Chapter 9
Androgen-Responsive Gene Expression in Prostate Cancer Progression

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Abstract Benign and cancerous prostate tissue is dependent upon androgens. Androgen ablation causes prostate tissue to undergo apoptosis which thereby provides the rationale of castration as a systemic therapy for advanced prostate cancer. The full-length androgen receptor is a ligand-activated transcription factor that regulates the expression of genes required for growth, function, and survival of prostate cells in response to androgen. Androgen binds to the androgen receptor which then translocates to the nucleus to bind to androgen response elements on target genes termed "androgen-responsive genes" (ARGs) to regulate their transcription and levels of expression. Identification and characterization of ARGs may provide an understanding of androgen receptor signaling, resistance mechanisms to current hormonal therapies, and reveal biomarkers for patient selection and sequential application of current and new therapies targeting the androgen axis. This review addresses differential expression of ARGs following androgen ablation treatment during progression of advanced prostate cancer.

Keywords Androgen-responsive gene • Prostate cancer • Androgen receptor • Castration-resistant prostate cancer (CRPC) • Gene expression

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9.1 Introduction

9.1.1 Prostate and Androgen

The prostate depends on androgen with androgen ablation therapy (castration) inducing programmed cell death and involution of the prostate. Androgen receptor mediates the effects of androgen and thereby regulates the development, function, and survival of prostate cells. Prostate cancer is also dependent upon androgens and this is why castration is a form of therapy for advanced disease. When androgen binds to the androgen receptor, the receptor becomes transactivated and translocates to the nucleus where it binds to androgen-response elements (AREs) in enhancer and promoter regions of target genes to regulate their transcription. The target gene is generally termed an "androgen-responsive gene" (ARG) and its levels of expression can be altered by addition or withdrawal of androgen. Expression of ARGs can be regulated at many different levels, such as transcription, RNA processing, RNA stability, protein translation, and protein stability [1]. ARGs are fundamental in normal prostate development, growth, survival, and function and many of these genes are speculated to be important in the progression of prostate cancer [2].

9.1.2 Androgen Receptor Mechanisms of Resistance

Resistance of prostate cancer to androgen ablation therapy is inevitable at least by current approaches. Initially based upon the reexpression of ARGs such as prostate-specific antigen (PSA) in castration-resistant prostate cancer (CRPC), there have since been many proposed mechanisms that involve resumed androgen receptor signaling that include overexpression and/or gene amplification of androgen receptor; gain-of-function mutations in the ligand-binding domain of androgen receptor; androgen receptor splice variants lacking the ligand-binding domain; ligand-independent activation by alterative signaling pathways; and/or increased levels of intracellular androgen [3–8].

9.1.3 Identification of Androgen-Responsive Genes

The quest to identify ARGs lies in their potential to reveal biomarkers for prognosis and patient selection for therapies as well as yield insight into novel approaches for the development of new treatments for prostate cancer. Identification of ARGs has encompassed numerous models including prostate cancer cell lines, rodents, and/or human xenografts. It is estimated that 1.5–4.3% of genes are responsive to androgen in LNCaP cells [9]. This percentage includes the genes directly or indirectly (as a

consequent event) regulated by androgen receptor. In 2009, 1,785 human genes, 583 rat genes, and 993 mouse genes were considered to be ARGs based on a literature search [10]. Most ARGs identified have functions in binding (72%) or catalytic activity (36%) according to gene ontology (GO) analysis from the Androgen-Responsive Gene Database website (http://argdb.fudan.edu.cn/index_info.php). There are many other cellular functions mediated by androgen receptor signaling. Understanding the changes in expression of ARGs between benign and cancerous prostate and ultimately in lethal CRPC may facilitate the development of therapeutic approaches to target androgen receptor signaling. Expression patterns of ARGs may provide indications of time, duration, and doses of therapies required to inhibit androgen receptor.

9.1.4 Prostate-Specific Antigen: The Prototype ARG

The best characterized ARG is prostate-specific antigen (*PSA/KLK3*). The *PSA* gene contains several functional AREs in its enhancer and promoter regions and its transcription is highly inducible by androgen [11–14]. Although cellular levels of PSA may decrease in cancer compared to normal prostate tissue, especially in poorly differentiated tumors, levels of PSA in the serum enables the early detection of prostate cancer. It is important to note that serum levels of PSA do not correlate to tissue levels in any stages of prostate cancer [15–17]. Serum level of PSA is also a useful early indication of biochemical failure and recurrence of prostate cancer after primary therapy as well as after secondary and tertiary therapies. After primary therapy, 30% of patients will have biochemical failure and require secondary treatment, which involves androgen ablation by chemical or surgical castration. For those patients with metastatic disease, approximately 75–80% respond to androgen ablation therapies [18]. However, androgen ablation therapy is only palliative with ultimate failure and progression to lethal CRPC [19]. New approaches involving chemotherapy, immunotherapy, or new hormonal approaches recently approved by the FDA have improved overall survival by approximately 2–5 months for men with CRPC [7, 20–24]. Current approaches of androgen ablation and androgen receptor blockade are considered to fail by mechanisms that lead to resumed transcriptionally active androgen receptor and concomitant expression of ARGs, or at least a subset of ARGs.

9.2 Approaches of Gene Expression Analyses

9.2.1 Model Systems for Samples

Gene expression profiles have been carried out in human prostate cancer cell lines treated with and without androgen such as dihydrotestosterone (DHT) or R1881,

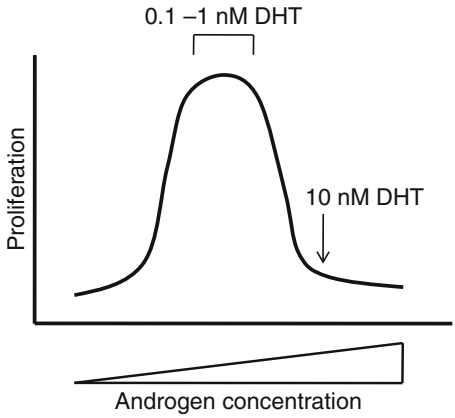

Fig. 9.1 Effect of androgen concentration on LNCaP cell proliferation. The maximal proliferative response by androgen, DHT as an example in this diagram, is between 0.1 and 1 nM for in vitro LNCaP cell culture. The cells stop proliferating when DHT is higher than 10 nM

a synthetic androgen. Most of the cell lines used were originally derived from metastatic disease. LNCaP, derived from lymph node metastatic tumor, is the most frequently analyzed cell line for transcriptome changes in response to androgen. The frequent use of LNCaP cells is probably because it is a well-differentiated human prostate cancer cell line that expresses a functional androgen receptor and can be grown as a xenograft in murine hosts to mimic several aspects of human disease such as becoming castration resistant when the host is castrated [25]. LNCaP cells have a mutation in the ligand-binding domain at T877A making the receptor more promiscuous and activated by other steroids and some antiandrogens [26–30] which may impact gene expression signatures. It is important to note that androgen not only causes growth and survival but also causes differentiation of prostate cells. This is an important concept when studying ARGs as different subsets of genes may be expressed depending on whether cells are proliferating or become quiescent with differentiation. LNCaP cells display biphasic response on proliferation with increasing concentrations of androgen. The cells proliferate in response to DHT ranging between 0.1 and 1 nM. However, proliferation is inhibited and cells become more differentiated when DHT concentration is 10 nM or higher [31–33]. A simple diagram of the biphasic proliferative response is shown in Fig. 9.1. This biphasic proliferative effect may occur at slightly varying concentration ranges because of different cell line types, cell culture passage number, culture conditions prior to androgen treatment, and treatment duration [31, 34–36]. These variables may be responsible for the relatively few numbers of ARGs that are commonly detected amongst different gene expression profiling studies [37]. The spectrum of expression of ARGs over different concentrations of androgen still remains relatively uncharacterized with few studies that have examined ARGs using the relevant concentrations of androgens that would be encountered under castrate conditions.

Advantages of using cell lines include that they are relatively homogeneous, easily accessible, and stringent control of experimental conditions can be achieved thereby providing potentially highly reproducible data. However, cell lines growing as a monolayer on plastic culture dishes and passaged many times may not accurately

reflect the complexity of tumor environment nor represent different stages of disease. Therefore human xenografts and rodents have been explored as more complex models. Rodents with intact tissue structure including cell–cell interaction between different types of cells, such as epithelial cells, stromal cells, and blood cells make them suitable as an in vivo model for gene expression profiling analyses. However, these too may fall short and do not necessarily reflect the complexity of tumor progression in humans. Of note are the physiological differences between rodents and humans in the structure of the prostate. Unlike human prostate separating into zones, rodent prostate consists of distinct lobes. Rat ventral lobe is the most commonly studied because the epithelial cells in the ventral lobe undergo involution in response to androgen ablation [1]. Mouse dorsolateral lobe is the most commonly studied because the gene expression pattern in these two lobes is more similar to human prostate than other lobe [38]. Of note, the most characterized ARG in humans, *PSA*, is not expressed in rodents. This allows monitoring of serum PSA in mice carrying human xenografts to be a relatively accurate indication of tumor growth since only the human prostate tissue would be accountable for levels of PSA in the blood.

9.2.2 Clinical Samples

Difficulties in obtaining clinical samples from patients before and after castration from both the prostate as well as distant sites that are suitable to measure levels of RNA have led to fewer gene expression studies with clinical tissues. Generally, clinical samples are categorized based on disease stages, treatment type, treatment duration, location of tissues (primary prostate or metastatic tumor), and androgen responsiveness (androgen sensitive or castration resistant). Several factors need to be considered when using clinical samples for transcriptome analyses. First, the sample quality and sample handling affect gene expression analyses. Freshly frozen tissues and paraffin-fixed tissues (and fixing duration) provide different RNA quality (stability and degradation) [39]. Second, heterogeneity of cell populations in a tumor sample may complicate the gene expression analyses. Therefore, enrichment of epithelial cells or tumor cells is usually achieved by laser capture microdissection. The importance of microdissection is emphasized for those clinical samples with a Gleason Score greater or equal to 7. This is because of the often potential lethality of a Gleason Grade of 4 or more. Combination of RNA from both fields, such as a Gleason 4 with a Gleason 3 from a Gleason Score 7 tumor may confound interpretation of data since Gleason Grade 3 is considered generally to be relatively benign [40–42].

9.2.3 Methods of Analysis

Different platforms for genome-wide expression profiling have varying sensitivities to detect ARGs. Dehm and Tindall [9] summarized the following percentages of

genes that were detected to be regulated by androgen in LNCaP cells depending upon the approach: serial analysis of gene expression (SAGE) analyses yielded 1.5–2.1%; oligonucleotide array analyses, 2.8–3.7%; and cDNA array analyses, 4.3%. However, it is important to consider the total number of genes detected by each platform which may vary considerably.

Depending on the methods used to generate gene expression profiles, starting materials such as total RNA or mRNA (polyA$^+$ RNA) may result in different detection sensitivities due to RNA extraction manipulation and influence of non-transcripts. Reverse-transcribed cDNA or amplified RNA is used as the source for detection by different profiling methods. Other considerations include that tumor samples from individual patients are usually pooled to obtain enough material and to have an average expression value, but this approach may mask the variation between individuals. The choice of reference sample for comparison and to present data as fold change is also a critical factor that influences the data obtained, interpreting changes in expression, and for comparison amongst different studies. Ultimately data validation is carried out by quantitative polymerase chain reaction, tissue microarray, or immunohistochemistry due to the propensity of high-throughput approaches to generate false positives and less than optimal dynamic ranges of some approaches which may underestimate fold-change.

9.3 ARGs

9.3.1 ARGs Detected Using Human Cells Lines

Human prostate cell lines have been used in a large number of ARG expression studies. Reviews from Dehm and Tindall [9] and Clegg and Nelson [1] comprehensively discuss the ARGs and their functions in prostate cancer and provide a list of the studies about gene expression profiling using human prostate cell lines or rodent models. As mentioned in Sect. 9.2.1, different cell lines may respond differently to varying concentrations of androgen, and even in the same cell lines, especially the extensively studied LNCaP cells. Such differences may be contributed from the strains of LNCaP cells or variations in culture and/or experimental conditions [37, 43]. For example, the presence or absence of serum in the control samples not treated with androgen will have profound effect on overall differences in the expression patterns obtained. This is because serum would provide cholesterol precursors for potential de novo androgen synthesis as well as contains a variety of growth factors and cytokines that may impact androgen receptor activity and gene expression.

Velasco et al. [37] cross-compared expression patterns of four in vitro studies and revealed that only 13 ARGs were common amongst four different studies: *BCHE*, *CDK8*, *CTBP1*, *DHCR24*, *FKBP5*, *FN1*, *HERC3*, *PSA/KLK3*, *LIFR*, *MMP16*, *NDRG1*, *PIK3R3*, and *RAB4A*. Interestingly, an oligonucleotide microarray and

LongSAGE analyses in LNCaP cells treated with R1881 also detected differential expression of 7 of these genes: *DHCR24*, *FKBP5*, *PSA/KLK3*, *MMP16*, *NDRG1*, *PIK3R3*, and *RAB4A* [44, 45]. One study that analyzed ARG identified in vitro for relevance in clinical samples revealed that transcripts of *CAMK2N1* and *GLO1* were significantly increased in the primary lesions from patients that later had biochemical failure [46].

Importantly LNCaP cells have been used to identify genes potentially associated with CRPC. The majority of CRPC involves osseous metastases and hence creating in vitro conditions that tried to mimic CRPC was attempted by culturing LNCaP cells with osteoblast-conditioned media [47]. In the absence of androgen, osteoblast-derived factors activate androgen receptor transcriptional activity and increase proliferation of LNCaP cells [47, 48]. The gene expression signature that was identified in LNCaP cells in response to osteoblast-conditioned media was enriched with ARGs such as *PSA/KLK3*, *ACPP*, and cell cycle-related genes that included *ASNS*, *AURKB*, *BUB1*, *BUB1B*, *CCNA2*, *CCNB1*, *CCNB2*, *CCNE2*, *CDC2*, *CDC6*, *CDC7*, *CDC20*, *CDC25C*, *CDKN3*, *CHEK1*, *DEPDC1*, *ESPL1*, *GTSE1*, *HCAP-G*, *MCM5*, *PLK1*, *SMC4L1*, and *STK6*. Blocking interleukin-6, which is abundant in osteoblast-conditioned media and also known to activate the androgen receptor in the absence of androgen [49], decreased expression of *AURKB*, *CCNA2*, *CCNB1*, *CCNB2*, *CDC2*, and *PSA/KLK3* in response to osteoblast-conditioned media [47]. Importantly, this gene expression signature could identify both clinical metastases and CRPC. All of the cyclins identified in response to osteoblast-conditioned media (*CCNA2*, *CCNB1*, *CCNB2*, and *CCNE2*) and most of the CDCs (*CDC2*, *CDC6*, *CDC20*, *CDKN3*, and *CDC25C*) were upregulated in clinical samples of metastases and the genes overlapping with CRPC clinical samples were *CCNB1*, *CDC20*, *CHEK1*, *ESPL1*, *HCAP-G*, *SMC4L1*, and *STK3* [47]. Later additional in vitro validation that androgen receptor regulates a distinct transcriptional program in androgen-insensitive cells was generated by comparing sublines of LNCaP cells [50]. Importantly, androgen receptor was shown to selectively upregulate M-phase cell-cycle genes in androgen-independent cells including *CCNA2*, *CCNE2*, *CDC2*, *CDC20*, *CDC25C*, *CDKN3*, and *UBE2C* [50]. Some of these genes known to be regulated by androgen receptor were associated with high expression of androgen receptor splice variant in bone metastases such as *CCNA2*, *CDC20*, *CDK1*, *HSP27*, *C-MYC*, *UBE2C*, and *UGT2B17*, while other known ARGs such as *PSA/KLK3*, *KLK2*, *NKX3.1*, *FKBP5*, and *TMPRSS2* were not associated with metastases containing high level of splice variant [51].

9.3.2 ARGs Discovered Using In Vivo Models

More than a decade ago, dysregulation of ARGs was demonstrated in human xenografts including CWR22 and the CWR22-R1 xenograft derived from CWR22 cells. These studies revealed that some genes normally regulated by androgens (e.g., *FKBP5* and *S100P*), that had reduced levels of expression in response to

castration were reexpressed when the tumors began to grow again in the absence of testicular androgens [52, 53]. One of the largest in vivo study in terms of three million tags generated was from a LongSAGE analyses using the LNCaP Hollow Fiber model. This study uniquely used serial samples from the same mouse from before and after castration to reveal differential expressed genes in response to androgen ablation and with CRPC. The differential expressed genes identified in CRPC included genes known to be altered in response to androgen (*ABHD2*, *BM2*, *BTG1*, *C19orf48*, *CAMK2N1*, *CXCR7*, *EEF1A2*, *ELOVL5*, *ENDOD1*, *ENO2*, *HSD17B4*, *MAOA*, *MDK*, *NKX3.1*, *ODC1*, *P4HA1*, *PCGEM1*, *PGK1*, *PSA/KLK3*, *SELENBP1*, *TMEM66*, *TPD52*, and *TRPM8*) as well as those involved in androgen receptor signaling (*CCNH*, *CUEDC2*, *FLNA*, and *PSMA7*) and androgen biosynthesis and metabolism (*DHCR24*, *DHRS7*, *ELOVL5*, *HSD17B4*, and *OPRK1*) [54]. This library (GSE18402) is freely available at Gene Expression Omnibus and provides a resource where anyone can mine the data to determine if their gene of interest changes expression in vivo in the LNCaP Hollow Fiber model in response to castration and in CRPC.

It is important to note that the LNCaP hollow fiber model has genomic similarity to clinical samples in terms of differential gene expression during hormonal progression [55]. This model provided evidence for the reactivation of the androgen receptor signaling pathway in CRPC by hierarchical two-dimensional clustering algorithm based on similarity of expression patterns of 1,092 ARGs. Pathway-based characterization of gene expression revealed activation of Wnt/beta-catenin signaling pathway and interaction with androgen receptor in CRPC [55].

9.4 ARG Analyses Using Clinical Patient Tumor

9.4.1 Clinical Sample Analyses

Gene expression profiling may be a useful tool to categorize tumor subtypes since the changes at molecular level are correlated with tumor progression [56–59]. ARGs may have important roles in the development of castrate resistance since androgen receptor protein is detected in most CRPC [37]. Profiling the expression of ARGs at different stages of prostate cancer will help elucidate the mechanism(s) leading to terminal CRPC as well as provide biomarkers for prognosis and patient selection for therapies. Several studies on the gene expression analyses have been carried out to compare androgen-responsive and castrate-resistant tumors [16, 60–63]. Studies by Holzbeierlein et al. [60] and Chandran et al. [16] analyzed tumor specimens without separating different types of cells to examine gene expression, while studies from Best et al. [61] and Tamura et al. [63] analyzed epithelial cells separated by laser capture microdissection. Heterogeneity of gene expression levels must be considered. For example, although the majority of lethal CRPC still express PSA, the levels are extremely variable [64]. Tissue levels of expression of *PSA/KLK3* and *AMACR* can vary considerably from high expression to non-detectable within tumors

from the same patient [16, 65]. Also there is no correlation between expression of PSA and androgen receptor [66]. Expression levels of a gene may also be high in the primary tumor and downregulated in the metastatic tumor such as seen with *FOS* and *JUNB* [16].

It is important to note whether change in levels of expression of an ARG is in direct response with change in occupancy of androgen receptor on an ARE on the ARG locus or if it is a downstream effect. The time from treatment to sample collection may affect gene expression. Studies from Holzbeierlein et al. [60] demonstrated that differential expression of ARGs was directly regulated by androgen after 3 months of androgen ablation therapy based upon in vitro validation that 22.8% of those genes had differential expression in LNCaP cultures after 36 h of androgen withdrawal. There are additional complexities that may be encountered with patient tissue as described by Mostaghel et al. [17] showing evidence that after short-term suppression of androgen, even though the serum testosterone levels are decreased, the intraprostatic levels may not be reduced. Hence, patients receiving androgen ablation therapy may not have complete suppression of androgen in the prostate microenvironment which would have profound effects on expression of ARGs. Therefore, duration of hormonal therapy and the residual levels of tissue androgen need to be considered for gene expression analyses on patient samples.

Finally the definition of CRPC needs to be consistent between analyses for correct interpretation of the data. Different criteria for clinical castrate resistance have been employed. For example, Best et al. [61] defined CRPC as patients with two rising PSA levels and at least one new lesion found in bone or progressive measurable disease, whereas Tamura et al. [63] defined CRPC as patients with three rising PSA levels.

9.4.2 ARGs and Androgen Ablation Therapy

Despite the potential incomplete suppression of androgen within prostate tissues, the expression of ARGs is altered after short-term (1–9 months) androgen ablation therapy [17, 60]. Notably, there was a higher percentage of genes downregulated than those that were upregulated following androgen ablation therapy. These studies showed that 56% of the altered genes were downregulated while 44% of the altered genes were upregulated in response to androgen ablation treatment, even though the numbers of altered genes, profiling platform, analysis criteria, tissues sources, treatment agents, and treatment duration were different between the two studies [17, 60]. Comparison between these two studies revealed only nine ARGs that were in common and were differentially regulated after short-term castration in benign versus malignant prostate. One ARG, *TFF1*, was upregulated in response to castration, while six others (*ABCC4*, *CAMK1*, *DCXR*, *SORD*, *SPON2*, and *TMPRSS2*) were downregulated [17, 60]. *GSTM1* (involved in metabolism) was upregulated in the tumor [60], but downregulated in benign prostate [17] after castration, whereas *FOLH1* was downregulated in the tumor but upregulated in benign prostate after castration.

TFF1, *DCXR*, and *SORD* are involved in carbohydrate metabolism and these alterations consequently affect the cell proliferative capacity [16, 17]. CAMK1 has a role in calmodulin-dependent protein kinase cascade but its exact role in prostate cancer in response to androgen ablation requires investigation. ABCC4, a member of ABC-type multidrug transporters, transports a number of molecules. Its gene expression was highly upregulated in malignant compared to benign prostate and it is suggested to be involved in tumor progression [67]. Consistent with patient samples, expression of *SPON2* was reduced by castration and then highly expressed in CRPC in the LNCaP hollow fiber model [54]. SPON2 protein is a proposed serum biomarker for prostate cancer [68]. *TMPRSS2* encodes a serine protease and is one of the best characterized ARGs with bona fide AREs [69, 70]. Expression of *TMPRSS2* is decreased following short-term castration [17, 60]. The discovery of the *TMPRSS2-ERG* fusion gene has increased interest in this ARG with this fusion gene expressed in ~50% of primary prostate cancers [71, 72]. Comparison of gene expression profiles between *TMPRSS2-ERG* fusion-positive tumor cells to fusion-negative cells after androgen ablation treatment revealed that most of the differentially expressed genes are probably targets of ERG. Hence, these differentially expressed genes in fusion-positive tumor cells after short-term castration are indirect consequent of androgen receptor regulation. Moreover, these differentially expressed genes are associated with cell cycle and mitosis, indicating the *TMPRSS2-ERG* fusion has influence on proliferation-related genes that are potentially regulated by androgen receptor [73, 74].

The fact that only nine genes overlapped in the two gene expression studies (less than 5% of the genes detected), flags the importance of experimental design and/or highlights how different approaches of androgen ablation therapy may impact gene expression. Lehmusvaara et al. [74] reported that gonadotropin-releasing hormone (GnRH) agonist (goserelin) and anti-androgen (bicalutamide) treatments result in different gene expression profiles even though the clinical outcomes are similar. In their studies, only 16% of the differentially expressed genes were common in both treatments. *DCXR* and *TMPRSS2*, included in the list of the nine common genes found between Holzbeierlein et al. [60] and Mostaghel et al. [17] studies, were also found to be downregulated after bicalutamide treatment. Lack of high frequency of common genes amongst different treatments may be due to different targets at the molecular level. Moreover, some genes responding to androgen ablation were not detected in the above gene expression profiling analyses but were found in the prostate tumor after androgen ablation in studies from other researchers. Examples include beta1C (*b1C*) integrin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) death ligand [75, 76]. Expression of *b1C* integrin was upregulated after 1 month of androgen ablation therapy using goserelin and bicalutamide but was not differentially expressed after 3- or 6-month periods of androgen ablation therapy [75]. Expression of *TRAIL* and its receptor (*TRAIL-R*) was upregulated in patients with advanced prostate cancer after androgen ablation therapy, but expression was lower in CRPC. These results suggest that androgen ablation therapy may be effective only initially with tumors susceptible to apoptosis, and longer periods of androgen ablation enable the cells to escape apoptosis [76]. Such a scenario has been put

forth as an underlying hypothesis that intermittent androgen suppression may lead to increased apoptosis as compared to continuous androgen ablation [77, 78].

9.4.3 ARGs Change in CRPC

Eight of the nine common genes differentially expressed after short-term castration (*TFF1*, *CAMK1*, *DCXR*, *SORD*, *SPON2*, *TMPRSS2*, *GSTM1*, and *FOLH1*) were not detected in CRPC. The one exception was *ABCC4* which decreased expression in CRPC [16]. However, comparing all the differentially expressed genes after androgen ablation therapy from Holzbeierlein et al. [60] and Mostaghel et al. [17] studies with differential gene expression analyses from CRPC samples in studies from other groups (list in Table 9.1), a few number of genes reversed their expression in CRPC. For example, *CYB561*, *NTN4*, and *RARRES1* had reversed expression in CRPC compared with tumor samples treated with androgen ablation [60]. *CYB561* was upregulated, while *NTN4* and *RARRES1* were downregulated in CRPC. *CYB561* (cytochrome b561) encodes a secretory vesicle-specific electron transport protein [79]. *NTN4* (Netrin 4) is a secreted protein and involved in anti-angiogenesis and has tumor suppressive activity in breast cancer [80]. *RARRES1* (retinoic acid receptor responder 1) also has tumor suppressive activity and plays an important role in tumorigenesis [81, 82]. The function of CYB561 is currently unclear in prostate cancer. The reversed expression of *NTN4* and *RARRES1* and their function as tumor suppressors suggest that they have important roles in prostate cancer during the transition from androgen sensitive to castration resistant.

Although the frequency of the same ARGs responding to androgen ablation and castration resistance is very low based on current gene expression profiling analyses, many studies have demonstrated that androgen receptor function is reactivated in CRPC as upregulation of androgen receptor was detected in CRPC, compared to androgen-sensitive tumors [16, 60, 62, 63]. This observation is consistent with studies that have examined xenograft models [83]. Although androgen receptor was not differentially expressed between androgen ablation treated prostate and untreated prostate [17, 60], upregulation of its expression and reactivation of its transcriptional activity in CRPC tumors indicate its critical role in CRPC [44, 52, 54]. The recent discovery of constitutively active splice variants of androgen receptor that lack ligand-binding domain, especially V567es that seems to be expressed exclusively in response to androgen ablation [51, 84], may provide an important marker of CRPC and indication of eminent failure to current therapies that target the ligand-binding domain of full-length androgen receptor. These variants appear to regulate a unique transcriptome that is different from full-length androgen receptor. A recent study demonstrated that androgen receptor splice variants regulate expression of cell cycle genes in the absence of full-length androgen receptor, while the full-length androgen receptor mainly regulates genes associated with macromolecular synthesis, metabolism, and differentiation in CRPC [85]. Importantly, their studies revealed that expression of splice variant of androgen receptor, but not the full-length

Table 9.1 Profiling of androgen-responsive genes in clinical patient samples

Study	Samples	Method
Holzbeierlein et al. [60]	(a) Benign prostate tissues (peripheral zone) of cancer patients (b) Primary prostate cancer (radical prostatectomy; no other therapy) (c) Primary prostate cancers [3-month androgen ablation therapy (goserelin + flutamide)] (d) Metastatic prostate cancer (3 of 9 progressed after 5–10 year of androgen ablation therapy) (e) LNCaP +/− R1881 (0.1 nM for 24 h and remove R1881 for 36 h)	Total RNA Affymetrix U95 oligonucleotide array (~54,000 genes and EST)
Best et al. [61]	(a) Primary androgen-dependent tumor (untreated) (b) Primary androgen-independent tumor (different hormonal treatments plus on clinical trial for docetaxel and thalidomide) *Laser capture microdissection for epithelial cells	Total RNA Affymetrix U133A oligonucleotide array (18,400 transcripts)
Varambally et al. [2]	(a) Benign prostate tissues (b) Clinically localized, i.e., primary cancer (c) Metastatic hormone-refractory cancer (androgen ablation, and/or chemotherapy and/or radiation therapy) [66]	Proteomics: – High-throughput immunoblot (1,354 proteins; 521 at expected molecular weight) – Tissue microarray
Stanbrough et al. [62]	(a) Primary androgen-dependent tumor (b) Metastatic androgen-independent tumors from bone marrow *Laser capture microdissection for tumor cells from primary tumor *Select bone marrow metastatic samples with high tumor content and without erythroid and myeloid cells	Total RNA Affymetrix U133A array (22,283 probes)
Mostaghel et al. [17]	(a) Prostate tissues from healthy men treated with or without 1-month Acyline (placebo, acyline or acyline + testosterone) (b) Prostate tumor from localized prostate cancer (androgen deprivation therapy for 0, 3–6, or 6–9 months) *Laser capture microdissection for epithelial cells	Total RNA Custom cDNA array (~6,700 unique cDNA clones)
Chandran et al. [16]	(a) Normal prostate tissues (b) Primary tumor tissues (c) Metastatic androgen ablation-resistant samples (androgen ablation) *Genes mainly expressed in stromal cells were removed	Total RNA – Affymetrix HGU95av2, HGU95b, HGU95c oligonucleotide array – CodeLink oligonucleotide array

(continued)

Table 9.1 (continued)

Study	Samples	Method
Tamura et al. [63]	(a) Normal prostatic epithelial cells (b) Primary hormone-sensitive prostate cancer (no treatment) (c) Primary or metastatic hormone-refractory prostate cancer (androgen ablation) *Laser capture microdissection for cancer or epithelial cells	Total RNA Genome-wide cDNA array (36,864 cDNA)
Romanuik et al. [46]	(a) Primary androgen-dependent prostate tumor (no treatment) (b) Primary tumor without recurrent within 5 years (c) Primary tumor with recurrent disease within 5 years (biochemical failure) (d) Lymph node metastatic androgen-independent tumors *Laser capture microdissection for cancer or benign epithelial cells	Total RNA QRT-PCR (27 genes)

androgen receptor, was strongly correlated with expression of the cell cycle gene *UBE2C* in CRPC.

Interestingly, although downregulation of *TMPRSS2* was detected in prostate tissues (benign or malignant) following short-term castration, no differential expression was detected in clinical CRPC compared to primary tumors in gene expression profiling analyses. Instead, upregulation or restoration of expression of androgen receptor regulated *TMPRSS2-ERG* fusion, together with androgen receptor reactivation, were detected in other studies of CRPC [73]. Increased levels of *TMPRSS2-ERG* fusion may contribute to the progression to CRPC by altering genes associated with cell proliferation under regulation by androgen receptor. On the other hand, TMPRSS2-ERG fusion can inhibit androgen receptor signaling. Yu et al. [71] proposed a model showing that TMPRSS2-ERG interferes with the balance between androgen receptor-mediated differentiation and EZH2-mediated dedifferentiation of prostate cells as well as inhibits androgen receptor signaling, thus leading to a selective pressure for development of CRPC [71]. Of note, non-androgen receptor-mediated signaling pathway, such as phosphatidylinositol 3-kinase/Akt pathway involving cell survival and proliferation, may also contribute to CRPC development and the aggressive phenotype of tumor cells [60, 63, 86].

Similar to gene expression profiles of tumors after androgen ablation treatment, the expression profiles for CRPC showed very few common genes amongst different studies. However, several cellular functions including coordinated ARGs and response to androgen were altered in CRPC. These included altered expression of steroidogenic enzymes of androgen biosynthetic and metabolic pathways in CRPC as compared to androgen-sensitive tumors [16, 17, 60, 62, 87, 88]. Examples include *AKR1C3*, *HSD3B2*, and *SRD5A1* for biosynthesis and *AKR1C1*, *AKR1C2*, *UGT2B15*, and *UGT2B17* for metabolism. Increased expression of *CYP17A1* and *HSD17B3* was also detected in CRPC [89]. The enzyme CYP17A1 produces androgens from progestins and the enzyme HSD17B3 converts testosterone from androstenedione [89]. The altered expression of *SRD5A* genes (*SRD5A1* and *SRD5A2*), encoding 5-alpha-reductases, was detected in CRPC clinical samples as well as in the DuCaP cell line [89, 90]. Increased expression of these enzymes in CRPC provides the availability of androgen and conversion of low-level androgen to testosterone and DHT to allow reactivation of androgen receptor to ultimately overcome androgen ablation therapies. Identification of these genes enables development of therapeutic strategies to ultimately reduce levels of residual androgens in CRPC. One example of such a therapy is abiraterone, an inhibitor of CYP17A1 enzyme that blocks biosynthesis of androgen [91]. Mostaghel and Nelson [92] raised several questions regarding the targeting steroidogenic enzymes for CRPC treatment. The treatment outcome may be affected by the choice of a particular steroidogenic enzyme to inhibit duration of androgen ablation therapy in CRPC patients and the specific tissues/sites of targeting, e.g., adrenal gland or prostate tumor [92].

Other altered cellular functions of identified ARGs involve cell–cell interaction and cell adhesion. Upregulation of *SPP1*, *FN1*, or *CDH11*, all involved in cell adhesion, was detected in CRPC [16, 61, 62]. Osteopontin (*SPP1*) promotes metastasis [16] and cadherin11 (*CDH11*) contributes to metastasis [93]. The role of fibronectin-1 (*FN1*) in prostate cancer is unclear. However, induction of apoptosis by tumor necrosis factor-alpha can be blocked by fibronectin in LNCaP cells [94].

9.5 Summary

Comparison of gene expression profiling studies of cell lines, xenografts, and clinical tissues revealed that only a few of the differentially expressed ARGs were common amongst the different samples. These ARGs included *UBE2C*, *SPON2*, *FKBP5*, *TMPRSS2*, and *KLK3* (PSA). Although the majority of differentially expressed ARGs were different, three major cellular functions were commonly affected by androgen during progression of prostate cancer and were (1) androgen biosynthesis and metabolism; (2) cell cycle and proliferation; and (3) cell adhesion (Fig. 9.2). ARGs with differential expression included *CYP17A1* and *SRD5A* (steroidogenic enzymes), *UBE2C* (cell cycle), *TMPRSS2* (cell proliferation), and *SPP1* and *CDH11* (cell adhesion). These ARGs are potential candidates as diagnostic biomarkers or

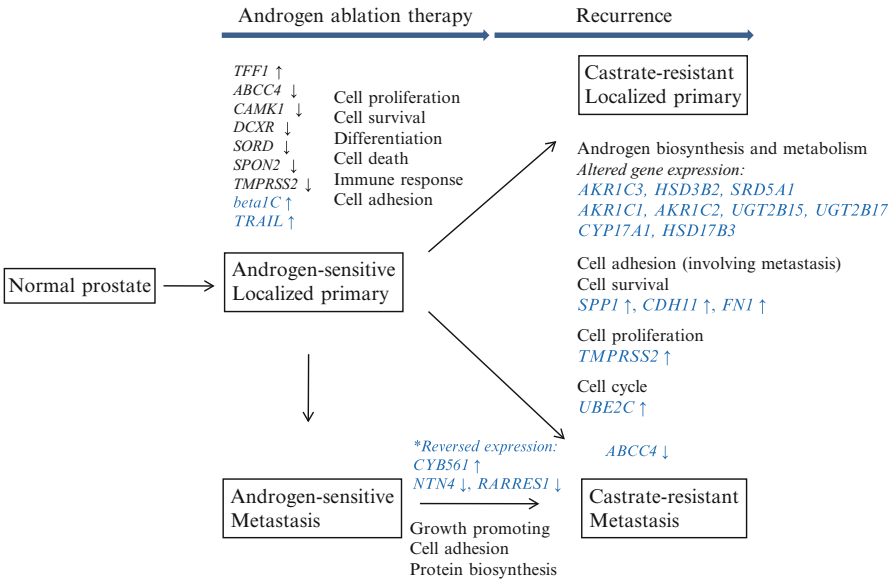

Fig. 9.2 ARG change leading to disease stage change (under and following androgen ablation therapy) in clinical patients. Altered cellular functions and ARGs are listed. (*black*: common genes found between studies; *blue*: detected but may not be common between different studies). *Asterisk* indicates reversed expression detected in CRPC by comparing castrate-resistant metastatic samples with androgen-sensitive metastatic samples from Holzbeierlein et al. [60] studies

therapeutic targets. Evaluation of expression of these and other ARGs may provide insight of androgen receptor activity in prostate cancer and be utilized for personalized therapies by shedding light on efficacy of hormonal therapies, sequencing of therapies, selection of patients for clinical trials, and mechanisms of resistance.

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