Androgen receptor interacting proteins in prostate cancer development and therapy resistance

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Running title: AR cofactors in carcinogenesis

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Abstract

Endocrine therapy for prostate cancer is based on the use of drugs that diminish androgen concentration and androgen receptor (AR) signaling inhibitors and is limited by the functional consequences of AR point mutations and increased expression of constitutively active receptors. Many coactivators (> 280) interact with different AR regions. Most studies have determined the expression of coactivators and their effects in the presence of increasing concentrations of androgen or the anti-androgen enzalutamide. The p160 group of coactivators (SRC-1, SRC-2, and SRC-3) is highly expressed in prostate cancer and contributes to ligand-dependent activation of the receptor in models that represent therapy-sensitive and therapy-resistant cell lines. The transcriptional coactivators p300 and CBP are implicated in the regulation of a large number of cellular events, such as proliferation, apoptosis, migration, and invasion. AR coactivators may also predict biochemical and clinical recurrence. The AR coactivator expression, which is enhanced in enzalutamide resistance, includes GREB1 and GATA2. Several coactivators also activate AR-unrelated signaling pathways, such as those of insulin-like growth factors that inhibit apoptosis in cancer cells. They are expressed in multiple models of resistance to therapy and can be targeted by various inhibitors in vitro and in vivo. The role of glucocorticoid receptor in endocrine therapy-resistant prostate cancer has been previously documented. Specific coactivators may interact with glucocorticoid receptor, thus contributing to therapy failure.
Introduction

Medical therapy for non-organ-confined prostate cancer (PCa) is based on inhibition of ligand-induced androgen receptor (AR) activity. Historically, *in vitro* and *in vivo* experiments have been designed to identify compounds that can inhibit androgen activity. They were followed by clinical trials that resulted in the widespread use of the nonsteroidal drugs hydroxyflutamide and bicalutamide in therapy for many years. PCa endocrine therapy is not curative and tumor progression has been studied using multiple models. Current PCa research could benefit from novel cell lines and patient-derived xenografts, resulting in the detection of multiple changes in the AR structure. Mutations in the AR ligand-binding domain may lead to agonistic effects of these drugs, stimulation of tumor cell proliferation, and *in vivo* growth. Previous findings on the role of AR in advanced PCa stimulated the chemical search for novel AR signaling inhibitors such as enzalutamide, apalutamide, or darolutamide, which improved the survival of PCa patients. However, these drugs cannot cure PCa and the mechanisms involved in cancer progression have been investigated. Thus, AR mutations have also been detected in specimens from patients treated with enzalutamide. Other researchers have focused on the appearance of the constitutively active ARs that have been frequently detected in tissues obtained from patients during therapy. Several reviews have discussed in detail the issues related to point mutations and receptors activated in the absence of AR. In this review, we address other aspects of AR action that may be important in the future, particularly in drug development. Future studies should consider the clinical relevance of AR-interacting proteins and their expression in tumor tissues.

Translational research on PCa has focused on AR coactivators, a large group of proteins that interact with receptors. They may influence selective ligand binding since it is known that AR in certain situations is activated by steroids other than testosterone and dihydrotestosterone. More than 280 coactivators have been reported in literature. An important issue related to a particular coactivator is the determination of its relative importance for a specific function in PCa and examination of its expression at different stages of the disease (Figure 1). Some coactivators target a smaller group of genes so bioinformatic analysis can help in their identification. Small-molecule inhibitors can target coactivators and their development is important for improving current therapeutic options.

Previous studies have discussed whether some coactivators exclusively interact with AR. Although it has been suggested that such interactions occur, most studies have analyzed the effects of cofactors that bind to multiple steroid receptors. Importantly, cofactor expression
and function are increasingly being investigated in association with drug resistance. The AR contains a variable N-terminal region and conserved DNA- and ligand-binding domains. In general, coactivators interact with one or more receptor domains and are mentioned in this review according to this interaction but also according to the importance of specific processes relevant to cancer progression.

Relevance of the N-terminal region of the AR for coactivation

The AR transcription activation function is present in the N-terminal region (activation function 1) and ligand-binding domain (activation function-2). The coactivators SRC-1 and -3 and CBP, whose functions are discussed in detail in the manuscript, interact with the N-terminus of AR. Therefore, approaches aimed at inhibiting AR function by blocking the N-terminus may be considered. Ralaniten (EPI-002) is an antagonist of activation function-1. Ralaniten downregulates the transcriptional activity of AR. Interestingly, it blocked the interaction between AR and CBP but not SRC coactivators. Under conditions in which SRC overexpression was achieved, ralaniten remained active. The N-terminal region of the AR consists of a different number of glutamine repeats. Variable length of the polyglutamine region did not affect the inhibition of transcriptional activity by EPI-002. Similarly, mutations in the N-terminal region of the receptor do not compromise the effects of the drug. In contrast to enzalutamide, ralaniten inhibits AR-V7 constitutive activity in enzalutamide-resistant cells. These results were also confirmed in vivo. The growth of enzalutamide-resistant LNCaP95 xenografts expressing AR-V7 is attenuated by this novel drug. Therefore, EPI-002 is a potential compound for use in clinical trials for PCa. The next-generation analog of ralaniten EPI-7170 was applied in combination with enzalutamide in PCa cells positive for AR-V7. This approach yielded a synergistic growth inhibitory effect. A phase I study was conducted with the EPI-506 derivative, which established a safety profile, but was limited by the oral bioavailability of the drug.

The p160 group of coactivators in PCa

The p160 coactivators of the AR, to which SRC-1, SRC-2, and SRC-3 belong (known as NCOA1, NCOA2, and NCOA3, respectively), are functional at different stages of prostate carcinogenesis and interact strongly with the N-terminal region of the AR. These coactivators recruit histone acetyltransferases and methyltransferases to specific promoters and enhancer
regions to facilitate transcription (structure shown in Figure 2). They are involved in the regulation of multiple cellular processes in PCa and act via multiple mechanisms.

SRC-1 is expressed in many steroid-responsive tissues and tumors and is necessary for achieving a full hormonal response. SRC-1 is phosphorylated by mitogen-activated protein kinases (MAPK) to promote receptor activation. Functional SRC-1 is needed for AR activation in LNCaP cells and in their derivative C4-2, which displays high AR expression and represents a more malignant phenotype. SRC-1 coactivator is involved in the progression of PCa. Inhibition of its expression revealed reduced proliferation in two AR-positive cell lines but no effect in PC-3 cells. This coactivator also affects cell migration and invasion. The effect of SRC-1 knockdown could be reversed by the inhibition of protein kinase D1, the expression of which was inversely correlated with that of SRC-1. Protein kinase D1 is repressed by AR. AR coactivators have been investigated in ligand-independent AR activation. Thus, SRC-1 is one of the cofactors required for AR activation by interleukin-6 which occurs through the N-terminal region. SRC-1 overlapping peptides interact specifically with AR and could therefore be used as a therapeutic approach in PCa. These peptides inhibited androgen-dependent PCa cells and their sublines.

In general, AR coactivators can be characterized according to their regulation by androgens and are known to be androgen-induced and androgen-suppressed. SRC-2 is highly expressed after androgen ablation and promotes castration-resistant PCa. In an animal model, SRC-2 has been shown to induce early stage tumors and cancer progression. Its downstream signaling pathways include PI3 kinase/Akt and MAPK. In clinical samples, higher SRC-2 expression has been reported in recurrent tumors. SRC-2 inhibits the SIRT3 promoter. Conversely, SRC-2 depletion enhances SIRT3 expression. SIRT3 is regulated in the mitochondrial matrix and contains a mitochondrial processing peptide. In the same publication, it was revealed that SRC-2 coactivator enhances mitochondrial aconitase (ACO2), which up-regulates mitochondrial citrate and facilitates *de novo* lipogenesis.

SRC-3 belongs to the p160 group of proteins and regulates the steroid receptor activity and cellular proliferation. *In vitro* observations have been confirmed *in vivo* in experiments in which inducible short hairpin RNA expression decreased tumor growth. The anti-apoptotic effects of SRC-3 in PCa could be explained by its interaction with the activating protein-2 and regulation of the components of the insulin-like growth factor-1 pathway. Furthermore, it promotes cellular migration and invasion through regulation of matrix metalloproteinases. SRC-3 may contribute to PCa progression by stimulation of Akt phosphorylation. Inhibition of SRC-3 recruitment in human PCa was achieved by HOXC8. HOXC8 is a homeobox gene
which thus plays an important role in the regulation of AR signaling. Interestingly, HOXC8 may promote tumor cell invasiveness when AR activity is downregulated. More recently, the role of SRC-3 in tumor immunity has been studied.\textsuperscript{20} It was found to be highly expressed in regulatory T cells (Tregs) and B cells. Immune cells depleted of SRC-3 generated anti-tumor immunity. Therapeutic approaches against SRC-1 and SRC-3 have been established by the administration of gossypol to cells representing various cancers including those of the breast and prostate.\textsuperscript{21} Treatment with gossypol affects cancer cells but not normal cells. Taken together, the results in which the inhibition of the activity of coactivators has been reported indicate that their role is also important in a large number of human tumors, not only in those that are dependent on hormonal steroids. For example, SRC-3 downregulation inhibits the growth of pancreatic ductal adenocarcinoma, confirming its role in many oncogenic signaling pathways.\textsuperscript{22}

Consequently, targeted therapies may be developed to inhibit transcriptional coactivators in PCa. SRC-3 has also been implicated in the regulation of neuroendocrine differentiation in PCa.\textsuperscript{23} Neuroendocrine PCa has been the subject of many investigations, particularly because of the absence of appropriate therapies and poor prognosis. The localization of SRC proteins in PCa may be influenced by cholesterol.\textsuperscript{24} These studies provide an additional explanation as to how cholesterol supports androgenic signaling in PCa and nuclear translocation of receptors. Animals with hypercholesterolemia have displayed higher serum androgen concentrations. TRAMP mice with global SRC-3 knockout did not develop neuroendocrine transdifferentiation.

Further studies of major interest in PCa will focus on speckle-type POZ protein (SPOP) and its interaction with SRC-3. Initially, SPOP was described as a protein that mediates transcriptional repression and interacts with components of corepressor complexes. SPOP interacts directly with SRC-3 and promotes ubiquitination and proteolysis in cancer cells. Therefore, wild-type SPOP is considered a tumor suppressor in PCa. SPOP mutations have been observed in approximately 15\% of the PCa cases. The functions of SPOP may be more complex in other cancers. Consequently, the appearance of such mutations may lead to higher expression of SRC-3 and increased proliferation of cancer cells.\textsuperscript{25} SPOP mutations also support the activation of AR signaling and PI3 kinase/mTOR as evidenced in a mouse model of PCa.\textsuperscript{26} Morphologically, these mutations lead to the development of early high-grade prostate intraepithelial neoplasia, most likely in association with alterations in other oncogenes and/or tumor suppressors. By 12 months of age, most animals develop invasive and poorly
differentiated cancer. pS6 and phosphorylated 4eBP1, which are markers of mTOR pathway activation, were highly expressed. SPOP mutant organoids had a higher rate of formation and irregular borders with differences in size. SPOP mutations were not observed in tumors lacking ETS rearrangements. Another molecule downregulated by SPOP in a manner similar to that of SRC-3 is the ERG oncoprotein. As expected, clinically relevant SPOP mutants could not decrease ERG levels, thus potentiating tumor progression. SPOP mutations co-occur with deletions of the chromatin-remodeling protein (CHD)1. Interestingly, prostate tumors with these features respond to abiraterone treatment.28

SPOP mutations lead to the stabilization of the tripartite motif (TRIM)24 protein, which supports cellular proliferation induced by low androgen concentrations.29 The TRIM24 bromodomain and AR-interacting motif are essential for growth enhancement. SPOP-mediated degradation of TRIM24 may be antagonized by TRIM28.30 Therefore, TRIM24 and 28 interaction is important for PCa progression. Coexpression of TRIM24 and 28 in PCa may result in worse clinical outcomes. TRIM33 is an oncogenic coactivator that drives prostate tumor growth by stabilizing AR from Skp2-mediated degradation.31 This coactivator prevents cell cycle arrest and apoptosis.

Multiple functions of p300/CBP and mediator complex transcriptional coactivators in PCa

Considerable efforts have been made to investigate the regulation of AR-mediated cellular events by the coactivators p300 and CBP.32,33 They modulate multiple cellular events such as proliferation, apoptosis, migration, and invasion. The regulation of AR activity by interleukin-6 is dependent on functional p300.34 These coactivators are upregulated by androgen ablation.35,36 P300 binding and acetylation of AR are increasingly observed under conditions in which the tumor suppressor PTEN is deleted.37 AR phosphorylation at serine 81 promotes p300 binding. The effects of p300 and CBP in PCa are not limited to androgen-sensitive cell lines because they enhance the migration and invasion of AR-negative PC-3 cells. Small-molecule inhibitors of p300 and CBP have been used in experimental therapies for several cancers, including PCa (Figure 3).38 Studies in PCa cell lines have been carried out with C646. However, modifications of this compound are necessary to increase its stability. GNE-049 is a small-molecule bromodomain inhibitor that is selective for p300/CBP and has been tested in vitro and in vivo.39 It affects a large number of AR-regulated genes and decreases the growth of cells that are resistant to endocrine therapy. A-485 inhibits the AR program in androgen-sensitive and castration therapy-resistant cells.38 It can selectively target the catalytic activity of histone
acetyltransferases, suggesting a possible innovation in PCa therapy. FT-6876 is a bromodomain inhibitor that causes a decrease in AR target gene expression, which is consistent with tumor growth inhibition. Treatment of castration therapy-resistant PCa with the new p300/CBP inhibitor CCS 1477 affected a considerable number of AR target genes. P300 could be considered a valid target in chemotherapy-resistant PCa. This observation is based on findings in docetaxel-resistant cells, in which the levels of p300 are higher than those in control cells. The inhibition of p300 expression affects colony formation, migration, and invasion. Docetaxel-resistant cells show higher expression of mesenchymal markers, such as N-cadherin and vimentin. AR activity is also regulated by RUNX2. Importantly, the transcription factor SNAI2, which regulates epithelial-to-mesenchymal transition and invasion, is co-regulated by RUNX2 and AR. SNAI2 expression is correlated with RUNX2 expression. Several biological processes, such as immune responses, angiogenesis, and epithelial-to-mesenchymal transition are regulated by transforming growth factor-β. Its intermediary molecule Smad3 promotes the expression and activity of AR. Treatment of enzalutamide-resistant cells with p300/CBP inhibitors resulted in anti-androgen effects and down-regulation of ribosomal proteins. High levels of ribosomal proteins, as a consequence of gene amplification, were observed in cells in which c-Myc was amplified.

In addition to p300, other coactivators may also be involved in the regulation of non-steroidal activation of AR. Nuclear factor kappa B (NF kappa B) regulates several proinflammatory cytokines, such as interleukin-6. NF kappa B is regulated by the coactivator MYST1 which interacts with sirtuin 1. Downregulation of MYST1 activates the cleavage of PARP and caspase 3 which leads to apoptosis. In PC-3 cells, which are modified to express AR, depletion of MYST1 causes G2M growth arrest. Therefore, it could be concluded that MYST1 coregulates AR and NF kappa B to regulate multiple cellular functions.

The elements of the mediator (MED) complex play an important role in the regulation of AR transcriptional activity. The transcriptional coactivator MED1 may have a particularly important role in the interaction with AR in PCa. MED1 is phosphorylated at T1457 by cyclin-dependent kinase (CDK)7. In this context, down-regulation of CDK7 leads to the inhibition of AR-mediated transcriptional amplification, thus representing a novel approach for the treatment of PCa.

AR coactivators and prediction of cancer recurrence
Nuclear four and half LIM domain protein (FHL)2, together with other oncogenic factors such as filamin, supports the progressive growth of PCa. Interaction between FHL2, AR, and filamin is particularly important for understanding the function of variant AR. Nuclear FHL2 expression was detected only in castration-resistant PCa samples. FHL2 predicts PCa recurrence risk, as evidenced in a study in which a number of other variables were analyzed. Activation of the AR by low concentrations of androgens may lead to increased expression of FHL2. Its levels are elevated in tumors with a high mutation rate of p53. FHL2 has been described as a coactivator that suppresses FOXO1 pro-apoptotic activity. Therefore, this mechanism may be important for better understanding PCa progression. A coactivator that is overexpressed in high Gleason score tumors and castration therapy-resistant PCa is the long noncoding RNA LINC00675. Its knockdown suppresses tumor formation and attenuates enzalutamide resistance. It modulates the interaction of the AR with mouse double minute (MDM)2 and binds to the receptor. The inhibition of LINC00675 could be combined with treatment with AR signaling inhibitors.

BAF53A is a protein member of the SWI/SNF complex that regulates gene expression by gene-specific chromatin remodeling of multiple target genes. In human PCa tissues, the expression of BAF53A mRNA was found to be elevated in several publicly available databases. The depletion of BAF53A significantly decreased in growth rate of tumor cells. DHX15 is an RNA helixase involved in stimulating PCa progression through the upregulation of AR. Coactivator expression is correlated with Gleason score and biochemical recurrence. It regulates AR activity through Siah2-mediated ubiquitination. Downregulation of DHX15 in the C4-2 derivative of LNCaP cells resulted in inhibition of xenograft growth. DHX15, like many other coactivators, is involved in the potentiation of AR activation at low androgen concentrations.

The expression of AR also correlates with that of YAP1, which is an element of the Hippo pathway that is involved in the regulation of cellular migration. YAP1 expression is considered a prognostic factor that predicts early PCa recurrence. High YAP1 expression has been observed in therapy-resistant PCa. YAP1 and AR colocalize in hormone-naive and castration therapy-resistant PCa. Androgens have been shown to promote YAP1 nuclear abundance and activity.

Determination of AR coactivator output and its role in the regulation of enzalutamide responsiveness in PCa.
The importance of studying the expression and specific action of coactivators in PCa cellular models was highlighted by Lee et al. They showed 100-fold heterogeneity in the activation of AR downstream genes in human PCa cell lines. The most important recent studies on coactivators have been conducted in PCa resistant to inhibitors of AR signaling. The medical treatment of patients with metastatic therapy-resistant disease is an unmet medical need. In addition, it is important to identify coactivators with specific functions which are not compensated by other coregulatory proteins. Cells with a higher AR output responded more strongly to low doses of androgen, which is relevant for castration resistance and reduced sensitivity to enzalutamide. It is important to analyze the consequences of the inhibition of AR coregulatory proteins in multiple models including cell lines, therapy-resistant sublines, and patient-derived xenografts. GREB1 is up-regulated in cells with higher AR transcriptional activity. It is regulated by androgens, enhances AR DNA binding, and promotes the recruitment of p300, which is involved in several cellular processes in PCs. GREB1 overexpression increased AR activity in a dose-dependent manner. On the other side, enzalutamide resistance can be delayed by GREB1 knock-down in vitro. Inhibition of GREB1 in enzalutamide-resistant cells restores their sensitivity to the drug. An increase in GREB1 expression has been documented during enzalutamide treatment, which is consistent with its causative role in the development of treatment resistance. Its oncogenic role has also been investigated in ovarian cancer, in which estrogen regulation has been documented.

Because of their large number and possibilities to interact with each other, the consequences of inhibition of a coactivator of interest may not necessarily be observed. Liu et al. reported 22 (out of 181) clinically relevant coactivators that drive PCa progression. These coactivators were selected based on their ability to contribute to the activation of cancer-specific signaling pathways. For each androgen-regulated gene, two-four coregulators affect androgen responsiveness. Interestingly, these authors showed for the first time that WDR77 cooperates with p53 to modulate the response to androgens. WDR77 is a component of the arginine methyltransferase PRMT5-cotaining complex, which modifies arginine to dimethylarginine in several spliceosomal proteins.

Another important regulator of AR signaling GATA2 (GATA-binding protein). The protein is a member of the GATA family of pioneer transcription factors and is an actionable therapeutic target. GATA2 and AR expression are correlated in PCs. When androgen levels are low, GATA2 increases AR expression, most likely by binding to the AR promoter. Consequently, GATA2 elevated the levels of the AR target gene KLK2. It colocalizes with AR and forkhead box protein A1 (FOXA1). FOXA1 and GATA2 strongly enhance each other’s transcriptional
program whereas FOXA1 is an up-stream regulator of GATA2. It is involved in regulating the transcriptional activity of full-length and variant ARs. Interestingly, these authors described a negative feedback loop, since androgens cause repression of GATA2. GATA2 stimulates enzalutamide-induced transcription by facilitating AR loading at the enzalutamide-responsive gene loci. Silencing GATA2 leads to inhibition of the enzalutamide-induced genes NR3C1 and SLC7A11. Consistent with these findings, the GATA2 inhibitor K7174 impaired the growth of PCA cells in response to enzalutamide. GATA2 may also be involved in PCA progression, independent of AR. This effect is achieved through upregulation of insulin-like growth factor 2, a well-established anti-apoptotic driver in cancer. Interestingly, a drug used in clinical medicine for vasodilatation (dilazep) has been identified in silico as a potential inhibitor of GATA2. It suppresses genes regulated by AR and c-Myc. In the future, clinical trials with dilazep in PCa should be conducted.

Interestingly, KDM1A (LSD1) acts as a transcriptional repressor by demethylating histone H3 lysine 4 and as a nuclear receptor coactivator. It is responsible for the binding of FOXA1 chromatin to AR. Therefore, inhibition of LSD1 diminishes PCa cell growth and synergizes with AR antagonist treatment in vivo. Interestingly, LSD1 interaction with the AR has been studied in kidney cancer in which it was found that the inhibitor paraglyline enhances the effects of enzalutamide.

c-Myc, AR, and cofactor network

The regulation of various cellular events in PCa is largely dependent on the c-Myc-AR axis. While c-Myc inhibits apoptosis, it stimulates glycolysis and cell cycle progression. A complex relationship exists between AR and c-Myc expression in PCa. Overexpression of c-Myc antagonizes the expression of AR target genes. In contrast, c-Myc expression is up-regulated by androgenic hormones. The androgen-regulated lysine methylase KDM4B causes AR-dependent transcription of c-Myc mRNA. Its expression is increased in enzalutamide-resistant prostate tumors. KDM4B enzymatic activity is necessary to increase AR stability by inhibiting its ubiquitination. High KDM4B levels have been observed in high-grade PCa specimens. Inhibition of KDM4B enhanced the antagonistic effect of enzalutamide in xenografts. Thus, treatment with KDM4B inhibitors may re-establish PCa sensitivity to AR signaling inhibitors. Increased c-Myc in response to androgen deprivation contributes to castration-resistant PCa, whereas decreased c-Myc contributes to responses to therapy with higher doses of androgen which could be considered in patients with AR gene amplification or in those with DNA repair
deficiency. Suppression of global AR activity is associated with the redistribution of coactivators. C-Myc-induced AR transcription via alteration of histone modifications at the c-Myc binding site within the AR gene is upregulated by RNF8. RNF8 promotes the recruitment of wild-type or variant ARs to the prostate-specific antigen promoter.

Interaction of AR coactivators with other cellular proteins

AR coactivators may interact also with other proteins. For example, the co-chaperone Cdc37, which is up-regulated in early PCa, interacts with Vav3 to promote receptor activity and tumor growth. Disruption of the Vav3-Cdc37 interaction inhibits Vav3 enhancement of AR transcriptional activity and AR N-C interaction, which is required for activation. The Vav3 Dbl homology domain disrupted the interaction of AR V7 with the coactivators Src1 and Vav2, leading to decreased cellular proliferation and anchorage-dependent growth, increased apoptosis, and decreased migration. The phosphorylation of the AR is regulated by Ack1 tyrosine kinase. Furthermore, SRA stem-loop interacting RNA binding protein associates with AR and the interaction is modulated by Ack1.

AR coactivators and tumor cell metabolism

Krebs cycle is suppressed in normal epithelial cells but not in cancer cells. The main difference between normal and cancer cells is that in benign epithelium glucose and aspartate are involved in citrate synthesis, whereas in tumor cells there is prominent oxidative phosphorylation and lipid synthesis. This metabolic switch is mediated by AR. Oxidation of citrate and production of ATP may occur before appearance of morphological changes in the tissue. Metabolic substrates such as lactate are necessary to sustain malignant cell growth and may be provided by the tumor microenvironment. Lactase production is stimulated by cytokines such as interleukin-6 and transforming growth factor-β.

AR coactivators are involved in regulating cancer metabolism. KDM8/JMJD5 is a histone lysine demethylase/dioxygenase, which is a dual coactivator of AR and pyruvate kinase (PK)M2. Increased KDM8 expression was observed in enzalutamide-resistant cells. KDM8 is a regulator of glycolytic genes and is a target for PCa therapy. Tumor metabolism is also affected by KDM4B, which has been implicated in c-Myc-regulated cellular events. Its expression leads to the activation of Warburg metabolism and metabolic genes such as lactate dehydrogenase A (LDHA). Androgen positively influences AMPK signaling, which increases
peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC). This transcriptional coactivator is an important regulator of mitochondrial function and oxidative phosphorylation. It may be particularly interesting to investigate the role of AR coactivators in lipid metabolism. Several steroid receptors are considered lipid sensing factors that affect different aspects of lipid metabolism.

Coactivators and AR-mediated growth inhibition

Treatment of PCa cells with higher androgen doses leads to growth inhibition and upregulation of cell cycle inhibitors such as p21 and p27. Transducin-beta-like-related protein (TBLR)1 interacts with AR and could show both coactivator and corepressor properties. Its expression in the nucleus is higher in benign tissues than in cancerous tissues. Thus, TBLR1 is a co-activator associated with androgen-mediated growth inhibition. Similarly, TERT2 binds to AR, and its loss is associated with PCa. TERT2 knockdown increases LNCaP cell proliferation, migration, and wound healing. Consistent with the functional data, it was demonstrated that decreased TERT expression in tumor tissues is associated with reduced survival.

Glucocorticoid receptor in advanced PCa and its role in therapy resistance

Other steroid receptors are expressed in the prostate epithelium and tumor microenvironment, however functional studies on some of them have not established a relationship with tumor cell growth. In contrast, recent investigations on PCa have focused on the glucocorticoid receptor (GR). GR expression decreases in early PCa but is elevated during metastatic progression. It has been shown that GR inhibition enhances sensitivity to enzalutamide in PCa. Thus, GR expression increases in a number of preclinical models used to study enzalutamide or abiraterone resistance. Progression-free survival is reduced in patients with higher GR expression. Other studies have demonstrated that GR-mediated therapy resistance can be reversed by BET inhibitors that bind to bromodomains. It has been recognized that the mechanism of escape from endocrine therapy involves GR activation. Other studies have shown that GR depletion delays the progression towards castration resistance. The anti-apoptotic glucocorticoid-induced kinase SGK-1 is also involved in this process. Therefore, the SGK-1 inhibition had a negative effect on PCa cell viability. Conversely, SGK-1-Flag overexpression reduced the time required for tumor initiation in vivo.
Consistent with these findings, docetaxel resistance could be reversed by inhibition of GR in PCa cell lines, associated with the downregulation of genes of the Bcl-2 family.\textsuperscript{91} The interaction of GR with β-catenin may be relevant to docetaxel resistance and cellular stemness.\textsuperscript{92} Furthermore, an increase in sensitivity to radiation therapy was reported after inhibition of GR.\textsuperscript{93} Mechanistically, elevation of miR-99a/100, which may be a result of treatment with mifepristone is required to achieve the effect of radiation therapy.

Based on laboratory findings, selective GR modulators have been developed for PCa.\textsuperscript{94} In contrast to mifepristone, they do not influence AR signaling. A common problem associated with classic endocrine therapy in PCa is compensatory activation of the anti-apoptotic PI3 kinase pathway. Targeting the PI3 kinase pathway with the pan-Akt inhibitor ipatasertib leads to the inhibition of GR expression and activity through cell cycle arrest. Moreover, GR inhibition is necessary to establish sensitivity to pan-Akt inhibitors.\textsuperscript{95} Expression of the GR and AR signature gene mono amine oxidase A (MAO-A) was found in primary tissue cultures after treatment with glucocorticoids as well as in tissues from patients obtained after neoadjuvant chemotherapy with docetaxel or mitoxantrone. A positive correlation between MAO-A and pathways associated with mitochondrial activity such as oxidative phosphorylation, adipogenesis, and fatty acid metabolism has been previously demonstrated. MAO-A can be targeted in PCa, leading to an increase in the efficacy of anti-androgen therapy.\textsuperscript{96}

Based on these results, one can expect GR-interacting proteins to have specific functions in PCa progression. GR coactivators may lower the IC\textsubscript{50} required for the induction of receptor transcriptional activity. The upregulation of selected coactivators under conditions in which high levels of GR are measured may have implications for the development of new drugs for PCa treatment. However, the GR-cofactor interaction in PCa has not been sufficiently investigated. This is in contrast to the AR-cofactor interaction that has been studied in benign prostate epithelium, pre-malignant lesions, early and metastatic PCa, and therapy resistance. If critical GR partners in PCa tissue can be identified in the future, small-molecule antagonists could be used to prevent or delay therapy resistance.

Summary and conclusions

To improve therapeutic strategies against AR coactivators, it is crucial to focus on those proteins that are highly expressed in PCa. Furthermore, knockdown strategies have been shown to be involved in important cellular functions in cancer development and progression.
Pharmacological studies have led to the identification of inhibitors of coactivator functions with improved pharmacokinetics and pharmacodynamics characteristics. Recently, several laboratories have performed *in vivo* studies in order to confirm the potential application of small-molecule inhibitors in clinical settings. However, clinical studies with these compounds have to be designed keeping in mind that patients should be carefully selected based on their clinical status, specific molecular characteristics of the tumor, and previous therapies.

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Figure legends

**Figure 1.** Selected cofactors with important functions at different stages of prostate carcinogenesis. Androgen receptor coactivators have distinct functions during tumor initiation and progression as well as in therapy resistance.

**Figure 2.** General structure of SRC coactivators. Regions responsible for protein protein interactions, binding to nuclear receptors, and intrinsic activation domains (AD) are shown.

**Figure 3.** Possibilities to inhibit p300/CBP in prostate cancer. Compounds with different mechanisms of action have been developed and tested in cell lines and patient-derived xenografts; HAT - histone acetyltransferase, AR – androgen receptor, MMP – matrix metalloproteinase, BD – bromodomain, PDX – patient-derived xenograft. Arrows indicate p300/CBP targeting.
INITIATION
Cdc 37
INDIRECTLY:
SPOP

PRIMARY LESION
SRC-1
Cdc 37

METASTATIC SPREAD
GATA 2
SRC - 1
SRC - 2
SRC - 3
FHL 2
YAP 1
KDM4B
p300/CBP

THERAPY RESISTANCE
GREB 1
SRC - 1
SRC - 2
SRC - 3
FHL 2
DHX 15
p300 / CBP
RUNX2

Figure 1
STEROID RECEPTOR COACTIVATOR

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- PROTEIN PROTEIN INTERACTIONS
- PROTEASOME - DEPENDENT TURNOVER
- CONSERVED PART: BINDING TO NUCLEAR RECEPTORS
- INTRINSIC ACTIVATION DOMAINS FOR INTERACTION WITH CBP / p300

CELL GROWTH

CELL DIFFERENTIATION
Figure 3

Inhibitors

**IN VITRO**

C646: p300, HAT, AR, INVASION, MMP-2,9

GNE - 049: p300, CBP, BD, AR, 2D Models, PDX

A-485: p300, CBP, CATALYTIC INHIBITOR, AR, XENOGRAFT

**IN VIVO**

CCS 1477: p300, CBP, BD, AR, C-MYC, 22Rv1, XENOGRAFT