Molecular Science

AIMS Molecular Science, 3(2): 280-299.
DOI: 10.3934/molsci.2016.2.280
Received 22 April 2016,
Accepted 5 June 2016,
Published 7 June 2016

http://www.aimspress.com/journal/Molecular

Review

Androgen receptor and prostate cancer

Karen E. Livermore*, Jennifer Munkley, and David J. Elliott

Institute of Genetic Medicine, Newcastle University, Newcastle-upon-Tyne, UK

*** Correspondence:** Email: k.e.livermore@newcastle.ac.uk.

Abstract: Androgens play a key role in the development and progression of prostate cancer, and androgen deprivation therapy (ADT) is the first line treatment for advanced disease. Although ADT is initially successful in controlling prostate cancer, many patients eventually become resistant to therapy and progress to develop lethal castration-resistant prostate cancer (CRPC). Androgens drive prostate cancer cell growth via the androgen receptor (AR), which is a transcription factor essential for prostate cancer cell viability, proliferation and invasion and has important roles in a range of signalling pathways. The progression to CRPC is thought to involve persistence of AR signalling and reprogramming of the AR transcriptional landscape to allow tumour cells to continue to grow despite low levels of circulating androgens. During this time AR activity can be maintained through activating mutations, gene amplification, AR splice variants or signalling crosstalk with other pathways. CRPC is highly aggressive and ultimately lethal, meaning there is an urgent need to understand the mechanisms that drive this form of the disease and to develop new therapeutic targets. This review discusses the role of the AR signalling in some of the many mechanisms and pathways that contribute to the development of prostate cancer and the progression to castrate resistant disease.

Keywords: prostate; prostate cancer; androgens; androgen receptor; castrate resistant prostate cancer

1. The prostate gland

The prostate is a small glandular organ situated within the pelvic cavity of males, beneath the bladder and surrounding the urethra. The main function of the prostate is to produce prostatic fluid, a component of semen which protects and enhances the survival of sperm cells. Structurally, the mature prostate is divided into four distinct zones, the transition zone, the central zone, the peripheral zone and a fibromuscular stroma (Figure 1a). The transition, central and peripheral zones contain highly organised glandular epithelium structures separated by a fibromuscular stromal network [1].

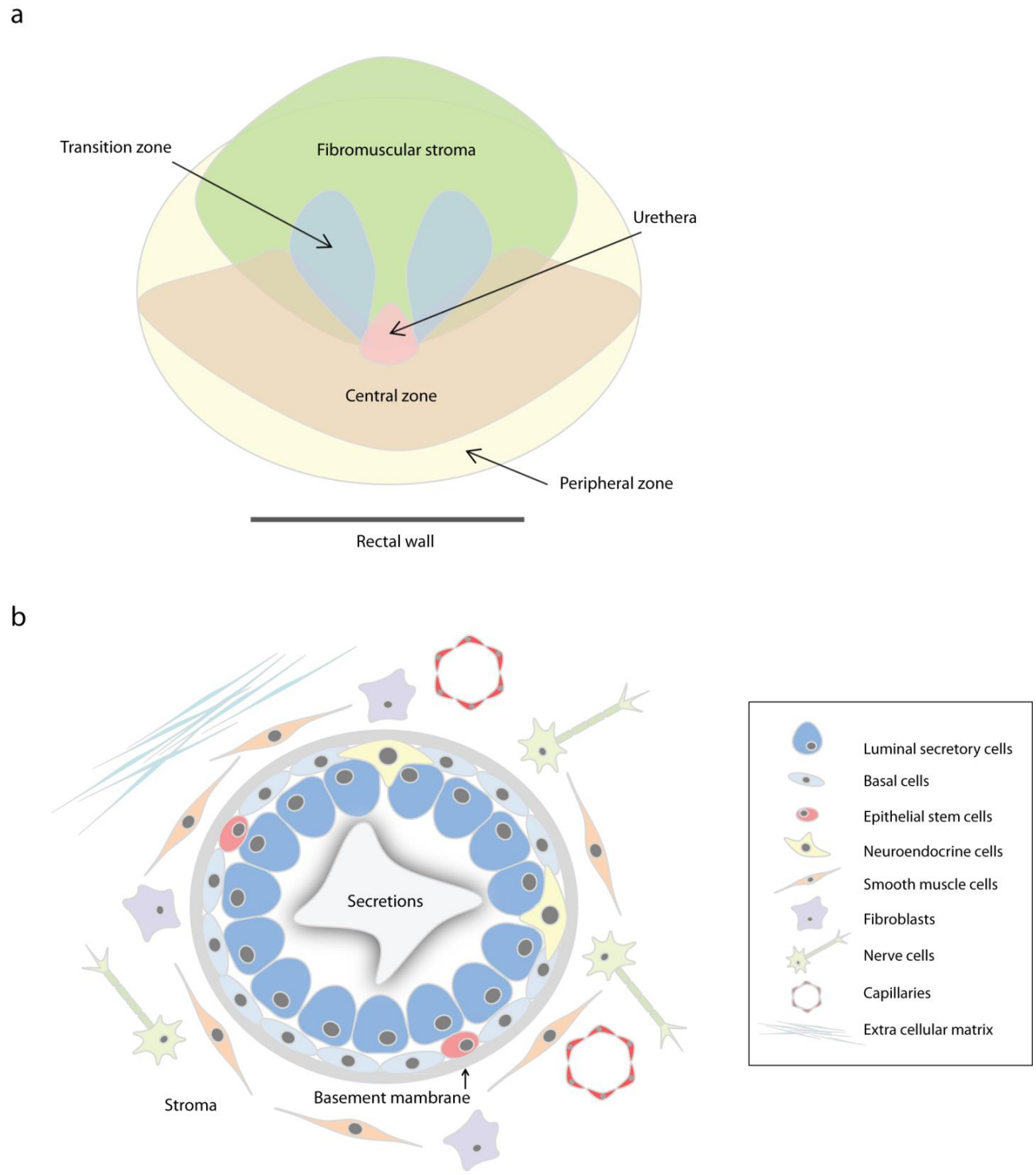

Figure 1. (a) Zonal anatomy of the prostate—the prostate is divided into four distinct zones; three glandular zones (peripheral zone, central zone and transition zone) and a fibromuscular stroma. **(b) Cells within with the prostate gland**—arranged within the basement membrane of prostate glands are basal cells, luminal secretory cells and a small number of neuroendocrine cells. The fibromuscular stroma contains smooth muscle cells, fibroblasts, nerve cells, blood vessels within an extracellular matrix.

Arranged within the basement membrane of these glandular epithelium structures are transient stem cells (~1%), basal cells (40%), luminal secretory cells (60%) and a small number of neuroendocrine cells. The surrounding fibromuscular stromal tissue contains smooth muscle cells, fibroblasts, nerve cells, blood vessels, extracellular matrix and lymphatics (Figure 1b) [2].

The size of the prostate can increase with age, resulting in a condition termed benign prostatic hyperplasia (BPH). This non-malignant condition is common in men >60 years [3] and is characterised by progressive hyperplasia of glandular and stromal tissues within the transitional zone of the prostate [4]. BPH is not usually serious, but the increased growth can sometimes impact on the urethra causing discomfort and leading to complication such as acute urinary tract infections (UTI) and urinary retention (AUR) [5,6].

2. Prostate cancer

Prostate cancer (PCa) is the second most common type of non-skin cancer in men, after lung cancer. There were an estimated 1.1 million new cases of PCa in 2012 worldwide, accounting for around 15% of all new cancer diagnoses [7]. In the United Kingdom, there are over 47,000 new PCa cases diagnosed each year with around 10,000 deaths and it is predicted that approximately 1 in every 5 men will be diagnosed with PCa during his lifetime [8]. PCa incidence rates increase with age and are highest in men ≥65 years. PCa incidence is expected to rise with an increasingly aging population.

PCa is a heterogeneous disease and the process of initiation is not fully understood. As with many cancers, two main models of tumour initiation and progression have been proposed. The clonal evolution model involves multiple genetic and epigenetic changes within a single cell of origin which confer a selective growth and survival advantage to produce a dominant clone. Genetic instability within the expanding tumour population produces further mutant cells creating tumour cell heterogeneity [9]. The cancer stem cell model suggests that the tumour originates from a small sub-population of tumour initiating cells that have retained the ability to self-renew, generating heterogeneity through differentiation [10,11]. For either model of cancer development, the complex heterogeneity of the disease creates a major challenge for treatment.

PCa diagnosis usually involves measurement of serum prostate-specific antigen (PSA) levels, a digital rectal examination, and a needle core biopsy sampling. PSA is a serine protease which is secreted almost exclusively by the epithelial cells of the prostate [12]. PSA is commonly used as a biomarker of PCa as disruption of the prostatic epithelium allows PSA to leak into the circulating blood stream [13]. However, PSA use as a PCa biomarker is controversial as it does not distinguish between PCa and other non-malignant conditions such as BPH, infection or chronic inflammation [14]. Several new PCa biomarkers are currently being investigated, including the use of tumour specific PSA isoforms [15] and PSA glycan signatures [16], as well as non-invasive urine-based biomarkers such as detection of prostate cancer antigen 3 (PCA3) RNA [17] and TMPRSS2:ERG fusion transcripts [18]. These new approaches may prove to be more reliable at detecting PCa, thus helping to reduce the over diagnosis and over treatment issues associated PSA screening.

The Gleason grading system is used in combination with PSA screening to categorise hematoxylin and eosin (H&E) stained prostatic tissue sections from biopsy samples. The morphology and structural arrangement of carcinoma cells help separate prostate tumours into five basic grades, from grade 1 (well-differentiated, small uniform glands) to grade 5 (poorly-differentiated, occasional gland

formation) [19]. These grades are used to generate an average Gleason score indicating the clinical stage of the tumour, possibility of progression to metastatic disease, and patient's treatment/survival prognosis [20].

3. Androgen receptor signalling

Androgens play a key role in the growth and function of the prostate. Androgens are a group of steroid hormones of which testosterone is the most prevalent in males. Testosterone is primarily produced in the testes by the Leydig cells (90%), although small amounts are also produced by the adrenal glands (10%). Testosterone production is regulated through the hypothalamic-pituitary-gonadal (HPG) axis. Pulses of GnRH (gonadotropin-releasing hormone) are secreted from the hypothalamus to stimulate the release of LH (luteinising hormone) and FSH (follicle-stimulating hormone) from the anterior pituitary gland, this in turn stimulates the synthesis of testosterone. Circulating testosterone levels regulate the further production of GnRH to create a feedback loop [21].

The AR is a nuclear steroid hormone receptor which functions as a ligand dependant transcription factor. The human *AR* gene is located on the X chromosome (Xq11-12) and spans >90-kb of DNA [22]. Eight coding exons in the *AR* gene [23] encode a 110-kDa protein with four functionally distinct domains: an N-terminal domain (NTD), a DNA-binding domain (DBD), a small hinge region and a ligand-binding domain (LBD). The first large *AR* gene exon encodes the highly variable NTD, which contains several regions of repetitive DNA sequences (CAG tri-nucleotide repeat) [24]. The highly conserved DBD contains two zinc finger domains and is encoded by exons 2 and 3 [25], whilst exons 4 to 8 encode the C-terminal LBD. The AR protein contains two trans-activation domains, the hormone independent activation function 1 (AF1) is located within the NTD and the hormone-dependent activation function 2 (AF2) within the LBD (Figure 2a). Not all cell types within the prostate gland are AR-positive. Whilst the luminal secretory cells express high levels of AR [26], the majority of basal cells, neuroendocrine cells, and stem cells are AR-negative and function independently of androgens [27].

In the prostate, testosterone is converted to dihydrotestosterone (DHT) by 5α-reductase enzymes [28]. The action of DHT is dependent upon binding to the androgen receptor (AR). In the prostate DHT has a 10-fold higher binding affinity for the AR than testosterone [29]. In its inactive form, the AR is located in the cytoplasm bound to heat shock proteins (specifically HSP90) and other chaperone molecules [30]. In the "genomic signalling" AR pathway, binding of DHT to the ligand binding domain (LBD) of the AR induces a series of conformational changes that dissociate the AR from the HSPs and chaperone molecules. These changes promote AR phosphorylation and its translocation to the nucleus, where the activated AR interacts with co-activators and binds as a dimer to androgen response elements (AREs) found in the promoter regions of target genes [31]. The AR controls transcription of many genes which are involved in cell growth and survival [32] as well as prostate-specific antigen (PSA) [33] (Figure 3a).

This classical AR genomic signalling pathway depends on AR nuclear translocation and DNA binding for transcription and cell proliferation, a process which occurs over several hours. In contrast, the "non-genomic AR signalling pathway" involves interactions within minutes between AR and intracellular signalling molecules in the cytoplasm. Activated AR can interact directly with the p85α regulatory subunit of PI3K (phosphoinositol 3-kinase) [34], SH3 (Src homology 3) domain of Src [35] and Ras/Raf-1 [36] leading to MAPK/ERK (mitogen-activated protein kinase/extracellular signal

regulated kinase) activation and subsequent cell proliferation. Non-genomic AR signalling may also enhance AR genomic activity. AR activated kinases can directly phosphorylate AR even in the absence of ligand binding [37], creating an autocrine feedback loop (Figure 3b).

4. Androgen receptor in prostate cancer

In 1941, Huggins and Hodges were the first to demonstrate the androgen dependency of PCa growth and progression [38]. Androgens and the AR have since been portrayed as the crucial players in both localised and advanced disease [39] and have been the major target for therapeutic treatment of PCa for many years.

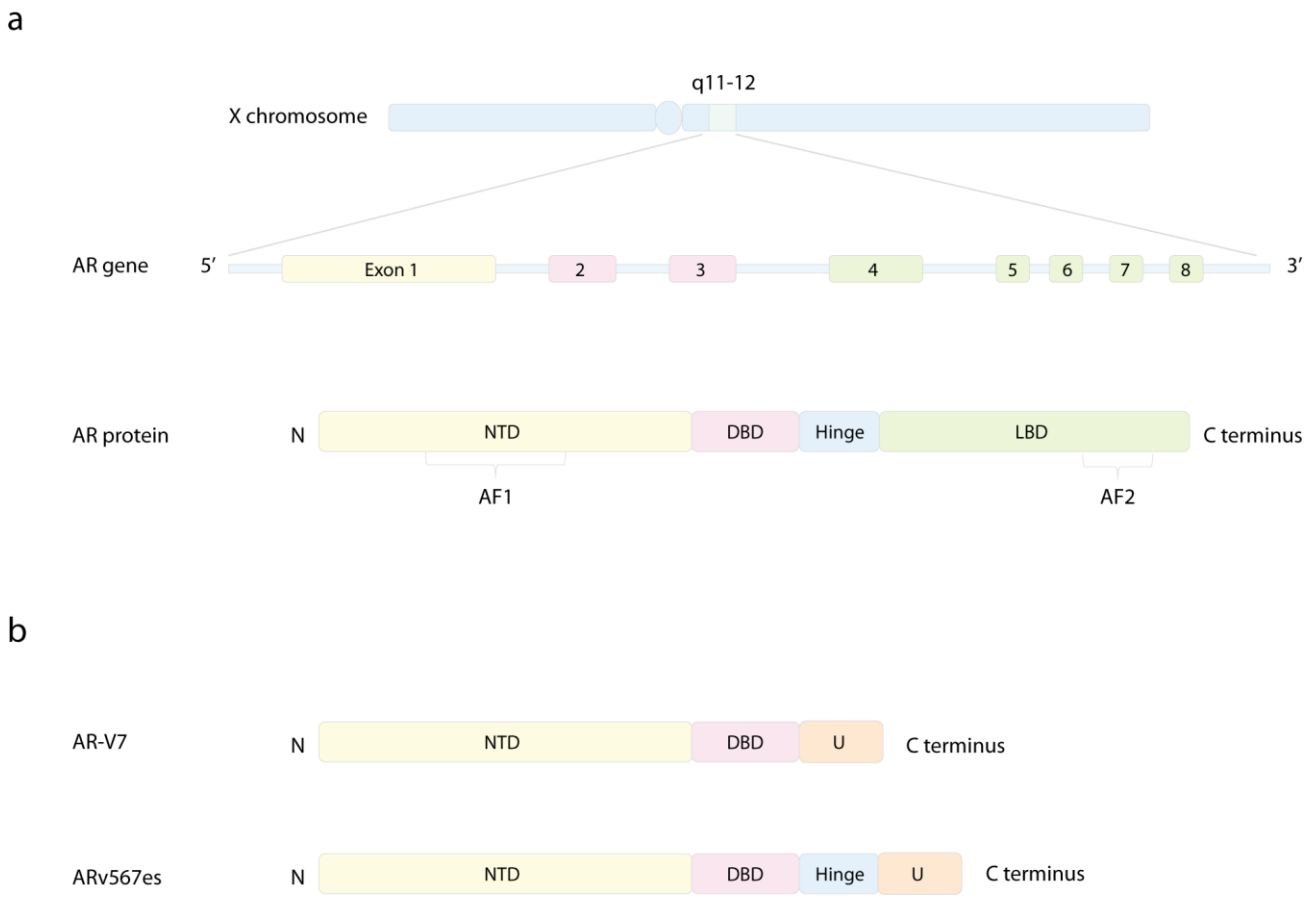

Figure 2. (a) The androgen receptor gene—the *AR* gene is located on the X chromosome (Xq11-12) and has eight coding exons. The full length AR protein has four functionally distinct domains: an N-terminal domain (NTD), a DNA-binding domain (DBD), a small hinge region and a ligand-binding domain (LBD). These four domains include two trans-activation domains, AF1 and AF2. **(b) Common cancer associated androgen receptor splice variants**—the most common AR splice variants AR-V7 and Arv567es lack a functional LBD and are constitutively active. U—unique variant specific C terminal sequence.

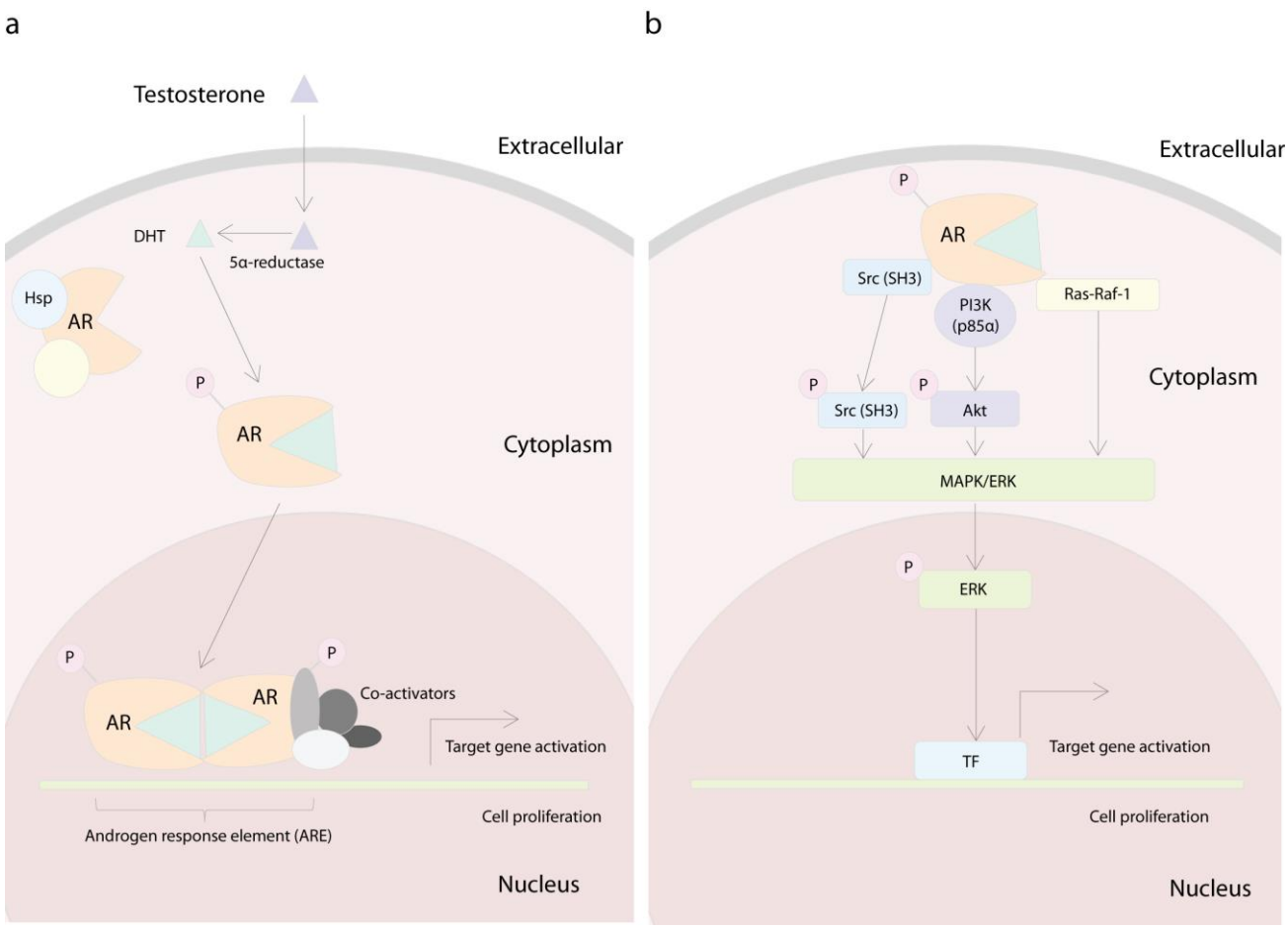

Figure 3. (a) Classical genomic androgen receptor signalling in the prostate—testosterone enters the prostate cell where it is converted to DHT by the enzymes 5α-reductase. Inactive AR is located in the cytoplasm bound to heat shock proteins (Hsp) and other chaperone molecules. Binding of DHT to the AR induces a series of conformational changes that dissociate the AR from the Hsps and promotes AR phosphorylation and translocation to the nucleus. In the nucleus the activated AR interacts with co-activators and binds as a dimer to androgen response elements (AREs) of target genes leading cell proliferation. **(b) Non-genomic androgen receptor signalling in the prostate**—activated AR can interact directly with numerous intracellular molecules including the p85α regulatory subunit of PI3K, the SH3 (Src homology 3) domain of Src and Ras/Raf-1 leading to MAPK/ERK activation and subsequent cell proliferation.

The current treatment options for localised PCa and locally advanced PCa include active surveillance, radical prostatectomy and types of radiation therapy such as external beam radiotherapy, permanent seed brachytherapy, high-intensity focused ultrasound (HIFU) or cryotherapy to remove or kill tumour cells. The main treatment options for advanced metastatic PCa are chemotherapy with docetaxel (Taxotere®) and androgen deprivation therapy (ADT). Reduced serum testosterone levels are achieved by surgically removing the testicles (orchidectomy) [40] or using a combination of GnRH agonists and antagonists such as goserelin (Zoladex®), leuprorelin acetate (Prostap®), triptorelin (Decapeptyl®) and degarelix (Firmagon®) to suppress the production of testosterone [41],

and AR antagonists such as bicalutamide (Casodex®), and flutamide (Eulexin®) which block AR function [42].

Despite an initial response to ADT, many patients go on to develop castration-resistant prostate cancer (CRPC) within a few years [43], in which the tumour cells develop mechanisms which allow them to continue to grow despite depleted androgen levels. This has led to the development of second generation AR signalling inhibitors such as Abiraterone (Zytiga®) and Enzalutamide (Xtandi®). Abiraterone is an irreversible inhibitor of the enzyme CYP17A1, which is designed to inhibit extragonadal testosterone synthesis from the adrenal glands and the tumour microenvironment [44]. Enzalutamide is an AR antagonist which works by binding to the LBD of the AR, thus inhibiting its translocation to the nucleus, chromatin binding and interactions with co-regulators [45]. Radium-233 (Xofigo®), a radiopharmaceutical agent has recently been approved for the treatment of CRPC patients with bone metastases [46]. Although these agents have been modestly successful at prolonging the overall survival of PCa patients, resistance mechanisms inevitably develop which will continue to drive disease progression. CRPC is highly aggressive and ultimately lethal, meaning there is an urgent need to understand the mechanisms that drive this form of the disease and to develop new therapeutic targets.

5. Splicing

Most human genes produce multiple mRNA isoforms that can be translated into a diverse range of proteins often with distinct functions and cellular localisation. Aberrant splicing as a result of defective splicing regulation has been associated with the onset and progression of many types of cancer, including PCa [47]. Over 200 genes are known to express PCa-specific splice variants, including the *AR* itself, *KLK3* (kallikrein 3) which encodes PSA [48], *KLF6* (kruppel-like factor 6) [49], *ACTN1* (actinin 1), *CALD1* (caldesmon 1), *VCL* (vinculin), *COL6A3* (collagen type VI 3), *TPM1* (tropomyosin 1) [50], *FGFR2* (fibroblast growth factor receptor 2) [51] and the tumour suppressor gene *TSC2* (tuberous sclerosis complex 2) [52]. Alternative promoters can also be important in producing mRNA isoforms: these include an androgen regulated alternative isoform of *TSC2* mRNA that has been shown to increase cell proliferation [53].

The up-regulation of several splicing regulators has been shown to alter the splicing profile of key genes involved in PCa. These include the RNA-binding proteins SAM68 (also known as KHDRBS1, KH domain containing, RNA binding, signal transduction associated 1) [54,55], SRSF1 (serine/arginine-rich splicing factor 1) [56] and DDX5 (DEAD (Asp-Glu-Ala-Asp) box helicase 5) [57]. SAM68 can alter signal dependent splicing and transcriptional activity of the AR [55].

6. Downstream regulated pathways

AR signalling has been directly linked to numerous processes known to be important in prostate cancer development and progression, including central metabolism and biosynthesis [32], lipid and cholesterol biosynthesis [58-60], fatty acid metabolism [61-63], response to ER stress [64,65], and most recently glycosylation [66].

Aberrant glycosylation is a prevalent feature in cancer and has been linked to PCa progression [66-71]. A number of glycosylating enzymes are AR regulated and over-expressed in PCa including UAP1, ST6GALNAC1 (ST6 alpha-N-acetyl-neuraminyl-2, 3-beta-galactosyl-1,

3-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1), GCNT1 (glucosaminyl (N-acetyl) transferase 1), GALNT7 (polypeptide N-acetylgalactosaminyltransferase 7), PGM3, CSGALNACT1 (chondroitin sulfate N-acetylgalactosaminyltransferase 1), ST6GAL1 (ST6 beta-galactosamide alpha-2,6-sialyltranferase-1) and EDEM3 (ER degradation enhancer, mannosidase alpha-like 3) [72]. Over-expression of UAP1, the last enzyme in the HBP pathway, has been observed in tumour tissue from PCa patients and correlates positively with AR expression [73]. Increased ST6GALNAC1 expression in PCa cells increases cell mobility and decreases cell adhesion [74] and GCNT1 expression is associated with the aggressive potential of PCa [75,76].

The PI3K-AKT signalling pathway is another important player in prostate cancer progression, and has been shown to be altered in 42% of primary and up to 49% of metastatic tumours [77]. Loss of the tumour suppressor PTEN, a negative regulator of the PI3K/AKT signalling pathway, has been identified in almost all advanced metastatic CRPC cases [78] together with mutations in PIK3CA, AKT1 and PIK3CA [79]. Reciprocal crosstalk between AR signalling and the PI3K pathway has been identified as possible mechanism underlying CRPC [80]. Expression of the PI3K regulatory sub-unit PIK3R1 is androgen regulated and repressed in PCa tissue, suggesting a transcriptional link between AR signalling and the PI3K pathway [81], and supporting combinatorial inhibition of AR and PI3K signalling to significantly reduce progression to CRPC. Another common feature of PCa is activation of the RAS/ERK1/2 signalling pathway, which is mutated in 43% of primary PCa tumours and 90% of PCa metastases [77]. Hyperactivation of RAS/ERK1/2 is thought to be due to loss of negative regulators of the pathway, including sprouty genes and PTPRR, which are both directly repressed by the AR [81,82]. Activation of RAS/ERK1/2 is thought to serve as a potentiating second hit to loss of PTEN to accelerate PCa progression [82].

There is increasing evidence for the role of the Wnt/β-catenin pathway in the progression to CRPC (reviewed in [83]). β-catenin (CTNNB1) interacts with the AR enhancing transcriptional activity by altering the sensitivity and the specificity of the receptor binding to ligands [84,85]. Increased expression of nuclear β-catenin has been observed in advanced metastatic and CRPC compared with primary PCa tumours [86,87]. Activating mutations in β-catenin [79,88] and recurrent alterations in APC [79] have been described in CRPC patients.

7. The development of castrate resistant prostate cancer

There are many mechanisms and alternative pathways associated with the androgen-independent growth observed in CRPC, the majority of which involve androgens and are mediated by the AR. Therefore, suppression of AR signalling remains the therapeutic goal in the treatment of prostate cancer.

7.1. Altered steroidogenesis

Despite the low serum testosterone levels obtained after ADT, intratumoral testosterone levels can remain sufficient enough to induce cancer progression, suggesting that altered steroidogenesis pathways have been activated. Several studies have now demonstrated that PCa cells are able to produce testosterone from different androgen precursors, such as cholesterol [89] and the adrenal androgen dehydroepiandrosterone (DHEA) [90]. In addition, several genes involved in testosterone biosynthesis become up-regulated in CRPCs [91,92]. These include *AKR1C3* (aldo-keto reductase

family 1, member C3), which encodes an enzyme which catalyses the conversion of androstenedione to testosterone, SRD5A1/2 (steroid-5-alpha-reductase, alpha polypeptide 1/2) which converts testosterone to DHT, *CYP17A1* (cytochrome P450 17A1) and *HSD17B6* (hydroxysteroid (17-Beta) dehydrogenase 6) [93].

7.2. AR amplification and hypersensitivity

Increased AR levels have been identified in CRPC cell lines [94] and occur in 20 to 30% of CRPC cases [95]. AR amplification allows tumour cells to become hypersensitive to low levels of testosterone. An excess in AR production can result from AR gene amplification, increased mRNA transcription or stabilisation of the mRNA or protein [96]. The mechanisms underlying AR hypersensitivity remain unclear but are thought to be a response mechanism to the selective pressure imposed within an androgen-depleted environment [97]. AR overexpression is the most frequent genetic alteration observed in CRPC, with AR copy number gain detected in up to 50% of patients [79,98,99]. Gene amplification and copy number variations in both *AR* and *CYP17A1* have been detected in circulating tumour cells (CTCs) and cell-free tumour DNA (ctDNA) from metastatic CRPC patients indicating a possible mechanism for the resistance to treatment with second generation therapies (abiraterone and enzalutamide) [100-103].

7.3. AR mutations and splice variants

AR mutations have been found in around 10% to 30% of CRPC patients [104]. The McGilll Androgen Receptor Gene Mutation Database (available at: http://androgendb.mcgill.ca) contains extensive details of 1110 AR mutations, 168 of which have been associated with PCa. The majority of mutations identified in CRPC are found within the LBD (49%) followed by the NTD (40%), DBD (7%) and hinge region (2%) [105]. The most frequent AR mutation is the point mutation T877A which substitutes a threonine for alanine at position 877. The T877A mutation is found within the LBD of the AR and occurs in around one-third of CRPC cases [106]. Mutations in the LBD broaden binding specificity resulting in activation by multiple endogenous hormones including estrogens, progesterone and even the androgen antagonist flutamide [107]. Mutations that occur in the NTD and DBD could modulate the receptors affinity for co-regulator and influence nuclear localisation [108].

A large number of constitutively active AR splice variants have been identified. The most prevalent of these AR isoforms are AR-V7 [109] and ARv567es [110], both of which lack a functional LBD but maintain a nuclear localization signal (NLS) (Figure 2b). These changes ensure a constitutive nuclear localisation and facilitates AR signalling in the absence of androgens, or in the presence of enzalutamide [111,112]. Patients with high expression levels of AR-V7 and ARv567es have particularly poor prognoses with significantly shorter survival rates [113]. AR-V7 mRNA transcripts have been detected in CTCs of metastatic CRPC patients and are highly predictive for resistance to treatment with abiraterone and enzalutamide [114,115], highlighting this molecule as a potential prognostic and predictive biomarker.

7.4. Co-activators and co-repressors

Many different proteins have been identified as co-regulators for AR. These proteins can

function to either enhance (co-activators) or repress (co-repressors) transcriptional activity of the AR. Expression of these co-regulatory proteins changes during the different stages of PCa progression, and can affect many cellular functions such as proliferation, apoptosis, migration, invasion and differentiation [116]. Increased expression of several AR co-activators has been observed during ADT, including of P300, CBP and Tip60 [117-119]. The P300/CBP pathway promotes androgen-independent IL-6 mediated AR activation [120], whilst Tip60 promotes cell proliferation by translocation of AR into the nucleus [121].

7.5. Ligand-independent activation

Although ADT works to repress AR signalling, there are a number of cytokines and growth factors that continue to stabilise the AR, enhancing transcriptional activity independently of ligand binding. Interleukin-6 (IL-6) is a multifunctional cytokine important for immune regulation and which regulates cell growth [122]. Androgens induce the expression of IL-6 in the androgen sensitive LNCaP PCa cell line [123]. Reciprocally, IL-6 can regulate AR activity in a ligand-independent and synergistic manner even in low concentrations of androgens [124,125]. Serum IL-6 levels are a significant prognostic factor in PCa and elevated IL-6 serum levels have been reported in CRPC patients [126]. The JAK-STAT (janus kinase/signal transducers and activators of transcription), MAPK and PI3K-AKT signalling pathways have been shown to be important in the AR activation by IL-6 [127,128].

The epidermal growth factor receptor (Her2/neu) is a receptor tyrosine kinase oncoprotein that plays a major role in cell growth and differentiation [129]. Gene amplification and over expression of the Her2/neu protein drive the progression of many types of cancers, including breast and ovarian cancers (Her2/neu gene amplification is found in ~25% of breast cancers) [130]. In prostate cancer, Her2/neu expression increases with progression to CRPC [131], promoting cell growth and survival in the absence of androgens through the activation of Akt (protein kinase B) [132].

The transcription factor nuclear factor kappaB (NF-κB) plays a critical role in cancer development and progression [133]. The AR is thought to activate NF-κB signalling in the absence of androgens and represses NF-κB in the presence of androgens [134]. Constitutive activation of NF-κB signalling in the absence of androgens significantly increases AR mRNA and protein levels, AR trans-activation activity and cell proliferation *in vitro* [135]. NF-κB2 (p52) interacts directly with the NTD of the AR, enhancing nuclear translocation, activation and enhances the recruitment of co-activators such as p300 to the promoter region of AR-dependent genes [136].

7.6. Neuroendocrine differentiation

Neuroendocrine cells in the prostate are rare and are found interspersed between the luminal secretory and basal cells within the prostate gland. Unlike the luminal secretory and basal cells, neuroendocrine cells are AR negative, non-proliferative, terminally differentiated cells [137,138]. They secrete a range of growth factors and hormones which can stimulate proliferation and inhibit apoptosis in the surrounding cells including chromogranin A (CgA), parathyroid hormone-related protein (PTHrp), bombesin (BBN), vascular endothelial growth factor (VEGF) and many more [139].

Neuroendocrine differentiation refers to the trans-differentiation of PCa cells toward a neuroendocrine phenotype, induced in response to the androgen-depleted environment created by

ADT [140]. The induction of neuroendocrine differentiation in PCa cells by androgen depletion is well documented *in vitro* [141] and in PCa xenografts in mice [142]. Neuroendocrine differentiation is significantly increased in CRPC [143], and relates to a more aggressive behaviour and less favourable prognosis [144]. A number of molecular signalling molecules and pathways have been shown to promote neuroendocrine trans-differentiation in prostate cancer cells including, IL-6 [145,146], isoform 1 of the TPD52 protein [147], Fyn kinase [148], Wnt-11 [149] and the PI3K-Akt-mTOR pathway [150]. However, the mechanisms involved are not fully understood.

Although AR is the main regulator and therapeutic target in PCa, other endocrine systems have also been linked to PCa development and tumour progression. Estrogen acting via its receptors (ERα and ERβ), can regulate proliferation, differentiation, apoptosis, EMT, invasiveness and chronic inflammation in prostate cancer cells (reviewed in [151]). Relaxin (H2), a peptide hormone secreted by the prostate, is up-regulated during progression to CRPC [152]. Over-expression of relaxin stimulates the PI3K-Akt signalling pathway leading to β-catenin stability, AR association and the subsequent transcription of target genes [153]. The tumour micro-environment also plays an important role in regulating PCa progression. In the normal prostate, signalling cross-talk between the stromal and epithelial compartments maintains cellular homeostasis. Stromal AR activity can regulate the composition of the prostate micro-environment. In particular, the AR activity of cancer-associated fibroblasts (CAFs) has been shown to promote PCa epithelial cell growth and invasion through the regulation of growth factors [154]. An important regulator which inhibits epithelial proliferation called transforming growth factor-β (TGF-β) is under androgenic control [155]. Over expression of TGF-β has been observed in prostate tumours isolated from patients following ADT [156]. Elevated levels of TGF-β in prostate stroma have been shown to promote prostate tumour growth and angiogenesis [157], and indirectly activate the AR in PCa cells [158].

8. Conclusion and future perspectives

PCa remains one of the leading causes of cancer-related death in men. Androgens and the AR are key players in the development and progression of this disease and have been the main target of therapeutic treatments for many years. ADT, the treatment for advanced PCa, works by reducing circulating testosterone levels and blocking AR signalling and is initially effective in halting tumour growth. Unfortunately there are a significant proportion of patients that go on to develop CRPC, in which PCa cells develop mechanisms which allow them to continue to grow despite depleted testosterone levels. The mechanisms underlying the development of CRPC are numerous and there are no doubt many more to discover before we will fully understand this disease. The high prevalence of AR pathway alterations observed in multiple patient cohort studies suggests that the majority CRPC tumours remain dependent of AR signalling for growth. Despite the recent development of new more potent treatments targeting AR signalling, CRPC remains terminal. Multiple mechanisms and alternative pathways have been associated with the androgen-independent growth observed in CRPC. Detailed knowledge of the genetic and biological background of tumours is therefore essential in understanding the drivers of disease progression and will assist in the development of effective biomarkers and patient treatments. Optimal treatment will likely require targeting AR signalling in combination with multiple other pathways specific to individual patients.

Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. McNeal JE (1981) The zonal anatomy of the prostate. *Prostate* 2: 35-49.
2. Mcneal JE (1988) Normal Histology of the Prostate. *Am J Surg Pathol* 12: 619-633.
3. Isaacs JT (1994) Etiology of benign prostatic hyperplasia. *Eur Urol* 25 Suppl 1: 6-9.
4. McNeal JE (1978) Origin and evolution of benign prostatic enlargement. *Invest Urol* 15: 340-345.
5. Berry SJ, Coffey DS, Walsh PC, et al. (1984) The development of human benign prostatic hyperplasia with age. *J Urol* 132: 474-479.
6. Briganti A, Capitanio U, Suardi N, et al. (2009) Benign Prostatic Hyperplasia and Its Aetiologies. *Eur Urol Suppl* 8: 865-871.
7. Ferlay J, Soerjomataram I, Dikshit R, et al. (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 136: E359-386.
8. Cancer Research UK (2013) Prostate cancer statistics.
9. Nowell PC (1976) The clonal evolution of tumor cell populations. *Science* 194: 23-28.
10. Collins AT, Berry PA, Hyde C, et al. (2005) Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 65: 10946-10951.
11. Visvader JE, Lindeman GJ (2012) Cancer stem cells: current status and evolving complexities. *Cell Stem Cell* 10: 717-728.
12. Ablin RJ, Soanes WA, Bronson P, et al. (1970) Precipitating antigens of the normal human prostate. *J Reprod Fertil* 22: 573-574.
13. Papsidero LD, Wang MC, Valenzuela LA, et al. (1980) A prostate antigen in sera of prostatic cancer patients. *Cancer Res* 40: 2428-2432.
14. Nadler RB, Humphrey PA, Smith DS, et al. (1995) Effect of inflammation and benign prostatic hyperplasia on elevated serum prostate specific antigen levels. *J Urol* 154: 407-413.
15. Romero Otero J, Garcia Gomez B, Campos Juanatey F, et al. (2014) Prostate cancer biomarkers: an update. *Urol Oncol* 32: 252-260.
16. Gilgunn S, Conroy PJ, Saldova R, et al. (2013) Aberrant PSA glycosylation--a sweet predictor of prostate cancer. *Nat Rev Urol* 10: 99-107.
17. Hessels D, Klein Gunnewiek JM, van Oort I, et al. (2003) DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. *Eur Urol* 44: 8-15.
18. Attard G, Clark J, Ambroisine L, et al. (2008) Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. *Oncogene* 27: 253-263.
19. Gleason DF, Mellinger GT (1974) Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *J Urol* 111: 58-64.
20. Mellinger GT, Gleason D, Bailar J 3rd (1967) The histology and prognosis of prostatic cancer. *J Urol* 97: 331-337.
21. Conn PM, Crowley WF Jr. (1994) Gonadotropin-releasing hormone and its analogs. *Annu Rev Med* 45: 391-405.
22. Lubahn DB, Joseph DR, Sullivan PM, et al. (1988) Cloning of human androgen receptor complementary DNA and localization to the X chromosome. *Science* 240: 327-330.

23. Gelmann EP (2002) Molecular biology of the androgen receptor. *J Clin Oncol* 20: 3001-3015.
24. Edwards A, Hammond HA, Jin L, et al. (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. *Genomics* 12: 241-253.
25. Verrijdt G, Haelens A, Claessens F (2003) Selective DNA recognition by the androgen receptor as a mechanism for hormone-specific regulation of gene expression. *Mol Genet Metab* 78: 175-185.
26. Maitland NJ, Frame FM, Polson ES, et al. (2011) Prostate cancer stem cells: do they have a basal or luminal phenotype? *Horm Cancer* 2: 47-61.
27. Mirosevich J, Bentel JM, Zeps N, et al. (1999) Androgen receptor expression of proliferating basal and luminal cells in adult murine ventral prostate. *J Endocrinol* 162: 341-350.
28. Radmayr C, Lunacek A, Schwentner C, et al. (2008) 5-alpha-reductase and the development of the human prostate. *Indian J Urol* 24: 309-312.
29. Saartok T, Dahlberg E, Gustafsson JA (1984) Relative binding affinity of anabolic-androgenic steroids: comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. *Endocrinology* 114: 2100-2106.
30. Cano LQ, Lavery DN, Bevan CL (2013) Mini-review: Foldosome regulation of androgen receptor action in prostate cancer. *Mol Cell Endocrinol* 369: 52-62.
31. He B, Kemppainen JA, Voegel JJ, et al. (1999) Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH(2)-terminal domain. *J Biol Chem* 274: 37219-37225.
32. Massie CE, Lynch A, Ramos-Montoya A, et al. (2011) The androgen receptor fuels prostate cancer by regulating central metabolism and biosynthesis. *EMBO J* 30: 2719-2733.
33. Cleutjens KB, van Eekelen CC, van der Korput HA, et al. (1996) Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. *J Biol Chem* 271: 6379-6388.
34. Sun M, Yang L, Feldman RI, et al. (2003) Activation of phosphatidylinositol 3-kinase/Akt pathway by androgen through interaction of p85alpha, androgen receptor, and Src. *J Biol Chem* 278: 42992-43000.
35. Migliaccio A, Castoria G, Di Domenico M, et al. (2000) Steroid-induced androgen receptor-oestradiol receptor beta-Src complex triggers prostate cancer cell proliferation. *EMBO J* 19: 5406-5417.
36. Liao RS, Ma S, Miao L, et al. (2013) Androgen receptor-mediated non-genomic regulation of prostate cancer cell proliferation. *Transl Androl Urol* 2: 187-196.
37. Peterziel H, Mink S, Schonert A, et al. (1999) Rapid signalling by androgen receptor in prostate cancer cells. *Oncogene* 18: 6322-6329.
38. Huggins C, Hodges CV (1972) Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *CA Cancer J Clin* 22: 232-240.
39. Harris WP, Mostaghel EA, Nelson PS, et al. (2009) Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion. *Nat Clin Pract Urol* 6: 76-85.
40. Klugo RC, Farah RN, Cerny JC (1981) Bilateral orchiectomy for carcinoma of prostate. Response of serum testosterone and clinical response to subsequent estrogen therapy. *Urology* 17: 49-50.

41. Labrie F, Dupont A, Belanger A, et al. (1986) Treatment of prostate cancer with gonadotropin-releasing hormone agonists. *Endocr Rev* 7: 67-74.
42. Labrie F, Belanger A, Dupont A, et al. (1993) Science behind total androgen blockade: from gene to combination therapy. *Clin Invest Med* 16: 475-492.
43. Karantanos T, Corn PG, Thompson TC (2013) Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches. *Oncogene* 32: 5501-5511.
44. de Bono JS, Logothetis CJ, Molina A, et al. (2011) Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med* 364: 1995-2005.
45. Tran C, Ouk S, Clegg NJ, et al. (2009) Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* 324: 787-790.
46. Kluetz PG, Pierce W, Maher VE, et al. (2014) Radium Ra 223 dichloride injection: U.S. Food and Drug Administration drug approval summary. *Clin Cancer Res* 20: 9-14.
47. Pal S, Gupta R, Davuluri RV (2012) Alternative transcription and alternative splicing in cancer. *Pharmacol Ther* 136: 283-294.
48. Heuze-Vourc'h N, Leblond V, Courty Y (2003) Complex alternative splicing of the hKLK3 gene coding for the tumor marker PSA (prostate-specific-antigen). *Eur J Biochem* 270: 706-714.
49. Narla G, DiFeo A, Fernandez Y, et al. (2008) KLF6-SV1 overexpression accelerates human and mouse prostate cancer progression and metastasis. *J Clin Invest* 118: 2711-2721.
50. Thorsen K, Sorensen KD, Brems-Eskildsen AS, et al. (2008) Alternative splicing in colon, bladder, and prostate cancer identified by exon array analysis. *Mol Cell Proteomics* 7: 1214-1224.
51. Erho N, Buerki C, Triche TJ, et al. (2012) Transcriptome-wide detection of differentially expressed coding and non-coding transcripts and their clinical significance in prostate cancer. *J Oncol* 2012: 541353.
52. Rajan P, Dalgliesh C, Carling PJ, et al. (2011) Identification of novel androgen-regulated pathways and mRNA isoforms through genome-wide exon-specific profiling of the LNCaP transcriptome. *PLoS One* 6: e29088.
53. Munkley J, Rajan P, Lafferty NP, et al. (2014) A novel androgen-regulated isoform of the TSC2 tumour suppressor gene increases cell proliferation. *Oncotarget* 5: 131-139.
54. Busa R, Paronetto MP, Farini D, et al. (2007) The RNA-binding protein Sam68 contributes to proliferation and survival of human prostate cancer cells. *Oncogene* 26: 4372-4382.
55. Rajan P, Gaughan L, Dalgliesh C, et al. (2008) The RNA-binding and adaptor protein Sam68 modulates signal-dependent splicing and transcriptional activity of the androgen receptor. *J Pathol* 215: 67-77.
56. Mavrou A, Brakspear K, Hamdollah-Zadeh M, et al. (2014) Serine-arginine protein kinase 1 (SRPK1) inhibition as a potential novel targeted therapeutic strategy in prostate cancer. *Oncogene* 34: 4311-4319.
57. Clark EL, Coulson A, Dalgliesh C, et al. (2008) The RNA helicase p68 is a novel androgen receptor coactivator involved in splicing and is overexpressed in prostate cancer. *Cancer Res* 68: 7938-7946.
58. Wu X, Daniels G, Lee P, et al. (2014) Lipid metabolism in prostate cancer. *Am J Clin Exp Urol* 2: 111-120.

59. Suburu J, Chen YQ (2012) Lipids and prostate cancer. *Prostaglandins Other Lipid Mediat* 98: 1-10.
60. Swinnen JV, Ulrix W, Heyns W, et al. (1997) Coordinate regulation of lipogenic gene expression by androgens: evidence for a cascade mechanism involving sterol regulatory element binding proteins. *Proc Natl Acad Sci U S A* 94: 12975-12980.
61. Swinnen JV, Esquenet M, Goossens K, et al. (1997) Androgens stimulate fatty acid synthase in the human prostate cancer cell line LNCaP. *Cancer Res* 57: 1086-1090.
62. Swinnen JV, Vanderhoydonc F, Elgamal AA, et al. (2000) Selective activation of the fatty acid synthesis pathway in human prostate cancer. *Int J Cancer* 88: 176-179.
63. Liu Y (2006) Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer. *Prostate Cancer Prostatic Dis* 9: 230-234.
64. Segawa T, Nau ME, Xu LL, et al. (2002) Androgen-induced expression of endoplasmic reticulum (ER) stress response genes in prostate cancer cells. *Oncogene* 21: 8749-8758.
65. Sheng X, Arnoldussen YJ, Storm M, et al. (2015) Divergent androgen regulation of unfolded protein response pathways drives prostate cancer. *EMBO Mol Med* 7: 788-801.
66. Munkley J, Mills IG, Elliott DJ (2016) The role of glycans in the development and progression of prostate cancer. *Nat Rev Urol* 13: 324-333.
67. Hakomori S (1996) Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. *Cancer Res* 56: 5309-5318.
68. Reis CA, Osorio H, Silva L, et al. (2010) Alterations in glycosylation as biomarkers for cancer detection. *J Clin Pathol* 63: 322-329.
69. Pinho SS, Reis CA (2015) Glycosylation in cancer: mechanisms and clinical implications. *Nat Rev Cancer* 15: 540-555.
70. Barfeld SJ, East P, Zuber V, et al. (2014) Meta-analysis of prostate cancer gene expression data identifies a novel discriminatory signature enriched for glycosylating enzymes. *BMC Med Genom* 7: 513.
71. Munkley J, Elliott DJ (2016) Hallmarks of glycosylation in cancer. *Oncotarget* in press.
72. Munkley J, Vodak D, Livermore KE, et al. (2016) Glycosylation is an androgen-regulated process essential for prostate cancer cell viability. *EBioMedicine* in press.
73. Itkonen HM, Engedal N, Babaie E, et al. (2015) UAP1 is overexpressed in prostate cancer and is protective against inhibitors of N-linked glycosylation. *Oncogene* 34: 3744-3750.
74. Munkley J, Oltean S, Vodak D, et al. (2015) The androgen receptor controls expression of the cancer-associated sTn antigen and cell adhesion through induction of ST6GalNAc1 in prostate cancer. *Oncotarget* 6: 34358-34374.
75. Sato T, Yoneyama T, Tobisawa Y, et al. (2016) Core 2 (beta-1, 6-N-acetylglucosaminyltransferase-1 expression in prostate biopsy specimen is an indicator of prostate cancer aggressiveness. *Biochem Biophys Res Commun* 470: 150-156.
76. Chen Z, Gulzar ZG, St Hill CA, et al. (2014) Increased expression of GCNT1 is associated with altered O-glycosylation of PSA, PAP, and MUC1 in human prostate cancers. *Prostate* 74: 1059-1067.
77. Taylor BS, Schultz N, Hieronymus H, et al. (2010) Integrative genomic profiling of human prostate cancer. *Cancer Cell* 18: 11-22.
78. Pourmand G, Ziaee AA, Abedi AR, et al. (2007) Role of PTEN gene in progression of prostate cancer. *Urol J* 4: 95-100.

79. Robinson D, Van Allen EM, Wu YM, et al. (2015) Integrative clinical genomics of advanced prostate cancer. *Cell* 161: 1215-1228.
80. Carver BS, Chapinski C, Wongvipat J, et al. (2011) Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. *Cancer Cell* 19: 575-586.
81. Munkley J, Lafferty NP, Kalna G, et al. (2015) Androgen-regulation of the protein tyrosine phosphatase PTPRR activates ERK1/2 signalling in prostate cancer cells. *BMC Cancer* 15: 9.
82. Schutzman JL, Martin GR (2012) Sprouty genes function in suppression of prostate tumorigenesis. *Proc Natl Acad Sci U S A* 109: 20023-20028.
83. Yokoyama NN, Shao S, Hoang BH, et al. (2014) Wnt signaling in castration-resistant prostate cancer: implications for therapy. *Am J Clin Exp Urol* 2: 27-44.
84. Truica CI, Byers S, Gelmann EP (2000) Beta-catenin affects androgen receptor transcriptional activity and ligand specificity. *Cancer Res* 60: 4709-4713.
85. Mulholland DJ, Cheng H, Reid K, et al. (2002) The androgen receptor can promote beta-catenin nuclear translocation independently of adenomatous polyposis coli. *J Biol Chem* 277: 17933-17943.
86. Rajan P, Sudbery IM, Villasevil ME, et al. (2014) Next-generation sequencing of advanced prostate cancer treated with androgen-deprivation therapy. *Eur Urol* 66: 32-39.
87. Chen G, Shukeir N, Potti A, et al. (2004) Up-regulation of Wnt-1 and beta-catenin production in patients with advanced metastatic prostate carcinoma: potential pathogenetic and prognostic implications. *Cancer* 101: 1345-1356.
88. Voeller HJ, Truica CI, Gelmann EP (1998) Beta-catenin mutations in human prostate cancer. *Cancer Res* 58: 2520-2523.
89. Mostaghel EA, Solomon KR, Pelton K, et al. (2012) Impact of circulating cholesterol levels on growth and intratumoral androgen concentration of prostate tumors. *PLoS One* 7: e30062.
90. Hamid AR, Pfeiffer MJ, Verhaegh GW, et al. (2012) Aldo-keto reductase family 1 member C3 (AKR1C3) is a biomarker and therapeutic target for castration-resistant prostate cancer. *Mol Med* 18: 1449-1455.
91. Stanbrough M, Bubley GJ, Ross K, et al. (2006) Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res* 66: 2815-2825.
92. Mohler JL, Gregory CW, Ford OH, 3rd, et al. (2004) The androgen axis in recurrent prostate cancer. *Clin Cancer Res* 10: 440-448.
93. Knuuttila M, Yatkin E, Kallio J, et al. (2014) Castration induces up-regulation of intratumoral androgen biosynthesis and androgen receptor expression in an orthotopic VCaP human prostate cancer xenograft model. *Am J Pathol* 184: 2163-2173.
94. Liu W, Xie CC, Zhu Y, et al. (2008) Homozygous deletions and recurrent amplifications implicate new genes involved in prostate cancer. *Neoplasia* 10: 897-907.
95. Edwards J, Krishna NS, Grigor KM, et al. (2003) Androgen receptor gene amplification and protein expression in hormone refractory prostate cancer. *Br J Cancer* 89: 552-556.
96. Edwards J, Krishna NS, Witton CJ, et al. (2003) Gene amplifications associated with the development of hormone-resistant prostate cancer. *Clin Cancer Res* 9: 5271-5281.
97. Holzbeierlein J, Lal P, LaTulippe E, et al. (2004) Gene expression analysis of human prostate carcinoma during hormonal therapy identifies androgen-responsive genes and mechanisms of therapy resistance. *Am J Pathol* 164: 217-227.

98. Barbieri CE, Bangma CH, Bjartell A, et al. (2013) The mutational landscape of prostate cancer. *Eur Urol* 64: 567-576.
99. Grasso CS, Wu YM, Robinson DR, et al. (2012) The mutational landscape of lethal castration-resistant prostate cancer. *Nature* 487: 239-243.
100. Shaffer DR, Leversha MA, Danila DC, et al. (2007) Circulating tumor cell analysis in patients with progressive castration-resistant prostate cancer. *Clin Cancer Res* 13: 2023-2029.
101. Salvi S, Casadio V, Conteduca V, et al. (2015) Circulating cell-free AR and CYP17A1 copy number variations may associate with outcome of metastatic castration-resistant prostate cancer patients treated with abiraterone. *Br J Cancer* 112: 1717-1724.
102. Azad AA, Volik SV, Wyatt AW, et al. (2015) Androgen Receptor Gene Aberrations in Circulating Cell-Free DNA: Biomarkers of Therapeutic Resistance in Castration-Resistant Prostate Cancer. *Clin Cancer Res* 21: 2315-2324.
103. Attard G, Swennenhuis JF, Olmos D, et al. (2009) Characterization of ERG, AR and PTEN gene status in circulating tumor cells from patients with castration-resistant prostate cancer. *Cancer Res* 69: 2912-2918.
104. Wallen MJ, Linja M, Kaartinen K, et al. (1999) Androgen receptor gene mutations in hormone-refractory prostate cancer. *J Pathol* 189: 559-563.
105. Gottlieb B, Beitel LK, Nadarajah A, et al. (2012) The androgen receptor gene mutations database: 2012 update. *Hum Mutat* 33: 887-894.
106. Gaddipati JP, McLeod DG, Heidenberg HB, et al. (1994) Frequent detection of codon 877 mutation in the androgen receptor gene in advanced prostate cancers. *Cancer Res* 54: 2861-2864.
107. Steketee K, Timmerman L, Ziel-van der Made AC, et al. (2002) Broadened ligand responsiveness of androgen receptor mutants obtained by random amino acid substitution of H874 and mutation hot spot T877 in prostate cancer. *Int J Cancer* 100: 309-317.
108. Steinkamp MP, O'Mahony OA, Brogley M, et al. (2009) Treatment-dependent androgen receptor mutations in prostate cancer exploit multiple mechanisms to evade therapy. *Cancer Res* 69: 4434-4442.
109. Hu R, Dunn TA, Wei S, et al. (2009) Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res* 69: 16-22.
110. Sun S, Sprenger CC, Vessella RL, et al. (2010) Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. *J Clin Invest* 120: 2715-2730.
111. Hu R, Lu C, Mostaghel EA, et al. (2012) Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. *Cancer Res* 72: 3457-3462.
112. Li Y, Chan SC, Brand LJ, et al. (2013) Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. *Cancer Res* 73: 483-489.
113. Hornberg E, Ylitalo EB, Crnalic S, et al. (2011) Expression of androgen receptor splice variants in prostate cancer bone metastases is associated with castration-resistance and short survival. *PLoS One* 6: e19059.
114. Antonarakis ES, Lu C, Wang H, et al. (2014) AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 371: 1028-1038.

115. Steinestel J, Luedeke M, Arndt A, et al. (2015) Detecting predictive androgen receptor modifications in circulating prostate cancer cells. *Oncotarget* 5.
116. Culig Z (2016) Androgen Receptor Coactivators in Regulation of Growth and Differentiation in Prostate Cancer. *J Cell Physiol* 231: 270-274.
117. Heemers HV, Sebo TJ, Debes JD, et al. (2007) Androgen deprivation increases p300 expression in prostate cancer cells. *Cancer Res* 67: 3422-3430.
118. Comuzzi B, Nemes C, Schmidt S, et al. (2004) The androgen receptor co-activator CBP is up-regulated following androgen withdrawal and is highly expressed in advanced prostate cancer. *J Pathol* 204: 159-166.
119. Halkidou K, Gnanapragasam VJ, Mehta PB, et al. (2003) Expression of Tip60, an androgen receptor coactivator, and its role in prostate cancer development. *Oncogene* 22: 2466-2477.
120. Debes JD, Schmidt LJ, Huang H, et al. (2002) p300 mediates androgen-independent transactivation of the androgen receptor by interleukin 6. *Cancer Res* 62: 5632-5636.
121. Shiota M, Yokomizo A, Masubuchi D, et al. (2010) Tip60 Promotes Prostate Cancer Cell Proliferation by Translocation of Androgen Receptor into the Nucleus. *Prostate* 70: 540-554.
122. Kishimoto T (1989) The biology of interleukin-6. *Blood* 74: 1-10.
123. Okamoto M, Lee C, Oyasu R (1997) Autocrine effect of androgen on proliferation of an androgen responsive prostatic carcinoma cell line, LNCAP: role of interleukin-6. *Endocrinology* 138: 5071-5074.
124. Hobisch A, Rogatsch H, Hittmair A, et al. (2000) Immunohistochemical localization of interleukin-6 and its receptor in benign, premalignant and malignant prostate tissue. *J Pathol* 191: 239-244.
125. Culig Z, Bartsch G, Hobisch A (2002) Interleukin-6 regulates androgen receptor activity and prostate cancer cell growth. *Mol Cell Endocrinol* 197: 231-238.
126. Twillie DA, Eisenberger MA, Carducci MA, et al. (1995) Interleukin-6: a candidate mediator of human prostate cancer morbidity. *Urology* 45: 542-549.
127. Chen T, Wang LH, Farrar WL (2000) Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. *Cancer Res* 60: 2132-2135.
128. Heinrich PC, Behrmann I, Haan S, et al. (2003) Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J* 374: 1-20.
129. Olayioye MA, Neve RM, Lane HA, et al. (2000) The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J* 19: 3159-3167.
130. Slamon DJ, Godolphin W, Jones LA, et al. (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244: 707-712.
131. Signoretti S, Montironi R, Manola J, et al. (2000) Her-2-neu expression and progression toward androgen independence in human prostate cancer. *J Natl Cancer Inst* 92: 1918-1925.
132. Wen Y, Hu MC, Makino K, et al. (2000) HER-2/neu promotes androgen-independent survival and growth of prostate cancer cells through the Akt pathway. *Cancer Res* 60: 6841-6845.
133. Karin M (2006) Nuclear factor-kappaB in cancer development and progression. *Nature* 441: 431-436.
134. Suh J, Payvandi F, Edelstein LC, et al. (2002) Mechanisms of constitutive NF-kappaB activation in human prostate cancer cells. *Prostate* 52: 183-200.

135. Zhang L, Altuwaijri S, Deng F, et al. (2009) NF-kappaB regulates androgen receptor expression and prostate cancer growth. *Am J Pathol* 175: 489-499.
136. Nadiminty N, Lou W, Sun M, et al. (2010) Aberrant activation of the androgen receptor by NF-kappaB2/p52 in prostate cancer cells. *Cancer Res* 70: 3309-3319.
137. Krijnen JL, Janssen PJ, Ruizeveld de Winter JA, et al. (1993) Do neuroendocrine cells in human prostate cancer express androgen receptor? *Histochemistry* 100: 393-398.
138. Bonkhoff H, Stein U, Remberger K (1995) Endocrine-paracrine cell types in the prostate and prostatic adenocarcinoma are postmitotic cells. *Hum Pathol* 26: 167-170.
139. Li Q, Zhang CS, Zhang Y (2016) Molecular aspects of prostate cancer with neuroendocrine differentiation. *Chin J Cancer Res* 28: 122-129.
140. Alberti C (2010) Neuroendocrine differentiation in prostate carcinoma: focusing on its pathophysiologic mechanisms and pathological features. *G Chir* 31: 568-574.
141. Yuan TC, Veeramani S, Lin FF, et al. (2006) Androgen deprivation induces human prostate epithelial neuroendocrine differentiation of androgen-sensitive LNCaP cells. *Endocr Relat Cancer* 13: 151-167.
142. Jongsma J, Oomen MH, Noordzij MA, et al. (1999) Kinetics of neuroendocrine differentiation in an androgen-dependent human prostate xenograft model. *Am J Pathol* 154: 543-551.
143. Hirano D, Okada Y, Minei S, et al. (2004) Neuroendocrine differentiation in hormone refractory prostate cancer following androgen deprivation therapy. *Eur Urol* 45: 586-592.
144. Sagnak L, Topaloglu H, Ozok U, et al. (2011) Prognostic significance of neuroendocrine differentiation in prostate adenocarcinoma. *Clin Genitourin Cancer* 9: 73-80.
145. Qiu Y, Robinson D, Pretlow TG, et al. (1998) Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3'-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells. *Proc Natl Acad Sci U S A* 95: 3644-3649.
146. Spiotto MT, Chung TD (2000) STAT3 mediates IL-6-induced neuroendocrine differentiation in prostate cancer cells. *Prostate* 42: 186-195.
147. Moritz T, Venz S, Junker H, et al. (2016) Isoform 1 of TPD52 (PC-1) promotes neuroendocrine transdifferentiation in prostate cancer cells. *Tumour Biol* in press.
148. Gururajan M, Cavassani KA, Sievert M, et al. (2015) SRC family kinase FYN promotes the neuroendocrine phenotype and visceral metastasis in advanced prostate cancer. *Oncotarget* 6: 44072-44083.
149. Uysal-Onganer P, Kawano Y, Caro M, et al. (2010) Wnt-11 promotes neuroendocrine-like differentiation, survival and migration of prostate cancer cells. *Mol Cancer* 9: 55.
150. Wu C, Huang J (2007) Phosphatidylinositol 3-kinase-AKT-mammalian target of rapamycin pathway is essential for neuroendocrine differentiation of prostate cancer. *J Biol Chem* 282: 3571-3583.
151. Kowalska K, Piastowska-Ciesielska AW (2016) Oestrogens and oestrogen receptors in prostate cancer. *Springerplus* 5: 522.
152. Thompson VC, Morris TG, Cochrane DR, et al. (2006) Relaxin becomes upregulated during prostate cancer progression to androgen independence and is negatively regulated by androgens. *Prostate* 66: 1698-1709.
153. Liu S, Vinall RL, Tepper C, et al. (2008) Inappropriate activation of androgen receptor by relaxin via beta-catenin pathway. *Oncogene* 27: 499-505.

154. Yu S, Xia S, Yang D, et al. (2013) Androgen receptor in human prostate cancer-associated fibroblasts promotes prostate cancer epithelial cell growth and invasion. *Med Oncol* 30: 674.
155. Roberts AB, Anzano MA, Wakefield LM, et al. (1985) Type beta transforming growth factor: a bifunctional regulator of cellular growth. *Proc Natl Acad Sci U S A* 82: 119-123.
156. Fuzio P, Ditonno P, Rutigliano M, et al. (2012) Regulation of TGF-beta1 expression by androgen deprivation therapy of prostate cancer. *Cancer Lett* 318: 135-144.
157. Yang F, Tuxhorn JA, Ressler SJ, et al. (2005) Stromal expression of connective tissue growth factor promotes angiogenesis and prostate cancer tumorigenesis. *Cancer Res* 65: 8887-8895.
158. Yang F, Chen Y, Shen T, et al. (2014) Stromal TGF-beta signaling induces AR activation in prostate cancer. *Oncotarget* 5: 10854-10869.

AIMS Press