up to this point, chromatin loop formation has been discussed in the context of AR-associated coactivator/transcription complexes consisting of pioneer factors, histone modifying enzymes, and the Mediator complex. To address the determinants of locus looping in the absence of AR was of particular interest in light of CRPC heterogeneity characterized by both AR-positive (AR+) and AR-negative (AR−) phenotypes [86, 87]. We therefore investigated the factors contributing to enhanced *UBE2C* expression in the AR− CRPC cell line PC-3 compared to the AR+ LNCaP cell line [88]. In the PC-3 cellular context, *UBE2C* expression was necessary for androgen-independent cell proliferation and G2/M phase transit, similar to previous results in abl cells [48]. We then characterized several cell type-specific *UBE2C* enhancers exhibiting robust looping to the *UBE2C* promoter and stronger occupancy of H3K4me1/2, FoxA1, MED1, MED17 (another Mediator complex subunit) [89], Pol II, and TATA-box binding protein (TBP) in PC-3 versus LNCaP cells. Importantly, silencing of either FoxA1 or MED1 resulted in decreased chromatin loop formation and *UBE2C* expression in PC-3 cells. It was further shown that PI3 K/Akt-mediated MED1 phosphorylation (pMED1) supports *UBE2C* expression and locus looping, as pMED1 ChIP and serial ChIP (reChIP) assays revealed higher pMED1 binding to *UBE2C* regulatory elements in PC-3 versus LNCaP cells and direct interactions between enhancer-associated FoxA1/pMED1 complexes and promoter-associated Pol II/TBP transcription complexes. Expressing phosphomutant MED1 in PC-3 cells resulted in a marked reduction in coactivator/transcription complex assembly at the *UBE2C* locus corresponding to decreased locus looping, *UBE2C* expression, and AR− CRPC cell proliferation. These results were reproducible only in the AR+ CRPC cell line abl but not the AR+ ADPC cell line LNCaP, suggesting that MED1/pMED1 activity is of central importance to CRPC (AR−/+) alone.

The findings identified a potential therapeutic target for CRPC in MED1, a critical determinant of CRPC-specific disease-relevant gene expression. By revealing an important role of PI3 K/Akt in the phosphorylation/activation of MED1, our work also provides a rationale for focusing therapeutic development efforts on this kinase signaling pathway. Phosphatase and tensin homolog (PTEN) loss, resulting in deregulated PI3 K/Akt activity, is a common genetic abnormality of CRPC cases [90, 91], further underscoring the need for future investigations of this treatment strategy. A recent paper from the Fondell group demonstrated that MED1 phosphorylation is mediated by both ERK and Akt, leading to its activation as well as its accumulation [92]. Gene expression analysis of MED1-overexpressing LNCaP cells further revealed that enhanced MED1 activity results not only in mitotic cell cycle progression via expression of *UBE2C*, but also in the enhanced expression of antiapoptotic and proinflammatory gene sets, providing evidence of the broad impact of active MED1 signaling on disease-relevant transcription programs. Thus, targeting of multiple kinase pathways could precipitate regression of advanced prostate cancer, acting partially through inhibition of MED1-mediated gene expression.

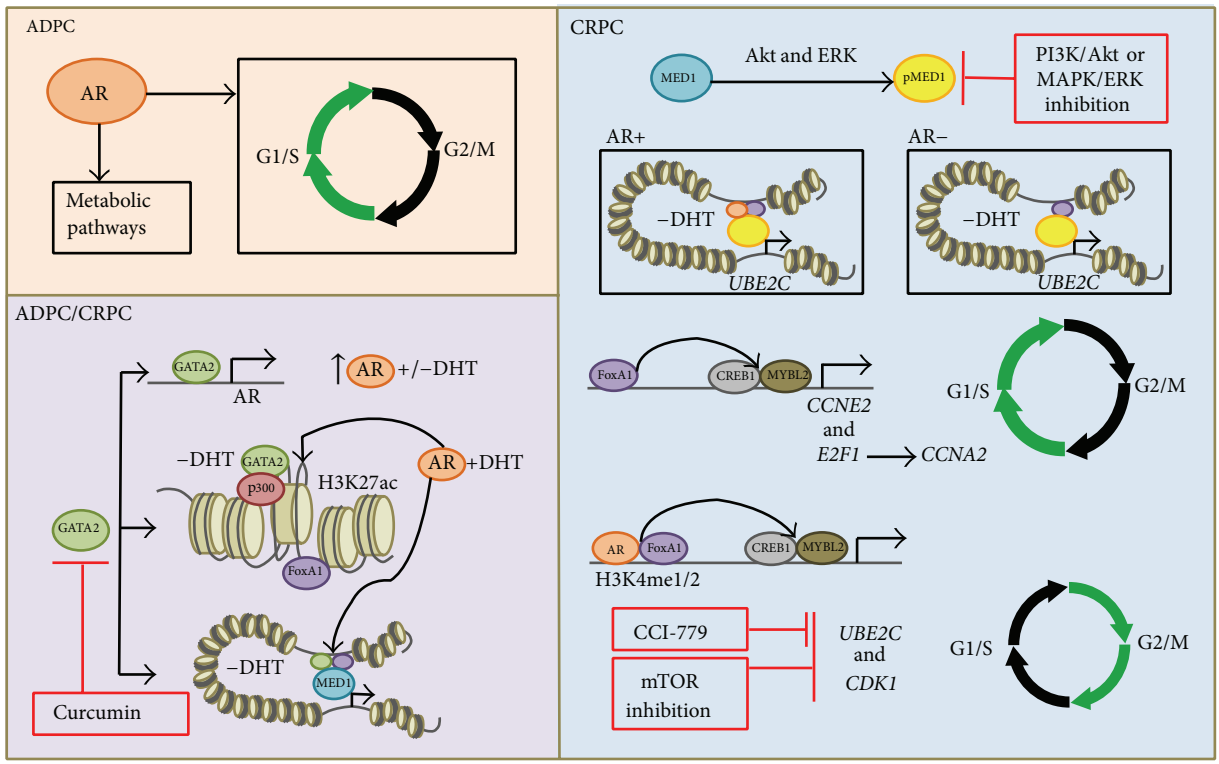

Figure 1: *Summary of relevant work*. In ADPC, androgen-stimulated AR activity drives gene expression profiles related to metabolism and androgen/AR regulates G1/S phase cell cycle progression. In both ADPC and CRPC, the pioneer factor GATA2 contributes to androgen-responsive gene expression via three mechanisms of action: regulating *AR* expression, promoting activation and accessibility of AR target gene regulatory elements, and aiding in the formation/maintenance of basal chromatin loops between AR target genes enhancers and promoters. In CRPC, Akt and ERK activity leads to MED1 phosphorylation/activation resulting in enhanced locus looping and expression of *UBE2C* in AR+ and AR− cell contexts. Finally, FoxA1 serves as a master cell cycle regulator in CRPC by facilitating AR-mediated expression of G2/M phase cell cycle genes and through a non-AR-associated role in driving G1/S phase progression.

6. Conclusions and Outlook

To briefly summarize (Figure 1), the efforts of our lab have contributed to a basic understanding of AR activity, supported by a host collaborating factors and contributing uniquely to various stages of prostate cancer progression [48]. In doing so, we have provided evidence to encourage the continued characterization of AR-mediated gene expression profiles throughout disease states in an effort to identify common as well as disease stage-specific drug targets acting downstream of this transcription factor. We have identified additional activities beyond AR with which the pioneer factor FoxA1 collaborates in determining the expression of both G2/M and G1/S phase cell cycle genes [68], encouraging the search for collaborating factors that may serve as drug targets to inhibit both AR-associated and non-AR-associated FoxA1 function. In determining the most basic means by which GATA2 contributes to AR genome-wide binding in prostate cancer [76], we have revealed a profound dependence of androgen/AR signaling on this pioneer factor, which has been the focus of previous investigations of novel therapeutic strategies in prostate cancer [79]. By providing evidence of the indispensible role of MED1 in both AR+ and AR− CRPC expression of the clinically relevant cell cycle gene *UBE2C* [88], we have introduced this factor as a potential therapeutic target for disparate molecular subtypes of advanced prostate cancer. Finally, our efforts have provided rationale for the development of novel prostate cancer therapeutics that target PI3 K/Akt signaling upstream of MED1 and mTOR signaling linked to both androgen-dependent and -independent AR activity and contributing to the expression of critical cell cycle genes [56].

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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