Seminars in Cancer Biology 35 (2015) 133–144

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

Therapy escape mechanisms in the malignant prostate

Frédéric R. Santer[a,*], Holger H.H. Erb[b], Rhiannon V. McNeill[c]

[a] Medical University of Innsbruck, Division of Experimental Urology, Department of Urology, Anichstrasse 35, A-6020 Innsbruck, Austria
[b] Yorkshire Cancer Research Unit, University of York, York YO10 5DD, United Kingdom
[c] Jack Birch Unit for Molecular Carcinogenesis, University of York, York YO10 5DD, United Kingdom

ARTICLE INFO

Article history:
Received 15 June 2015
Received in revised form 12 August 2015
Accepted 14 August 2015
Available online 21 August 2015

Keywords:
AR-targeting therapies
Therapy resistance mechanisms
Target modification
Bypass signalling
Histologic transformation
Cancer stem cells

ABSTRACT

Androgen receptor (AR) is the main target for prostate cancer therapy. Clinical approaches for AR inactivation include chemical castration, inhibition of androgen synthesis and AR antagonists (anti-androgens). However, treatment resistance occurs for which an important number of therapy escape mechanisms have been identified. Herein, we summarise the current knowledge of molecular mechanisms underlying therapy resistance in prostate cancer. Moreover, the tumour escape mechanisms are arranged into the concepts of target modification, bypass signalling, histologic transformation, cancer stem cells and miscellaneous mechanisms. This may help researchers to compare and understand same or similar concepts of therapy resistance in prostate cancer and other cancer types.

1. Introduction

1.1. Prostate tissue architecture

The prostate is an exocrine gland of the male reproductive system surrounding the prostatic urethra [1] and composed of acini, which are formed by a pseudo-stratified columnar epithelium comprising a terminally differentiated, secretory layer and a less differentiated basal compartment with regenerating cells [2]. A basement membrane separates the epithelial cells from the surrounding fibro-muscular stroma. Within the secretory layer, neuroendocrine cells represent a rare lineage, lack expression of androgen receptor (AR) and are thus not growth-dependent on androgens [3]. However, the prevalent lineage consists of AR-positive, luminal cells whose secretome is rich in prostate acid phosphatase (ACPP), kallikrein-related peptidase 3 (KLK3; also known as prostate specific antigen, PSA) and β-microseminoprotein (MSMB) [4].

Adult tissue stem cells (SC) reside within the basal layer and are able to regenerate the prostate through a differentiation process into secretory cells [2]. SC with a high expression of prominin 1 (PROM1) and integrin α_2 (ITGA2) give rise to rapidly proliferating, transit amplifying cells (PROM1$^-$, ITGA2^{hi}), which can further differentiate into committed basal cells (PROM1$^-$, ITGA2^{lo}) with a slower cell cycle. *In vitro* terminal differentiation of basal cells to a luminal phenotype is mainly triggered by androgens [5]. Additionally, basal cells can be differentiated into neuroendocrine cells *in vitro* [6].

1.2. Prostate cancer: Treatment options

The vast majority of all prostate cancer (PCa) cases are acinar adenocarcinomas and histologically appear as hyperplasia of luminal cells. Deferred treatment, radical prostatectomy, androgen deprivation therapy (ADT) and external beam radiation therapy are current options for localised PCa [7]. At a metastatic stage (M1), the disease becomes incurable and the mean survival is 21–54 months, depending on the treatment response [7]. A common first-line therapy option at this stage of the disease is androgen deprivation therapy (ADT), based on initial findings by Huggins and Hodges [8] helping to understand that PCa growth and progression is dependent on androgens. AR inactivation can be accomplished by several means. Surgical castration (orchiectomy) is only rarely performed these days. Chemical castration can be achieved by interfering with the hormonal signalling pathway of the hypothalamic–pituitary–gonadal axis, which regulates the production of testosterone. This consequently leads to hypogonadism with castrate testosterone levels below 20 ng/dl (1 nmol/l). Complete androgen blockade (CAB) can be reached in combination with chemical castration by the use of non-steroidal anti-androgens (e.g.

* Corresponding author. Tel.: +43 0 512 504 81801; fax: +43 0 512 504 24817.
E-mail address: frederic.santer@i-med.ac.at (F.R. Santer).

http://dx.doi.org/10.1016/j.semcancer.2015.08.005

hydroxyflutamide, bicalutamide) that compete with the natural ligand dihydrotestosterone (DHT) for binding to the AR.

Recurrence after ADT/CAB is termed castration-resistant prostate cancer (CRPCa) and is concurrent with a reactivation of the AR signalling axis. Treatment options at this stage of the disease include taxane-based chemotherapy, dendritic cell vaccination-based immunotherapy, the calcium mimic ^{223}Radium dichloride targeting bone metastases, and second-generation AR-targeting therapies [9,10]. However, resistance to therapy invariably occurs at these advanced stages of the disease, including *ab initio* resistance and/or cross-resistance to previously used drugs [11,12].

1.3. AR: Main target in PCa therapy

Luminal cells are essentially dependent on androgens for survival, or more precisely, dependent on active AR signalling. Inactivation of this signalling axis is the primary goal of ADT, CAB and second-generation agents enzalutamide and abiraterone. It is therefore not surprising that in the past most work elucidating resistance mechanisms against this therapy was focused on the AR signalling axis.

The gene encoding AR on Xq12 is organised into 8 exons [13] and structurally belongs to the nuclear steroid hormone receptor family. Exon 1 encodes the aminoterminal domain (NTD) and contains activation function 1 (AF-1; transcriptional units (TAU)-1 and -5). The DNA binding region consists of two zinc finger motifs, is encoded by exons 2 and 3, and is responsible for binding to androgen response elements (ARE) in promoter and enhancer regions of target genes. It is followed by a hinge region where a nuclear localisation signal is located, that is sterically unmasked upon ligand binding. Exons 4–8 comprise the ligand binding domain (LBD), as well as AF-2, which has only minor transcriptional activity [14]. AR interacts with an important number of co-integrators via the LXXLL motifs in the amino- and carboxy-terminal domains, controlling the full transcriptional activity/repression of AR [15].

The present review will summarise known therapeutic resistance mechanisms in the secretory and the basal prostatic layer and classify the therapy escape routes in the categories target modification, bypass signalling pathways, histologic transformation, cancer stem cells and miscellaneous mechanisms. This categorisation of tumour escape mechanisms has been adapted for PCa according to a recent publication based on resistance mechanisms to kinase inhibitors [16].

2. Target modification

2.1. Target overexpression and hypersensitive pathway

The most common molecular mechanism of endocrine therapy resistance is amplification of the AR gene locus. A number of studies have analysed paired patient biopsies before therapy, and after acquisition of castration resistance [17,18]. Amplification of the Xq12 locus, but also X-chromosome polysomy, have been documented in about 30% of all patients progressing to CRPCa, but were absent in the hormone-naïve specimens. These multiple copies of the AR gene result in high expression of AR mRNA and protein [18,19]. *In vitro* studies showed that AR transcriptional activity is regulated not only by the expression levels of both ligand and the receptor [20], but also as a consequence of increased receptor binding to chromatin [21]. In other words, ADT causes an amplification of the *AR* locus, in which a disturbed ratio of AR and its ligand results in a hypersensitive AR signalling pathway. Cells with such a high AR expression can deal with the low androgen concentrations during ADT, and give rise to new clonal outgrowth of castration resistant cells. However, *AR* gene amplification might not be exclusively driven by ADT. A recent study discovered an amplification of the *AR* locus in small foci of cells in hormone-naïve patients suggesting that ADT could select for the clonal expansion of an already existing *ab initio* resistant cell population [22]. Of clinical interest is the fact that patients with *AR* amplification and thus high AR levels could take advantage of a CAB approach, inhibiting AR directly instead of following a standard ADT protocol only [23]. Interesting to note is that selection of patients for this purpose could be done on the detection of *AR* amplifications in circulating tumour cells isolated from the patients' blood, thereby possibly eliminating the need for biopsies [24].

Another mechanism leading to a highly expressed and hypersensitive AR pathway is the stabilisation of AR, i.e. a longer half-life of the protein through decreased degradation or through increased translation. The Cullin-based E3 ligase SPOP can promote polyubiquitination and subsequent proteasomal degradation of AR, as well as of its co-activator NCOA3 [25,26]. Mutations in the substrate-binding cleft of SPOP impair ubiquitination of AR and NCOA3, thereby extending the turnover and leading to an accumulation of these proteins. PCa-specific mutations of SPOP are found in up to 15% of analysed specimens [27]. However, the frequency of SPOP mutations (3 out of 50 cases) seems to be lower in CRPCa [28], raising the question whether this mechanism contributes to castration resistance. Another E3 ligase, SIAH2, was found increasingly expressed in CRPCa and to selectively regulate the transcriptional activity of AR [29]. MID1, which is known to interact with active polyribosomes, has been shown to associate with AR mRNA [30]. Negative regulation by the AR signalling axis leads to high MID1 expression under androgen deprived conditions, favouring increased AR translation and expression. Usually, AR is a negative regulator of its own transcription under normal androgen concentrations [31]. However, the castrate androgen levels in CRPCa are sufficient to stimulate AR activity on enhancer elements, but not suppressor elements, resulting in an increased expression of AR and AR-repressed genes, which may drive progression to CRPCa [32]. In conclusion, multiple mechanisms can eventually result in high expression levels of AR, which are transcriptionally hypersensitive to the residual amounts of DHT in castrate patients (Fig. 1a).

2.2. Target mutation and promiscuous pathway

Base substitutions in the coding sequence of the AR gene are less frequently observed than amplifications of the locus. The latest study using exome sequencing revealed a mutation frequency of 10% in CRPCa specimens ($n = 50$) [28]. Clinically relevant mutations occur in the ligand-binding pocket [33]. For instance, a recent study discovered H874Y and T877A mutations in circulating cell-free DNA from patients that no longer responded to treatment with abiraterone, and a F876L amino acid substitution in one enzalutamide-resistant patient [34]. The latter mutation, F876L, was found in earlier experimental approaches to confer resistance to enzalutamide and ARN-509, an anti-androgen undergoing clinical trials [35,36]. The T877A mutation can also be detected in CRPCa patients that progressed on abiraterone treatment [37]. The driving force for the occurrence of many AR mutations in the LBD is broadened ligand specificity. Promiscuous activation of mutant AR by adrenal androgens, progesterone, cortisol and cortisone, β-estradiol, testosterone metabolites, and moreover, the anti-androgens hydroxyflutamide, bicalutamide and enzalutamide has been documented [36,38–43]. These agonistic properties of the AR antagonists may be the basis of the anti-androgen withdrawal syndrome described for all three drugs [44–46]. Furthermore, T877A generates an androgen-independent gain of function for nuclear import [47] and H874Y enhances binding to the co-activators NCOA1-3 [48]. Thus, base substitutions in the AR can be responsi-

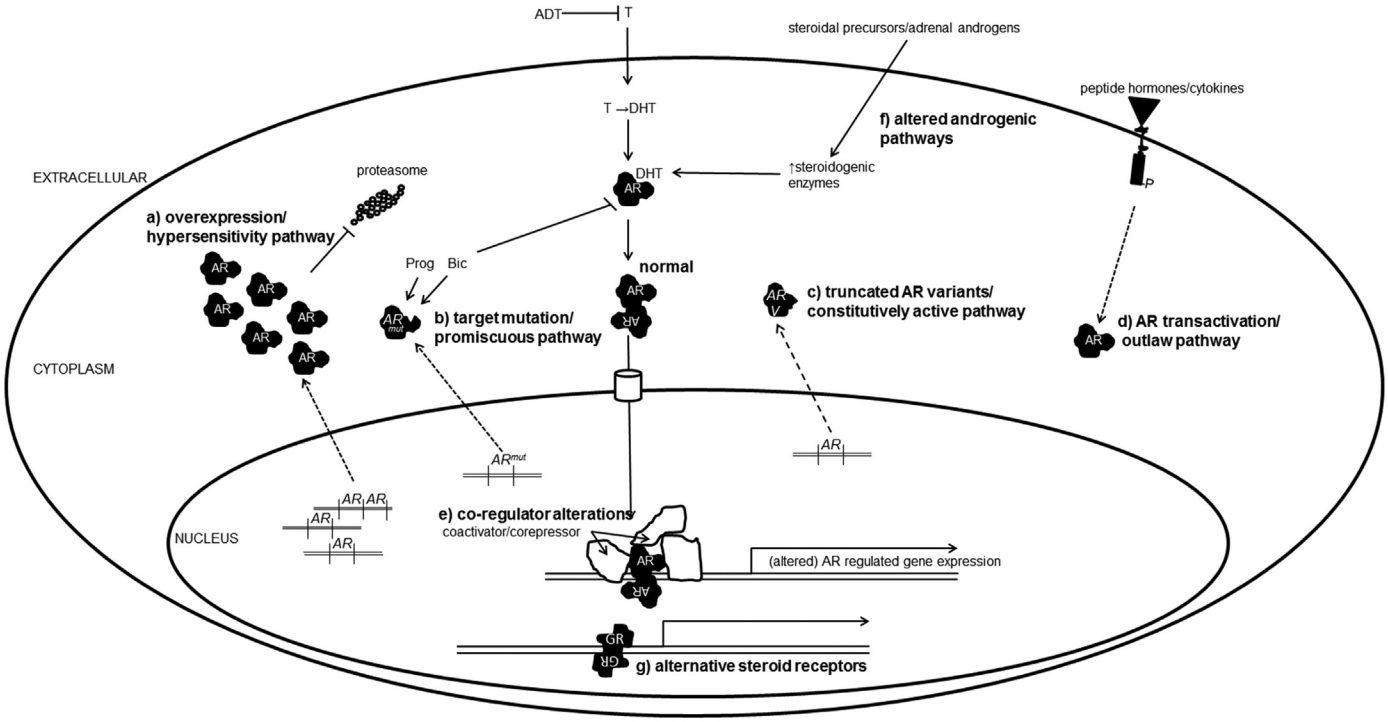

Fig. 1. Molecular mechanisms of AR-targeting therapy escape. Several molecular mechanisms leading to a reactivation of the AR-signalling axis and a progression to CRPCa have been described. These can be grouped in target modification (overexpression/hypersensitivity (a); target mutation/promiscuous (b); truncated AR variants/constitutively active (c)) and bypass (AR transactivation/outlaw (d); co-regulator alterations (e), altered androgenesis (f), alternative steroid receptors (g)) pathways. ADT, androgen deprivation therapy; T, testosterone; DHT, dihydrotestosterone; Bic, bicalutamide; Prog, progesterone.

ble for a reactivation of the AR signalling axis, through alternative steroidal and non-steroidal ligands under conditions where DHT is restricted (Fig. 1b).

2.3. Truncated AR variants and constitutively active pathway

C-terminal truncated AR variants may arise through proteolytic cleavage of full length AR, *AR* gene rearrangement, and by alternative splicing of the *AR* gene transcript [49–52]. To date, around 20 AR variants have been documented, which lack the LBD rendering these variants constitutively active even in the absence of its ligand [53]. Although there are conflicting reports whether AR variants require dimerisation with the full length receptor for nuclear import and transcriptional activity [54,55], there is consensus that resistance against the currently available therapies targeting the LBD of AR can be mediated by truncated AR variants [56,57]. The best investigated AR variant, AR-V7, can be increasingly detected in CRPCa tissues using AR-V7 specific antibodies [58,59]. Its expression is induced by ADT and predicts progression to CRPCa [60]. Furthermore, AR-V7 mRNA expression in circulating tumour cells correlates with primary resistance to abiraterone and enzalutamide [61]. These AR variants may induce a distinct gene expression signature compared to the full length receptor [62]. The transcriptional program of AR-Vs preferentially leads to expression of cell cycle regulatory genes, while full length AR represses that program and favours transcription of genes related to macromolecular synthesis, metabolism and differentiation. The recognition that LBD-deficient AR variants drive CRPCa has prompted the development of anti-androgens targeting the NTD of AR, which possesses the highest transcriptional activity [14]. In conclusion, truncated AR variants are constitutively active and can promote AR signalling without the need for a ligand (Fig. 1c).

3. Bypass signalling pathways

3.1. AR transactivation and outlaw pathway

Several studies show that AR can also be transactivated by growth factors, neuropeptides and cytokines. For instance, IL6 can cross-talk to the AR signalling axis and lead to the transcription of KLK3 under castrate androgen conditions [63]. AR transactivation in response to IL6 can be mediated by MAPK and STAT3 pathways, whereas signalling through PI3K-Akt suppresses this interaction [64]. Interestingly, STAT3 is able to heterodimerise with the N-terminal domain of AR upon IL6 signalling in LNCaP cells [65]. Activation of the MAPK pathway by IL6 leads to phosphorylation of the AR co-activator NCOA1 thereby enhancing AR transcriptional activity [66]. In addition, the kinase FER may phosphorylate AR at tyrosine 223 and participate in the crosstalk with AR in response to IL6/STAT3 signalling [67]. The IL6 transactivating effects on AR can be reversed by MAPK inhibition or with the anti-androgen bicalutamide [68]. Another example is the tyrosine kinase SRC which can phosphorylate AR thereby regulating AR's nuclear translocation, chromatin recruitment and transcriptional activity [69]. SRC is known to play important roles in the progression and recurrence of a number of cancer types [70]. SRC plays also a role in AR transactivation by the neuropeptide bombesin and promotes an overlapping but not identical AR transcriptional program [71]. Earlier it was shown that EGF, keratinocyte growth factor (KGF) and insulin-like growth factor-1 (IGF1) are also able to transactivate AR in the absence of androgens, which, like IL6 can be blocked by bicalutamide treatment [72]. In conclusion, several signalling pathways can cause transactivation of AR in the absence of its ligand or under low androgen concentrations and thus contribute to PCa progression (Fig. 1d).

3.2. Co-regulator alterations and aberrant transcription

AR transcriptional activity is controlled by numerous co-regulators, i.e. co-activators and co-repressors [15]. Aberrant expression of co-regulators has been associated with PCa progression, and in addition the expression of various co-regulators is directly regulated through androgenic action [73]. For instance, increased expression of the histone (lysine) acetyltransferase (HAT) EP300 has been detected in PCa cells after androgen deprivation [74]. EP300 is involved in transactivation of the AR through IL6 [75]. Moreover, bombesin is able to activate EP300 HAT activity via SRC and PRKCD pathways. This results in AR protein acetylation and an increased expression of AR-regulated genes [76]. A similar role has been documented for the close EP300 homologue CREB-binding protein (CREBBP), which is also increased after androgen withdrawal [77]. Protein kinase A (PKA), which is frequently over-expressed in PCa, leads to the transcription of AR regulated genes in response to cAMP [78]. This involves the phosphorylation of cAMP responsive element-binding protein (CREB) through PKA and the recruitment of EP300 and CREBBP. In cells with a high expression of CREBBP and EP300, the co-activators may act as a bridging molecule between AR and CREB, resulting in an enhanceosome-based cooperation [79]. Interestingly, PKA can also phosphorylate and enhance nuclear translocation and repressive function of the nuclear receptor co-repressor NCOR1 [80]. Although this would usually favour AR transcriptional inactivation, the situation may be different in a particular cellular context; exogenous overexpression of NCOR1 and NCOR2 can increase AR transcriptional activity, possibly due to an impaired AR/co-repressor interaction [81]. Another study found altered recruitment to AR but also loss of expression of NCOR1/2 in CWR22R castration-resistant cells compared to the androgen-dependent CWR22 [82]. BAG1 is an AR co-regulator whose expression levels are also increased in CRPCa [83]. Amplification of its locus was found in 7.4% of CRPCa samples, while analysis of treatment-naïve samples did not reveal gene aberrations. BAG1 binds to a novel identified sequence adjacent to the AF-2 of AR, termed binding function 3 (BF-3) [84]. BAG1 is important for fine-tuning AR transcriptional activity and for suppressing a sub-set of AR responsive genes. In conclusion, the changed expression profile of AR co-regulators caused by ADT has implications for the transcriptional activity and program of AR and can result in an aberrant expression of AR downstream genes promoting survival of the malignant cells (Fig. 1e).

3.3. Altered androgenic pathways

AKR1C3 encodes for an aldo–keto reductase that converts androstenedione, a weak adrenal androgen, into testosterone [85]. This steroidogenic enzyme, among others of androgen metabolism, was increasingly detected in mCRPCa bone samples compared to primary treatment-naïve PCa [86]. In other words, intra-tumoural upregulation of androgenic enzymes enables conversion of adrenal steroids into testosterone and DHT with levels that are sufficient to activate transcription through AR and drive castration resistance (also known as the front-door, conventional pathway, Fig. 1f) [87,88]. Interestingly, this intra-tumoural conversion of adrenal steroids is not only a mechanism of resistance against ADT; upregulation of AKR1C3 was also found in cells that are resistant to the second-line anti-androgen enzalutamide [89]. A recent study has shown that the transcription factor ERG, which is fused to the androgen-responsive TMPRSS2 in about 50% of PCa patients [90], is a direct positive regulator on the AKR1C3 promoter [91]. ERG is co-expressed with AKR1C3 in PCa tissue samples. This could have clinical implications and explain why TMPRSS2-ERG in circulating tumour cells is a predictive biomarker of sensitivity for CRPCa patients treated with the CYP17A1 inhibitor abiraterone acetate, which inhibits the formation of adrenal androgens [92]. Studies with a newly developed AKR1C3 inhibitor, SN33638, have been conducted in cell culture models [93]. However, the authors conclude that only a subset of CRPCa patients with high AKR1C3 expression could possibly benefit from such a therapy. Other steroidogenic enzymes may also be implicated in progression to CRPCa. A gain-of-function mutation in the HSD3B1 enzyme has been discovered in some CRPCa tissue specimens, which controls the rate-limiting step from dehydroepiandrosterone into DHT [94]. This mutation renders the enzyme resistant to ubiquitin-mediated proteasomal degradation, leading to its accumulation and accelerated synthesis of DHT. The expression levels and isozyme activity of 5α-steroid reductase, which converts testosterone into DHT, is shifted from isoform 2 (SRD5A2) to isoform 1 (SRD5A1) and 3 (SRD5A3) in CRPCa [95,96]. The mineralocorticoid deoxycorticosterone is also reduced by SRD5A1 and is able to activate the AR [97]. 5α-Dehydro-deoxycorticosterone was detected in eight out of 13 CRPCa tissues.

Another alternative steroidogenic route resulting from high expression and activity of SRD5A1 and AKR1C3 involves the conversion of androstenedione to 5α-androstanedione, and finally into DHT. This pathway bypasses the formation of testosterone (5α-androstanedione pathway). The shift from SRD5A2 to the SRD5A1 isoform in CRPCa samples may be a consequence of the fact that SRD5A1 has much higher affinity for androstenedione compared to SRD5A2 [98]. This pathway was shown to be the dominant pathway in CRPCa cell line models and metastatic tissue samples.

DHT may also be synthesised from cholesterol via the metabolites progesterone, allopregnanolone, androsterone and androstanediol, again by circumventing testosterone (back-door pathway) [99]. DHT synthesis through the back-door pathway requires the enzymes CYP17A1 and SRD5A, as well as a reverse oxidative step of androstanediol to DHT [100]. However, expression of CYP17A1 in PCa tissues seems to be low and infrequent [101,102], raising the question whether this back-door pathway actually plays a role in the acquisition of castration resistance.

3.4. Alternative steroid receptors

Glucocorticoids are frequently co-administered with abiraterone in order to counteract the drug-induced reductions in serum cortisol levels [103]. The glucocorticoid receptor (GR) however has been shown to drive a set of certain AR gene targets, including KLK3, in the absence of AR [104]. GR expression is increased in several PCa cell lines treated with the anti-androgens flutamide, bicalutamide and enzalutamide, and GR expression in metastatic tissue specimens isolated from the bone marrow correlates with the patients' response to enzalutamide [104,105]. A recent study demonstrated that GR transcriptional expression is inhibited through AR signalling via a negative androgen response element [106]. Thus, activation of the GR under conditions where AR is inactivated may give PCa cells a survival benefit and drive progression to castration resistance. Furthermore, a role for GR as a negative prognostic factor has already been described in ER-negative breast cancer [107]. This shows that steroid receptors may be able to compensate for the inactivation of another steroid receptor and thereby promote survival of the cell (Fig. 1g).

4. Histologic transformation

4.1. Neuroendocrine (trans-)differentiation

As stated above, neuroendocrine cells are rare secretory cells in normal prostatic tissue. Variable numbers of neuroendocrine cells can be detected in prostate adenocarcinomas by

immunohistochemical analysis, in part depending on the number of slides and various neuroendocrine antigen markers that are used [108]. The clinical prognostic significance of the presence and percentage of neuroendocrine cells in tumour specimens is unclear, although high grade and stage cancers express higher levels of chromogranin A (CHGA) than low grade or localised disease [109]. In contrast, there is no doubt that growth of malignant neuroendocrine-like cells is favoured by androgen deprivation and associated with acquisition of castration resistance, as well as loss of AR expression in a subset of CRPCa tissues [110,111]. However, due to a lack of a general marker it has been difficult to estimate the frequency of neuroendocrine differentiation in castration-resistant patients. Cancer cells with a neuroendocrine pattern may arise through a trans-differentiation process from luminal cells. *In vitro* evidence for a trans-differentiation process comes largely from studies performed with the androgen-responsive cell line LNCaP. The morphology of these cells changes to a neuroendocrine-like state upon growth in androgen-depleted medium and is accompanied by a secretion of several neuroendocrine markers [112]. The underlying mechanisms of this androgen deprivation-induced trans-differentiation include signalling via cAMP-activated PKA resulting in phosphorylation of RHOA [113,114], human achaete-scute homolog-1 (ASCL1) transcription factor [115], and receptor protein tyrosine phosphatase alpha (PTPRA) [116]. In addition, other factors and mechanisms such as IL6 through suppression of RE-1 silencing transcription factor (REST) [117], inhibition of NOTCH signalling leading to hypoxia [118], and cell-density-induced attenuation of CDK1/2 signalling [119] may induce neuroendocrine trans-differentiation.

Normal and malignant neuroendocrine cells do not express AR, and are unlikely to be affected by AR-targeting therapies [120]. It is therefore possible that while inactivation of AR decreases the number of luminal cells, malignant neuroendocrine cancer cells may repopulate the vacant space in the prostatic tissue and thus drive tumour recurrence. The pressure of AR-targeting therapy could select for a changed differentiation program in a common malignant progenitor cell to a neuroendocrine rather than a luminal phenotype. Most neuroendocrine cells express CD44, which is also a marker for the stem cell-containing, basal layer of the prostatic tissue [121]. Furthermore SOX2, an embryonic stem cell regulator that is repressed through AR signalling [122], is detected in basal cells of benign prostate hyperplasia specimens and in neuroendocrine tumours of murine and human origin, while being absent in primary PCa tissues [123]. CBX2 and EZH2, members of the polycomb group, are involved in epigenetic regulation of neuroendocrine differentiation [124] and are upregulated in metastatic CRPCa samples [125]. The fusion TMPRSS2-ERG can be detected in neuroendocrine cancers with a similar frequency to adenocarcinomas, showing that both cancer phenotypes have the same clonal origin [126]. When taken together neuroendocrine differentiation may be important in response to AR targeting therapies and progression to castration resistance, however due to a lack of adequate *in vitro* models and biomarkers, it remains an under-explored area (Fig. 2a).

4.2. Epithelial-to-mesenchymal transition

Epithelial-to-mesenchymal transition (EMT) as a mechanism of castration resistance has been studied infrequently in the past and clinical evidence for an EMT phenotype induced by AR-targeting therapies is sparse. Despite this, there are several *in vitro* and animal studies which have explored EMT as a resistance mechanism with interesting results. Recently, ADT-induced EMT was demonstrated in normal mouse prostate tissue and in the human LuCaP35 xenograft model [127]. In addition, increased mRNA expression of the mesenchymal markers Vimentin (VIM), ZEB1, Cadherin 11 (CDH11) and Fibronectin 1 (FN1) was detected in human tissue specimens from patients that underwent ADT. However, another study showed that DHT exposure led to EMT, higher migratory and invasive potential and activation of the EMT-inducing transcription factor Snail [128]. Interestingly, high AR expression can suppress androgen-induced EMT. This conflicting data could be explained by the observation that AR splice variants, that are induced through AR-targeting therapies (see above), have a distinctive transcriptional program compared to the full length receptor [62] and might be responsible for the transition to a mesenchymal phenotype. Overexpression of constitutively active variants AR-V7 and AR Q640X in LNCaP cells resulted in increased expression of mesenchymal markers [129]. Similarly androgen deprivation resulted in an upregulation of AR-V7 and mesenchymal markers, both of which can be reversed by the addition of DHT and reactivation of the full length receptor [130]. In general, EMT seems to be a reasonable explanation for cancers with a mesenchymal phenotype (Fig. 2b). However, to our knowledge there is as of yet no study demonstrating that this mesenchymal phenotype is a consequence of dedifferentiation of the epithelial phenotype or alternatively a clonal expansion from an already existing mesenchymal phenotype.

5. Cancer stem cells

In the previous sections we have discussed several mechanisms of therapy resistance, which may be well-explained by a stochastic model of cancer progression, i.e. that a random cell acquires a survival benefit in response to the treatment and gives rise to a new, resistant tumour bulk. However, evidence increasingly suggests that cancers are organised in a hierarchical manner, similar to normal tissues, and that resistant tumours arise from cancer stem cells that have adapted to the therapy environment.

5.1. Evidence for CSC in PCa

Since the first report in 1994 of a role for stem cells in human acute myeloid leukaemia subsequent reports have identified so-called cancer stem cells (CSC) in a variety of tumours such as breast, brain, colon, pancreas, lung, melanoma and glioblastoma [131]. CSC represent a small population of cells inside a tumour which share the characteristics of normal stem cells, i.e. self-renewal and the ability to recapitulate the heterogeneous phenotype of the parental cancers by differentiation [132]. However, the capacity to form a parental-like tumour is not sufficient to define a CSC; these tumours must also be serially transplantable. In contrast to initial beliefs, recent studies have suggested that around 25% of cancer cells may have CSC properties [133].

Small CSC populations have also been detected in PCa [134]. Although the main hypothesis is that prostate cancer arises from terminally differentiated luminal cells, there is growing evidence that PCa arises from more undifferentiated cells with a basal phenotype [135–138]. In limiting dilution assays it was demonstrated that androgen-independent tumours arise from AR-negative cells at a frequency of $1{:}10^5$–10^6 AR-positive cells [139]. Interestingly, AR-negative cells with very similar properties to stem cells have been found in the PCa cell line LNCaP, which is meant to represent luminal cancer cells [140]. In addition, the laboratory of Collins and Maitland could isolate a population from primary PCa cells with high clonogenic potential and the ability to differentiate into luminal cells (AR^+, $ACPP^+$, $ITGB2^+$), which were phenotypically identical to normal stem cells ($CD44^+$, $ITGA2^{hi}$, $PROM1^+$) [141]. PCa CSC have a unique miRNA expression profile overlapping with unfractionated advanced PCa and human embryonic SC [142]. Moreover, these cells express the cancer specific TMPRSS2:ETS fusion genes [143]. In further support of this, $CD44^+$ PCa xenograft

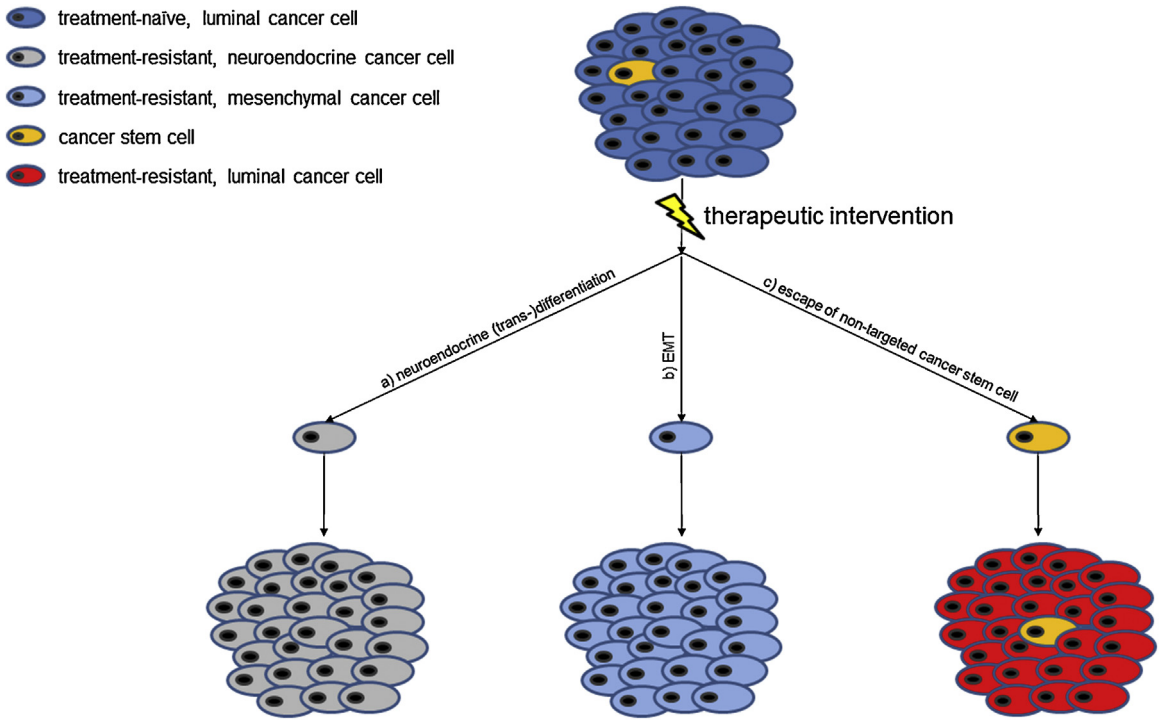

Fig. 2. Altered differentiation as therapy escape mechanisms. Histologic transformations of the treatment-naïve luminal tumour may occur in response to therapeutic intervention due to neuroendocrine (trans-)differentiation (a) and epithelial-to-mesenchymal transition (EMT, b). A small population of cancer stem cells within the treatment-naïve luminal tumour can give rise to a treatment-resistant luminal tumour via aberrant differentiation after/during therapeutic intervention (c).

cells possess enhanced tumour-initiating ability *in vivo* compared to the $CD44^-$ population and could be successively serially transplanted [144,145]. Lastly, one study demonstrated that tumours containing luminal, basal and neuroendocrine cells could be generated in mice by using AR- and p63-negative cells expressing the stem cell markers CD44, PROM1, nestin (NES) and KIT [146]. However, although there is evidence that supports PCa originating from CSC, until now there is no study to our knowledge that has shown that a single cell could form a tumour which both represents a patient's phenotype and which could be serially transplanted. Also, due to the high heterogeneity of PCa, the possibility that CSC exist in some but not all tumours should be considered.

5.2. Role of CSC in therapy resistance

ADT, radio- and chemotherapy efficiently target the highly proliferative and AR positive luminal cells representing the bulk (around 99%) of the tumour [138,141,147,148]. However, these therapies fail to target the small population of quiescent and AR-negative CSC, which are able to adapt to their new niche, resulting in the regrowth of a therapy-resistant and more aggressive tumour [149]. Quiescence is generally a challenging problem to target due to the fact that most therapeutics are designed to target highly proliferative cells. In addition, quiescence is not the only reason for prominent therapy resistance of CSC. High DNA repair activity is a property of CSC which protects them from DNA damage-inducing agents (e.g. Cisplatin) and radiotherapy [150]. PCa CSC sustained fewer lethal double-strand breaks than more differentiated cells, suggesting that CSC may have increased DNA repair activity and are therefore better equipped to deal with DNA damage [151]. Several studies reported that CSC as well as normal stem cells have an increased threshold for induction of mitochondrial apoptosis due to elevated expression of anti-apoptotic proteins (e.g. BCL2, BCL2L1 and MCL1) and members of the Inhibitor of Apoptosis family (e.g. BIRC5) and are therefore more resistant to cytotoxic agents [152–154]. We have recently shown increased MCL1 mRNA expression in PCa CSC [155]. The BCL2 Homology Domain-3 mimetic Obatoclax decreased the clonogenic efficiency of $CD44^+$ prostate cancer cells derived from a primary tumour supporting a role of the BCL2 family in the tumour forming fraction of PCa. In addition to the increased apoptotic threshold, CSC also have a high ability for drug efflux through proteins such as ATP-binding cassette (ABC) transporter family [156]. By using the Hoechst 33342 dye efflux assay, a stem cell-enriched population was isolated from benign and malignant prostate. This demonstrates that drug efflux transporters are also highly active in prostate CSC and normal SC, and consequently may be able to efflux chemotherapeutic drugs across the plasma membrane [157]. In conclusion, CSC have intrinsically high resistance against various therapies, and they seem a reasonable explanation for the high relapse of PCa after ADT and other therapies (Fig. 2c). However more research is necessary in order to prove the existence of these cells and to determine whether all tumours harbour CSC.

6. Miscellaneous mechanisms

A number of signalling pathways have been found to be deregulated in PCa and to contribute to PCa progression. Receptor and non-receptor kinases can circumvent the blocked AR pathway and stimulate the proliferation and survival of the androgen-deprived cells. As discussed above, this may also lead to AR transactivation. A recent quantitative phosphoproteomic study with mCRPCa samples revealed increased activation patterns for SRC, EGFR, RET and ALK kinases compared to treatment-naïve specimens, although with high inter-patient variability [158]. The IGF signalling pathway may also lead to PCa progression in patients undergoing ADT. It was suggested that the hypogonadal state during ADT may lead to insulin resistance and/or obesity and increased IGF-1 expression, driving proliferation and survival of the malignant cells [159]. Insulin resistance, hyperglycemia and metabolic syndrome are

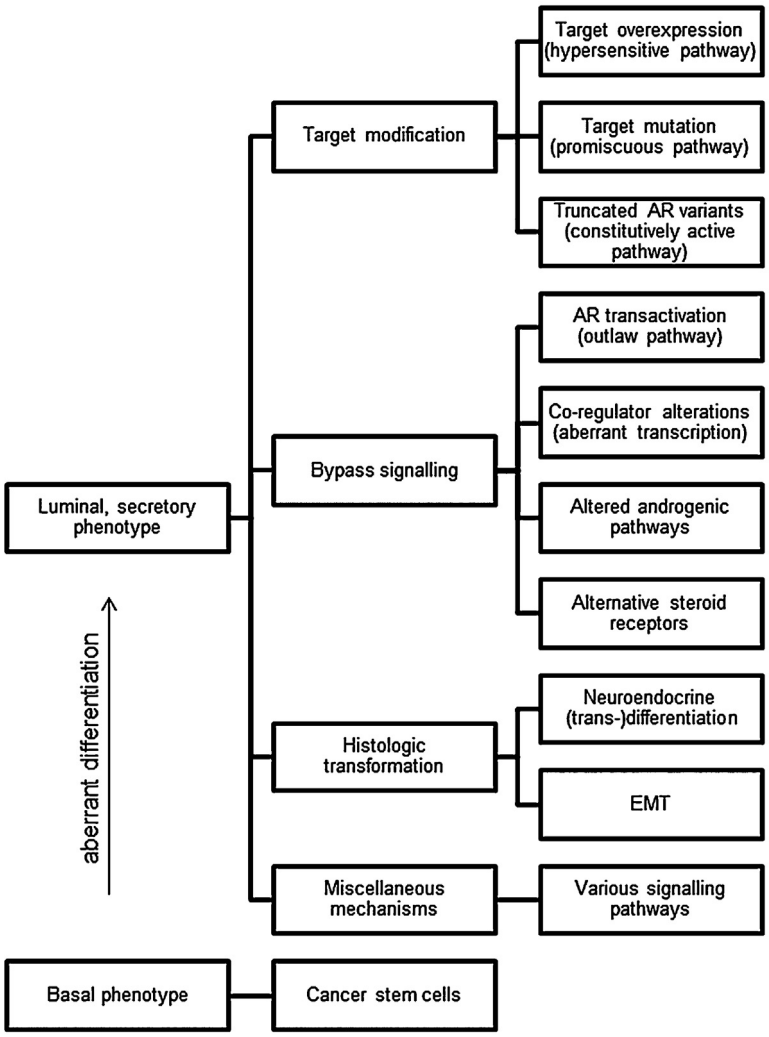

Fig. 3. Summary of the reviewed therapy escape mechanisms in PCa. Prostate adenocarcinoma appears as disease of malignant cells with a luminal phenotype, which are essentially dependent on androgenic signalling. Thus, the main therapy target is inactivation of AR. Various mechanisms confer acquired resistance to AR-targeting therapies. These include mechanisms modifying expression and transcriptional activity of AR (target modification), activation of compensatory pathways to circumvent the inhibited target (bypass signalling), phenotypic changes of the epithelial cells (histologic transformation) and miscellaneous pro-survival and pro-proliferative mechanisms. However, there is increasing data that those mechanisms originate in cancer stem cells with a basal phenotype, which give rise to an aberrantly differentiated luminal phenotype under the pressure of the AR-targeting therapy.

known endocrine complications of ADT [160]. Another mechanism leading to increased growth factor signalling is through inactivation of the phosphatase PTEN, which results in aberrant activation of the PI3K-Akt pathway. Loss of PTEN occurs in about 50% of all PCa patients [161] and its inactivation is sufficient to initiate PCa in mice [162]. In that particular mouse model it was shown that castration-resistant growth is an intrinsic property of *Pten*-null PCa cells, as a result of crosstalk between PI3K and AR [163]. Loss of PTEN expression is associated with poor survival and decreased time of patients on abiraterone treatment [164], and it was proposed that PCa patients with PTEN-deficiency might be better treated with PI3K- than AR-targeting therapies [165]. The metal ion-binding metallothionein (MT) superfamily has also been implicated in treatment-resistant prostate cancer. As there are unusually high levels of zinc in the prostate compared to the rest of the body [166], it is possible that this tissue already has high basal MT levels [167]. Carcinogenesis and treatment may further increase MT expression levels by factors such as hypoxia [168], reactive oxygen species [169] and androgen deprivation [170]. The MT protein is thought to play a role in a number of pathways where it acts to increase proliferation [171], reduce apoptosis [172] and scavenge free radicals [169], and thus increased expression may confer resistance to cells via these pathways [173]. However there are many MT protein isoforms and thus knowing exactly which ones are involved in resistance, and therefore which ones to target, currently proves troublesome. Another side effect of ADT is increased inflammation through infiltration of T-cells into the prostatic tissue [174]. Cytokines and chemokines produced by the infiltrating immune cells, such as Interleukins-1β, -6, and -8 may drive proliferation and survival of prostate cancer cells. In particular, serum IL6 levels are significantly elevated in CRPCa patients [175] and IL6 is known to upregulate expression of MCL1, a member of the BCL2 family, thus promoting PCa cell survival [176]. High expression of MCL1 and PCa cell survival is also a consequence of AR inactivation and subsequent cell cycle arrest [155]. In conclusion, extrinsic and intrinsic mechanisms can lead to increased growth factor production in the tumour environment and increased signalling in the tumour cell resulting in stimulatory effects supporting the cancer cell's survival and proliferation.

7. Conclusions

The present review has summarised and classified various PCa therapy escape mechanisms (Fig. 3). While some mechanisms occur more frequently, others are rare or their occurrence statistics are not available, mostly due to a lack of diagnostic detectability. Additionally, due to the heterogeneity of the disease, multiple mechanisms of therapy resistance may co-occur. It is important to note that the same or similar concepts of escape mechanisms as previously identified for ADT and CAB also confer resistance to the second-generation drugs enzalutamide and abiraterone. As a consequence, it remains difficult to select the best treatment option in order to eliminate the risk of *ab initio* resistance. A specific treatment sequence for CRPCa patients after chemotherapy failure is not available at the moment. The occurrence of various treatment resistances also gives a rationale for treating patients as early as possible with combination therapies, where ideally two or more different molecular targets are hit at the same time. For instance, a recent clinical phase III study shows promising results by treating patients with high-risk localised PCa with a combination of ADT and the microtubule-targeting agents docetaxel and estramustine compared to an ADT protocol only [177]. Also, the identification of CSC strongly argues for the use of therapeutic combination regimes. Ideally, this would include a therapy that targets the bulk of more differentiated, but highly proliferative cancer cells while the second aims to eliminate the more undifferentiated, CSC-containing population of the tumour. This concept would recommend for PCa that treatment-naïve tumours should be treated by the approved AR-targeting and/or radiation therapies in synergy with a protocol that targets the CSC-containing, basal layer of the prostate. In conclusion, the development of novel AR-targeting therapies is well-justified in order to better target luminal, androgen-dependent PCa. However, further research should also aim to develop therapeutic options to inactivate the CSC-containing, androgen-independent basal PCa phenotype.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Funding and acknowledgements

The authors would like to acknowledge the Austrian Science Fund (FWF, Project P26799-B23 to FRS) and thank Prof. Dr. Norman Maitland (University of York) for providing comments to the manuscript.

References

[1] McNeal JE. Normal and pathologic anatomy of prostate. Urology 1981;17:11–6.
[2] Isaacs JT. Prostate stem cells and benign prostatic hyperplasia. Prostate 2008;68:1025–34, http://dx.doi.org/10.1002/pros.20763.
[3] Krijnen JL, Janssen PJ, Ruizeveld de Winter JA, van Krimpen H, Schröder FH, van der Kwast TH. Do neuroendocrine cells in human prostate cancer express androgen receptor? Histochemistry 1993;100:393–8.
[4] Lilja H, Abrahamsson PA. Three predominant proteins secreted by the human prostate gland. Prostate 1988;12:29–38.
[5] Lang SH, Stark M, Collins A, Paul AB, Stower MJ, Maitland NJ. Experimental prostate epithelial morphogenesis in response to stroma and three-dimensional matrigel culture. Cell Growth Differ 2001;12:631–40.
[6] Rumpold H, Heinrich E, Untergasser G, Hermann M, Pfister G, Plas E, et al. Neuroendocrine differentiation of human prostatic primary epithelial cells in vitro. Prostate 2002;53:101–8, http://dx.doi.org/10.1002/pros.10129.
[7] Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, et al. EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer. Eur Urol 2014;65:467–79, http://dx.doi.org/10.1016/j.eururo.2013.11.002.
[8] Huggins C, Hodges CV. 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 1972;22:232–40.
[9] Scher HI, Fizazi K, Saad F, Taplin M-E, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012;367:1187–97, http://dx.doi.org/10.1056/NEJMoa1207506.
[10] Fizazi K, Scher HI, Molina A, Logothetis CJ, Chi KN, Jones RJ, et al. Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: Final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol 2012;13:983–92, http://dx.doi.org/10.1016/S1470-2045(12)70379-0.
[11] Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med 2014;371:424–33, http://dx.doi.org/10.1056/NEJMoa1405095.
[12] Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science 2009;324:787–90, http://dx.doi.org/10.1126/science.1168175.
[13] Lubahn DB, Brown TR, Simental JA, Higgs HN, Migeon CJ, Wilson EM, et al. Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity. Proc Natl Acad Sci U S A 1989;86:9534–8.
[14] Jenster G, van der Korput HAGM, van Vroonhoven C, van der Kwast TH, Trapman J, Brinkmann AO. Domains of the human androgen receptor involved in steroid binding, transcriptional activation, and subcellular localization. Mol Endocrinol 1991;5:1396–404, http://dx.doi.org/10.1210/mend-5-10-1396.
[15] Culig Z, Santer FR. Androgen receptor co-activators in the regulation of cellular events in prostate cancer. World J Urol 2012;30:297–302, http://dx.doi.org/10.1007/s00345-011-0797-6.
[16] Lovly CM, Shaw AT. Molecular pathways: Resistance to kinase inhibitors and implications for therapeutic strategies. Clin Cancer Res 2014;20:2249–56, http://dx.doi.org/10.1158/1078-0432.CCR-13-1610.
[17] Linja MJ, Savinainen KJ, Saramäki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 2001;61:3550–5.
[18] Ford OH, Gregory CW, Kim D, Smitherman AB, Mohler JL. Androgen receptor gene amplification and protein expression in recurrent prostate cancer. J Urol 2003;170:1817–21, http://dx.doi.org/10.1097/01.ju.0000091873.09677.f4.
[19] Qu X, Randhawa G, Friedman C, Kurland BF, Glaskova L, Coleman I, et al. A three-marker FISH panel detects more genetic aberrations of AR, PTEN and TMPRSS2/ERG in castration-resistant or metastatic prostate cancers than in primary prostate tumors. PLoS ONE 2013;8:e74671, http://dx.doi.org/10.1371/journal.pone.0074671.
[20] Waltering KK, Helenius MA, Sahu B, Manni V, Linja MJ, Jänne OA, et al. Increased expression of androgen receptor sensitizes prostate cancer cells to low levels of androgens. Cancer Res 2009;69:8141–9, http://dx.doi.org/10.1158/0008-5472.CAN-09-0919.
[21] Urbanucci A, Sahu B, Seppälä J, Larjo A, Latonen LM, Waltering KK, et al. Overexpression of androgen receptor enhances the binding of the receptor to the chromatin in prostate cancer. Oncogene 2012;31:2153–63, http://dx.doi.org/10.1038/onc.2011.401.
[22] Merson S, Yang ZH, Brewer D, Olmos D, Eichholz A, McCarthy F, et al. Focal amplification of the androgen receptor gene in hormone-naive human prostate cancer. Br J Cancer 2014;110:1655–62, http://dx.doi.org/10.1038/bjc.2014.13.
[23] Palmberg C, Koivisto P, Kakkola L, Tammela TL, Kallioniemi OP, Visakorpi T. Androgen receptor gene amplification at primary progression predicts response to combined androgen blockade as second line therapy for advanced prostate cancer. J Urol 2000;164:1992–5.
[24] Shaffer DR, Leversha MA, Danila DC, Lin O, Gonzalez-Espinoza R, Gu B, et al. Circulating tumor cell analysis in patients with progressive castration-resistant prostate cancer. Clin Cancer Res 2007;13:2023–9, http://dx.doi.org/10.1158/1078-0432.CCR-06-2701.
[25] Geng C, Rajapakshe K, Shah SS, Shou J, Eedunuri VK, Foley C, et al. Androgen receptor is the key transcriptional mediator of the tumor suppressor SPOP in prostate cancer. Cancer Res 2014;74:5631–43, http://dx.doi.org/10.1158/0008-5472.CAN-14-0476.
[26] An J, Wang C, Deng Y, Yu L, Huang H. Destruction of full-length androgen receptor by wild-type SPOP, but not prostate-cancer-associated mutants. Cell Rep 2014;6:657–69, http://dx.doi.org/10.1016/j.celrep.2014.01.013.
[27] Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat J-P, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 2012;44:685–9, http://dx.doi.org/10.1038/ng.2279.
[28] Grasso CS, Wu Y-M, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012;487:239–43, http://dx.doi.org/10.1038/nature11125.
[29] Qi J, Tripathi M, Mishra R, Sahgal N, Fazli L, Fazil L, et al. The E3 ubiquitin ligase Siah2 contributes to castration-resistant prostate cancer by regulation

of androgen receptor transcriptional activity. Cancer Cell 2013;23:332–46, http://dx.doi.org/10.1016/j.ccr.2013.02.016.
[30] Köhler A, Demir U, Kickstein E, Krauss S, Aigner J, Aranda-Orgillés B, et al. A hormone-dependent feedback-loop controls androgen receptor levels by limiting MID1, a novel translation enhancer and promoter of oncogenic signaling. Mol Cancer 2014;13:146, http://dx.doi.org/10.1186/1476-4598-13-146.
[31] Quarmby VE, Yarbrough WG, Lubahn DB, French FS, Wilson EM. Autologous down-regulation of androgen receptor messenger ribonucleic acid. Mol Endocrinol 1990;4:22–8, http://dx.doi.org/10.1210/mend-4-1-22.
[32] Cai C, He HH, Chen S, Coleman I, Wang H, Fang Z, et al. Androgen receptor gene expression in prostate cancer is directly suppressed by the androgen receptor through recruitment of lysine-specific demethylase 1. Cancer Cell 2011;20:457–71, http://dx.doi.org/10.1016/j.ccr.2011.09.001.
[33] McDonald S, Brive L, Agus DB, Scher HI, Ely KR. Ligand responsiveness in human prostate cancer: Structural analysis of mutant androgen receptors from LNCaP and CWR22 tumors. Cancer Res 2000;60:2317–22.
[34] Azad AA, Volik SV, Wyatt AW, Haegert A, Le Bihan S, Bell RH, et al. Androgen receptor gene aberrations in circulating cell-free DNA: Biomarkers of therapeutic resistance in castration-resistant prostate cancer. Clin Cancer Res 2015, http://dx.doi.org/10.1158/1078-0432.CCR-14-2666.
[35] Joseph JD, Lu N, Qian J, Sensintaffar J, Shao G, Brigham D, et al. A clinically relevant androgen receptor mutation confers resistance to second-generation antiandrogens enzalutamide and ARN-509. Cancer Discov 2013;3:1020–9, http://dx.doi.org/10.1158/2159-8290.CD-13-0226.
[36] Korpal M, Korn JM, Gao X, Rakiec DP, Ruddy DA, Doshi S, et al. An F876L mutation in androgen receptor confers genetic and phenotypic resistance to MDV3100 (enzalutamide). Cancer Discov 2013;3:1030–43, http://dx.doi.org/10.1158/2159-8290.CD-13-0142.
[37] Chen EJ, Sowalsky AG, Gao S, Cai C, Voznesensky O, Schaefer R, et al. Abiraterone treatment in castration-resistant prostate cancer selects for progesterone responsive mutant androgen receptors. Clin Cancer Res 2015;21:1273–80, http://dx.doi.org/10.1158/1078-0432.CCR-14-1220.
[38] Yoshida T, Kinoshita H, Segawa T, Nakamura E, Inoue T, Shimizu Y, et al. Antiandrogen bicalutamide promotes tumor growth in a novel androgen-dependent prostate cancer xenograft model derived from a bicalutamide-treated patient. Cancer Res 2005;65:9611–6, http://dx.doi.org/10.1158/0008-5472.CAN-05-0817.
[39] Culig Z, Hobisch A, Cronauer MV, Cato AC, Hittmair A, Radmayr C, et al. Mutant androgen receptor detected in an advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. Mol Endocrinol 1993;7:1541–50, http://dx.doi.org/10.1210/mend.7.12.8145761.
[40] Tan J, Sharief Y, Hamil KG, Gregory CW, Zang D-Y, Sar M, et al. Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. Mol Endocrinol 1997;11:450–9, http://dx.doi.org/10.1210/mend.11.4.9906.
[41] Taplin ME, Bubley GJ, Ko YJ, Small EJ, Upton M, Rajeshkumar B, et al. Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. Cancer Res 1999;59:2511–5.
[42] Zhao XY, Malloy PJ, Krishnan AV, Swami S, Navone NM, Peehl DM, et al. Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a mutated androgen receptor. Nat Med 2000;6:703–6, http://dx.doi.org/10.1038/76287.
[43] Culig Z, Stober J, Gast A, Peterziel H, Hobisch A, Radmayr C, et al. Activation of two mutant androgen receptors from human prostatic carcinoma by adrenal androgens and metabolic derivatives of testosterone. Cancer Detect Prev 1996;20:68–75.
[44] Rodriguez-Vida A, Bianchini D, Van Hemelrijck M, Hughes S, Malik Z, Powles T, et al. Is there an antiandrogen withdrawal syndrome with enzalutamide? BJU Int 2015;115:373–80, http://dx.doi.org/10.1111/bju.12826.
[45] Nieh PT. Withdrawal phenomenon with the antiandrogen casodex. J Urol 1995;153:1070–2 (discussion 1072–1073).
[46] Scher HI, Kelly WK. Flutamide withdrawal syndrome: Its impact on clinical trials in hormone-refractory prostate cancer. J Clin Oncol 1993;11:1566–72.
[47] Ni L, Llewellyn R, Kesler CT, Kelley JB, Spencer A, Snow CJ, et al. Androgen induces a switch from cytoplasmic retention to nuclear import of the androgen receptor. Mol Cell Biol 2013;33:4766–78, http://dx.doi.org/10.1128/MCB.00647-13.
[48] Duff J, McEwan IJ. Mutation of histidine 874 in the androgen receptor ligand-binding domain leads to promiscuous ligand activation and altered p160 coactivator interactions. Mol Endocrinol 2005;19:2943–54, http://dx.doi.org/10.1210/me.2005-0231.
[49] Libertini SJ, Tepper CG, Rodriguez V, Asmuth DM, Kung H-J, Mudryj M. Evidence for calpain-mediated androgen receptor cleavage as a mechanism for androgen independence. Cancer Res 2007;67:9001–5, http://dx.doi.org/10.1158/0008-5472.CAN-07-1072.
[50] Li Y, Hwang TH, Oseth LA, Hauge A, Vessella RL, Schmechel SC, et al. AR intragenic deletions linked to androgen receptor splice variant expression and activity in models of prostate cancer progression. Oncogene 2012;31:4759–67, http://dx.doi.org/10.1038/onc.2011.637.
[51] Nyquist MD, Li Y, Hwang TH, Manlove LS, Vessella RL, Silverstein KAT, et al. TALEN-engineered AR gene rearrangements reveal endocrine uncoupling of androgen receptor in prostate cancer. Proc Natl Acad Sci U S A 2013;110:17492–7, http://dx.doi.org/10.1073/pnas.1308587110.
[52] Liu LL, Xie N, Sun S, Plymate S, Mostaghel E, Dong X. Mechanisms of the androgen receptor splicing in prostate cancer cells. Oncogene 2014;33:3140–50, http://dx.doi.org/10.1038/onc.2013.284.
[53] Lu J, Van der Steen T, Tindall DJ. Are androgen receptor variants a substitute for the full-length receptor? Nat Rev Urol 2015;12:137–44, http://dx.doi.org/10.1038/nrurol.2015.13.
[54] Streicher W, Zengerling F, Laschak M, Weidemann W, Höpfner M, Schrader AJ, et al. AR-Q640X, a model to study the effects of constitutively active C-terminally truncated AR variants in prostate cancer cells. World J Urol 2012;30:333–9, http://dx.doi.org/10.1007/s00345-012-0842-0.
[55] Chan SC, Li Y, Dehm SM. Androgen receptor splice variants activate androgen receptor target genes and support aberrant prostate cancer cell growth independent of canonical androgen receptor nuclear localization signal. J Biol Chem 2012;287:19736–49, http://dx.doi.org/10.1074/jbc.M112.352930.
[56] Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM, et al. Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: Induction of steroidogenesis and androgen receptor splice variants. Clin Cancer Res 2011;17:5913–25, http://dx.doi.org/10.1158/1078-0432.CCR-11-0728.
[57] Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KAT, Dehm SM. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. Cancer Res 2013;73:483–9, http://dx.doi.org/10.1158/0008-5472.CAN-12-3630.
[58] Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res 2009;69:16–22, http://dx.doi.org/10.1158/0008-5472.CAN-08-2764.
[59] Qu Y, Dai B, Ye D, Kong Y, Chang K, Jia Z, et al. Constitutively active AR-V7 plays an essential role in the development and progression of castration-resistant prostate cancer. Sci Rep 2015;5:7654, http://dx.doi.org/10.1038/srep07654.
[60] Yu Z, Chen S, Sowalsky AG, Voznesensky OS, Mostaghel EA, Nelson PS, et al. Rapid induction of androgen receptor splice variants by androgen deprivation in prostate cancer. Clin Cancer Res 2014;20:1590–600, http://dx.doi.org/10.1158/1078-0432.CCR-13-1863.
[61] Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 2014;371:1028–38, http://dx.doi.org/10.1056/NEJMoa1315815.
[62] Hu R, Lu C, Mostaghel EA, Yegnasubramanian S, Gurel M, Tannahill C, et al. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. Cancer Res 2012;72:3457–62, http://dx.doi.org/10.1158/0008-5472.CAN-11-3892.
[63] Hobisch A, Eder IE, Putz T, Horninger W, Bartsch G, Klocker H, et al. Interleukin-6 regulates prostate-specific protein expression in prostate carcinoma cells by activation of the androgen receptor. Cancer Res 1998;58:4640–5.
[64] Yang L, Wang L, Lin H-K, Kan P-Y, Xie S, Tsai M-Y, et al. Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3, and MAPK, three distinct signal pathways in prostate cancer cells. Biochem Biophys Res Commun 2003;305:462–9.
[65] Ueda T, Bruchovsky N, Sadar MD. Activation of the androgen receptor N-terminal domain by interleukin-6 via MAPK and STAT3 signal transduction pathways. J Biol Chem 2002;277:7076–85, http://dx.doi.org/10.1074/jbc.M108255200.
[66] Ueda T, Mawji NR, Bruchovsky N, Sadar MD. Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells. J Biol Chem 2002;277:38087–94, http://dx.doi.org/10.1074/jbc.M203313200.
[67] Rocha J, Zouanat FZ, Zoubeidi A, Hamel L, Benidir T, Scarlata E, et al. The Fer tyrosine kinase acts as a downstream interleukin-6 effector of androgen receptor activation in prostate cancer. Mol Cell Endocrinol 2013;381:140–9, http://dx.doi.org/10.1016/j.mce.2013.07.017.
[68] Malinowska K, Neuwirt H, Cavarretta IT, Bektic J, Steiner H, Dietrich H, et al. Interleukin-6 stimulation of growth of prostate cancer in vitro and in vivo through activation of the androgen receptor. Endocr Relat Cancer 2009;16:155–69, http://dx.doi.org/10.1677/ERC-08-0174.
[69] Guo Z, Dai B, Jiang T, Xu K, Xie Y, Kim O, et al. Regulation of androgen receptor activity by tyrosine phosphorylation. Cancer Cell 2006;10:309–19, http://dx.doi.org/10.1016/j.ccr.2006.08.021.
[70] Summy JM, Gallick GE. Src family kinases in tumor progression and metastasis. Cancer Metastasis Rev 2003;22:337–58.
[71] Desai SJ, Ma A-H, Tepper CG, Chen H-W, Kung H-J. Inappropriate activation of the androgen receptor by nonsteroids: Involvement of the Src kinase pathway and its therapeutic implications. Cancer Res 2006;66:10449–59, http://dx.doi.org/10.1158/0008-5472.CAN-06-2582.
[72] Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, et al. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res 1994;54:5474–8.

[73] Heemers HV, Regan KM, Schmidt LJ, Anderson SK, Ballman KV, Tindall DJ. Androgen modulation of coregulator expression in prostate cancer cells. Mol Endocrinol 2009;23:572–83, http://dx.doi.org/10.1210/me.2008-0363.
[74] Heemers HV, Sebo TJ, Debes JD, Regan KM, Raclaw KA, Murphy LM, et al. Androgen deprivation increases p300 expression in prostate cancer cells. Cancer Res 2007;67:3422–30, http://dx.doi.org/10.1158/0008-5472.CAN-06-2836.
[75] Debes JD, Schmidt LJ, Huang H, Tindall DJ. p300 Mediates androgen-independent transactivation of the androgen receptor by interleukin 6. Cancer Res 2002;62:5632–6.
[76] Gong J, Zhu J, Goodman OB, Pestell RG, Schlegel PN, Nanus DM, et al. Activation of p300 histone acetyltransferase activity and acetylation of the androgen receptor by bombesin in prostate cancer cells. Oncogene 2006;25:2011–21, http://dx.doi.org/10.1038/sj.onc.1209231.
[77] Comuzzi B, Nemes C, Schmidt S, Jasarevic Z, Lodde M, Pycha A, et al. The androgen receptor co-activator CBP is up-regulated following androgen withdrawal and is highly expressed in advanced prostate cancer. J Pathol 2004;204:159–66, http://dx.doi.org/10.1002/path.1609.
[78] Merkle D, Hoffmann R. Roles of cAMP and cAMP-dependent protein kinase in the progression of prostate cancer: Cross-talk with the androgen receptor. Cell Signal 2011;23:507–15, http://dx.doi.org/10.1016/j.cellsig.2010.08.017.
[79] Kim J, Jia L, Stallcup MR, Coetzee GA. The role of protein kinase A pathway and cAMP responsive element-binding protein in androgen receptor-mediated transcription at the prostate-specific antigen locus. J Mol Endocrinol 2005;34:107–18, http://dx.doi.org/10.1677/jme.1.01701.
[80] Choi H-K, Yoo J-Y, Jeong M-H, Park S-Y, Shin D-M, Jang S-W, et al. Protein kinase A phosphorylates NCoR to enhance its nuclear translocation and repressive function in human prostate cancer cells. J Cell Physiol 2013;228:1159–65, http://dx.doi.org/10.1002/jcp.24269.
[81] Laschak M, Bechtel M, Spindler K-D, Hessenauer A. Inability of NCoR/SMRT to repress androgen receptor transcriptional activity in prostate cancer cell lines. Int J Mol Med 2011;28:645–51, http://dx.doi.org/10.3892/ijmm.2011.735.
[82] Godoy AS, Sotomayor PC, Villagran M, Yacoub R, Montecinos VP, McNerney EM, et al. Altered corepressor SMRT expression and recruitment to target genes as a mechanism that change the response to androgens in prostate cancer progression. Biochem Biophys Res Commun 2012;423:564–70, http://dx.doi.org/10.1016/j.bbrc.2012.06.005.
[83] Mäki HE, Saramäki OR, Shatkina L, Martikainen PM, Tammela TLJ, van Weerden WM, et al. Overexpression and gene amplification of BAG-1L in hormone-refractory prostate cancer. J Pathol 2007;212:395–401, http://dx.doi.org/10.1002/path.2186.
[84] Jehle K, Cato L, Neeb A, Muhle-Goll C, Jung N, Smith EW, et al. Coregulator control of androgen receptor action by a novel nuclear receptor-binding motif. J Biol Chem 2014;289:8839–51, http://dx.doi.org/10.1074/jbc.M113.534859.
[85] Khanna M, Qin KN, Wang RW, Cheng KC. Substrate specificity, gene structure, and tissue-specific distribution of multiple human 3 alpha-hydroxysteroid dehydrogenases. J Biol Chem 1995;270:20162–8.
[86] Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 2006;66:2815–25, http://dx.doi.org/10.1158/0008-5472.CAN-05-4000.
[87] Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res 2008;68:6407–15, http://dx.doi.org/10.1158/0008-5472.CAN-07-5997.
[88] Mostaghel EA, Page ST, Lin DW, Fazli L, Coleman IM, True LD, et al. Intraprostatic androgens and androgen-regulated gene expression persist after testosterone suppression: Therapeutic implications for castration-resistant prostate cancer. Cancer Res 2007;67:5033–41, http://dx.doi.org/10.1158/0008-5472.CAN-06-3332.
[89] Liu C, Lou W, Zhu Y, Yang JC, Nadiminty N, Gaikwad NW, et al. Intracrine androgens and AKR1C3 activation confer resistance to enzalutamide in prostate cancer. Cancer Res 2015;75:1413–22, http://dx.doi.org/10.1158/0008-5472.CAN-14-3080.
[90] Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8, http://dx.doi.org/10.1126/science.1117679.
[91] Powell K, Semaan L, Conley-LaComb MK, Asangani I, Wu Y-M, Ginsburg KB, et al. ERG/AKR1C3/AR constitutes a feed-forward loop for AR signaling in prostate cancer cells. Clin Cancer Res 2015, http://dx.doi.org/10.1158/1078-0432.CCR-14-2352.
[92] Danila DC, Anand A, Sung CC, Heller G, Leversha MA, Cao L, et al. TMPRSS2-ERG status in circulating tumor cells as a predictive biomarker of sensitivity in castration-resistant prostate cancer patients treated with abiraterone acetate. Eur Urol 2011;60:897–904, http://dx.doi.org/10.1016/j.eururo.2011.07.011.
[93] Yin YD, Fu M, Brooke DG, Heinrich DM, Denny WA, Jamieson SMF. The activity of SN33638, an inhibitor of AKR1C3, on testosterone and 17β-estradiol production and function in castration-resistant prostate cancer and ER-positive breast cancer. Front Oncol 2014;4:159, http://dx.doi.org/10.3389/fonc.2014.00159.
[94] Chang K-H, Li R, Kuri B, Lotan Y, Roehrborn CG, Liu J, et al. A gain-of-function mutation in DHT synthesis in castration-resistant prostate cancer. Cell 2013;154:1074–84, http://dx.doi.org/10.1016/j.cell.2013.07.029.
[95] Uemura M, Tamura K, Chung S, Honma S, Okuyama A, Nakamura Y, et al. Novel 5 alpha-steroid reductase (SRD5A3, type-3) is overexpressed in hormone-refractory prostate cancer. Cancer Sci 2008;99:81–6, http://dx.doi.org/10.1111/j.1349-7006.2007.00656.x.
[96] Titus MA, Gregory CW, Ford OH, Schell MJ, Maygarden SJ, Mohler JL. Steroid 5α-reductase isozymes I and II in recurrent prostate cancer. Clin Cancer Res 2005;11:4365–71, http://dx.doi.org/10.1158/1078-0432.CCR-04-0738.
[97] Uemura M, Honma S, Chung S, Takata R, Furihata M, Nishimura K, et al. 5αDH-DOC (5α-dihydro-deoxycorticosterone) activates androgen receptor in castration-resistant prostate cancer. Cancer Sci 2010;101:1897–904, http://dx.doi.org/10.1111/j.1349-7006.2010.01620.x.
[98] Chang K-H, Li R, Papari-Zareei M, Watumull L, Zhao YD, Auchus RJ, et al. Dihydrotestosterone synthesis bypasses testosterone to drive castration-resistant prostate cancer. Proc Natl Acad Sci U S A 2011;108:13728–33, http://dx.doi.org/10.1073/pnas.1107898108.
[99] Fukami M, Homma K, Hasegawa T, Ogata T. Backdoor pathway for dihydrotestosterone biosynthesis: Implications for normal and abnormal human sex development. Dev Dyn 2013;242:320–9, http://dx.doi.org/10.1002/dvdy.23892.
[100] Mostaghel EA. Beyond T and DHT – Novel steroid derivatives capable of wild type androgen receptor activation. Int J Biol Sci 2014;10:602–13, http://dx.doi.org/10.7150/ijbs.8844.
[101] Jernberg E, Thysell E, Bovinder Ylitalo E, Rudolfsson S, Crnalic S, Widmark A, et al. Characterization of prostate cancer bone metastases according to expression levels of steroidogenic enzymes and androgen receptor splice variants. PLoS ONE 2013;8:e77407, http://dx.doi.org/10.1371/journal.pone.0077407.
[102] Hofland J, van Weerden WM, Dits NFJ, Steenbergen J, van Leenders GJLH, Jenster G, et al. Evidence of limited contributions for intratumoral steroidogenesis in prostate cancer. Cancer Res 2010;70:1256–64, http://dx.doi.org/10.1158/0008-5472.CAN-09-2092.
[103] Auchus RJ, Yu MK, Nguyen S, Mundle SD. Use of prednisone with abiraterone acetate in metastatic castration-resistant prostate cancer. Oncologist 2014;19:1231–40, http://dx.doi.org/10.1634/theoncologist.2014-0167.
[104] Arora VK, Schenkein E, Murali R, Subudhi SK, Wongvipat J, Balbas MD, et al. Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. Cell 2013;155:1309–22, http://dx.doi.org/10.1016/j.cell.2013.11.012.
[105] Yemelyanov A, Bhalla P, Yang X, Ugolkov A, Iwadate K, Karseladze A, et al. Differential targeting of androgen and glucocorticoid receptors induces ER stress and apoptosis in prostate cancer cells. Cell Cycle 2012;11:395–406, http://dx.doi.org/10.4161/cc.11.2.18945.
[106] Xie N, Cheng H, Lin D, Liu L, Yang O, Jia L, et al. The expression of glucocorticoid receptor is negatively regulated by active androgen receptor signaling in prostate tumors. Int J Cancer 2015;136:E27–38, http://dx.doi.org/10.1002/ijc.29147.
[107] Pan D, Kocherginsky M, Conzen SD. Activation of the glucocorticoid receptor is associated with poor prognosis in estrogen receptor-negative breast cancer. Cancer Res 2011;71:6360–70, http://dx.doi.org/10.1158/0008-5472.CAN-11-0362.
[108] Epstein JI, Amin MB, Beltran H, Lotan TL, Mosquera J-M, Reuter VE, et al. Proposed morphologic classification of prostate cancer with neuroendocrine differentiation. Am J Surg Pathol 2014;38:756–67, http://dx.doi.org/10.1097/PAS.0000000000000208.
[109] Puccetti L, Supuran CT, Fasolo PP, Conti E, Sebastiani G, Lacquaniti S, et al. Skewing towards neuroendocrine phenotype in high grade or high stage androgen-responsive primary prostate cancer. Eur Urol 2005;48:215–21, http://dx.doi.org/10.1016/j.eururo.2005.03.018 (discussion 221–223).
[110] Hirano D, Okada Y, Minei S, Takimoto Y, Nemoto N. Neuroendocrine differentiation in hormone refractory prostate cancer following androgen deprivation therapy. Eur Urol 2004;45:586–92, http://dx.doi.org/10.1016/j.eururo.2003.11.032.
[111] Lipianskaya J, Cohen A, Chen CJ, Hsia E, Squires J, Li Z, et al. Androgen-deprivation therapy-induced aggressive prostate cancer with neuroendocrine differentiation. Asian J Androl 2014;16:541–4, http://dx.doi.org/10.4103/1008-682X.123669.
[112] Yuan T-C, Veeramani S, Lin F-F, Kondrikou D, Zelivianski S, Igawa T, et al. Androgen deprivation induces human prostate epithelial neuroendocrine differentiation of androgen-sensitive LNCaP cells. Endocr Relat Cancer 2006;13:151–67, http://dx.doi.org/10.1677/erc.1.01043.
[113] Kvissel A-K, Ramberg H, Eide T, Svindland A, Skålhegg BS, Taskén KA. Androgen dependent regulation of protein kinase A subunits in prostate cancer cells. Cell Signal 2007;19:401–9, http://dx.doi.org/10.1016/j.cellsig.2006.07.011.
[114] Jones SE, Palmer TM. Protein kinase A-mediated phosphorylation of RhoA on serine 188 triggers the rapid induction of a neuroendocrine-like phenotype

in prostate cancer epithelial cells. Cell Signal 2012;24:1504–14, http://dx.doi.org/10.1016/j.cellsig.2012.03.018.
[115] Rapa I, Volante M, Migliore C, Farsetti A, Berruti A, Vittorio Scagliotti G, et al. Human ASH-1 promotes neuroendocrine differentiation in androgen deprivation conditions and interferes with androgen responsiveness in prostate cancer cells. Prostate 2013;73:1241–9, http://dx.doi.org/10.1002/pros.22679.
[116] Zhang X-Q, Kondrikov D, Yuan T-C, Lin F-F, Hansen J, Lin M-F. Receptor protein tyrosine phosphatase alpha signaling is involved in androgen depletion-induced neuroendocrine differentiation of androgen-sensitive LNCaP human prostate cancer cells. Oncogene 2003;22:6704–16, http://dx.doi.org/10.1038/sj.onc.1206764.
[117] Zhu Y, Liu C, Cui Y, Nadiminty N, Lou W, Gao AC. Interleukin-6 induces neuroendocrine differentiation (NED) through suppression of RE-1 silencing transcription factor (REST). Prostate 2014;74:1086–94, http://dx.doi.org/10.1002/pros.22819.
[118] Danza G, Di Serio C, Rosati F, Lonetto G, Sturli N, Kacer D, et al. Notch signaling modulates hypoxia-induced neuroendocrine differentiation of human prostate cancer cells. Mol Cancer Res 2012;10:230–8, http://dx.doi.org/10.1158/1541-7786.MCR-11-0296.
[119] Pernicová Z, Slabáková E, Fedr R, Šimečková Š, Jaroš J, Suchánková T, et al. The role of high cell density in the promotion of neuroendocrine transdifferentiation of prostate cancer cells. Mol Cancer 2014;13:113, http://dx.doi.org/10.1186/1476-4598-13-113.
[120] Lin D, Wyatt AW, Xue H, Wang Y, Dong X, Haegert A, et al. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. Cancer Res 2014;74:1272–83, http://dx.doi.org/10.1158/0008-5472.CAN-13-2921-T.
[121] Palapattu GS, Wu C, Silvers CR, Martin HB, Williams K, Salamone L, et al. Selective expression of CD44, a putative prostate cancer stem cell marker, in neuroendocrine tumor cells of human prostate cancer. Prostate 2009;69:787–98, http://dx.doi.org/10.1002/pros.20928.
[122] Kregel S, Kiriluk KJ, Rosen AM, Cai Y, Reyes EE, Otto KB, et al. Sox2 is an androgen receptor-repressed gene that promotes castration-resistant prostate cancer. PLoS ONE 2013;8:e53701, http://dx.doi.org/10.1371/journal.pone.0053701.
[123] Yu X, Cates JM, Morrissey C, You C, Grabowska MM, Zhang J, et al. SOX2 expression in the developing, adult, as well as, diseased prostate. Prostate Cancer Prostatic Dis 2014;17:301–9, http://dx.doi.org/10.1038/pcan.2014.29.
[124] Clermont P-L, Lin D, Crea F, Wu R, Xue H, Wang Y, et al. Polycomb-mediated silencing in neuroendocrine prostate cancer. Clin Epigenetics 2015;7:40, http://dx.doi.org/10.1186/s13148-015-0074-4.
[125] Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 2002;419:624–9, http://dx.doi.org/10.1038/nature01075.
[126] Beltran H, Rickman DS, Park K, Chae SS, Sboner A, MacDonald TY, et al. Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. Cancer Discov 2011;1:487–95, http://dx.doi.org/10.1158/2159-8290.CD-11-0130.
[127] Sun Y, Wang B-E, Leong KG, Yue P, Li L, Jhunjhunwala S, et al. Androgen deprivation causes epithelial–mesenchymal transition in the prostate: Implications for androgen-deprivation therapy. Cancer Res 2012;72:527–36, http://dx.doi.org/10.1158/0008-5472.CAN-11-3004.
[128] Zhu M-L, Kyprianou N. Role of androgens and the androgen receptor in epithelial-mesenchymal transition and invasion of prostate cancer cells. FASEB J 2010;24:769–77, http://dx.doi.org/10.1096/fj.09-136994.
[129] Cottard F, Asmane I, Erdmann E, Bergerat J-P, Kurtz J-E, Céraline J. Constitutively active androgen receptor variants upregulate expression of mesenchymal markers in prostate cancer cells. PLoS ONE 2013;8:e63466, http://dx.doi.org/10.1371/journal.pone.0063466.
[130] Kong D, Sethi S, Li Y, Chen W, Sakr WA, Heath E, et al. Androgen receptor splice variants contribute to prostate cancer aggressiveness through induction of EMT and expression of stem cell marker genes. Prostate 2015;75:161–74, http://dx.doi.org/10.1002/pros.22901.
[131] Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: Accumulating evidence and unresolved questions. Nat Rev Cancer 2008;8:755–68, http://dx.doi.org/10.1038/nrc2499.
[132] Li F, Tiede B, Massagué J, Kang Y. Beyond tumorigenesis: Cancer stem cells in metastasis. Cell Res 2007;17:3–14, http://dx.doi.org/10.1038/sj.cr.7310118.
[133] Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. Science 2007;317:337, http://dx.doi.org/10.1126/science.1142596.
[134] Van Leenders GJ, Schalken JA. Stem cell differentiation within the human prostate epithelium: Implications for prostate carcinogenesis. BJU Int 2001;88(Suppl 2):35–42 (discussion 49–50).
[135] Nagle RB, Ahmann FR, McDaniel KM, Paquin ML, Clark VA, Celniker A. Cytokeratin characterization of human prostatic carcinoma and its derived cell lines. Cancer Res 1987;47:281–6.
[136] De Marzo AM, Meeker AK, Epstein JI, Coffey DS. Prostate stem cell compartments: Expression of the cell cycle inhibitor p27Kip1 in normal, hyperplastic, and neoplastic cells. Am J Pathol 1998;153:911–9, http://dx.doi.org/10.1016/S0002-9440(10)65632-5.
[137] Stoyanova T, Cooper AR, Drake JM, Liu X, Armstrong AJ, Pienta KJ, et al. Prostate cancer originating in basal cells progresses to adenocarcinoma propagated by luminal-like cells. Proc Natl Acad Sci U S A 2013;110:20111–6, http://dx.doi.org/10.1073/pnas.1320565110.
[138] Grisanzio C, Signoretti S. p63 in prostate biology and pathology. J Cell Biochem 2008;103:1354–68, http://dx.doi.org/10.1002/jcb.21555.
[139] Craft N, Chhor C, Tran C, Belldegrun A, DeKernion J, Witte ON, et al. Evidence for clonal outgrowth of androgen-independent prostate cancer cells from androgen-dependent tumors through a two-step process. Cancer Res 1999;59:5030–6.
[140] Qin J, Liu X, Laffin B, Chen X, Choy G, Jeter CR, et al. The PSA(-/lo) prostate cancer cell population harbors self-renewing long-term tumor-propagating cells that resist castration. Cell Stem Cell 2012;10:556–69, http://dx.doi.org/10.1016/j.stem.2012.03.009.
[141] Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res 2005;65:10946–51, http://dx.doi.org/10.1158/0008-5472.CAN-05-2018.
[142] Rane JK, Scaravilli M, Ylipaa A, Pellacani D, Mann VM, Simms MS, et al. MicroRNA expression profile of primary prostate cancer stem cells as a source of biomarkers and therapeutic targets. Eur Urol 2015;67:7–10, http://dx.doi.org/10.1016/j.eururo.2014.09.005.
[143] Polson ES, Lewis JL, Celik H, Mann VM, Stower MJ, Simms MS, et al. Monoallelic expression of TMPRSS2/ERG in prostate cancer stem cells. Nat Commun 2013;4:1623, http://dx.doi.org/10.1038/ncomms2627.
[144] Kroon P, Berry PA, Stower MJ, Rodrigues G, Mann VM, Simms M, et al. JAK-STAT blockade inhibits tumor initiation and clonogenic recovery of prostate cancer stem-like cells. Cancer Res 2013;73:5288–98, http://dx.doi.org/10.1158/0008-5472.CAN-13-0874.
[145] Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S, et al. Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene 2006;25:1696–708, http://dx.doi.org/10.1038/sj.onc.1209327.
[146] Gu G, Yuan J, Wills M, Kasper S. Prostate cancer cells with stem cell characteristics reconstitute the original human tumor in vivo. Cancer Res 2007;67:4807–15, http://dx.doi.org/10.1158/0008-5472.CAN-06-4608.
[147] Rane JK, Pellacani D, Maitland NJ. Advanced prostate cancer – A case for adjuvant differentiation therapy. Nat Rev Urol 2012;9:595–602, http://dx.doi.org/10.1038/nrurol.2012.157.
[148] Oldridge EE, Pellacani D, Collins AT, Maitland NJ. Prostate cancer stem cells: Are they androgen-responsive? Mol Cell Endocrinol 2012;360:14–24, http://dx.doi.org/10.1016/j.mce.2011.07.008.
[149] Lang SH, Anderson E, Fordham R, Collins AT. Modeling the prostate stem cell niche: An evaluation of stem cell survival and expansion in vitro. Stem Cells Dev 2010;19:537–46, http://dx.doi.org/10.1089/scd.2009.0291.
[150] Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006;444:756–60, http://dx.doi.org/10.1038/nature05236.
[151] Frame FM, Pellacani D, Collins AT, Simms MS, Mann VM, Jones GD, et al. HDAC inhibitor confers radiosensitivity to prostate stem-like cells. Br J Cancer 2013;109:3023–33, http://dx.doi.org/10.1038/bjc.2013.691.
[152] Konopleva M, Zhao S, Hu W, Jiang S, Snell V, Weidner D, et al. The anti-apoptotic genes Bcl-X(L) and Bcl-2 are over-expressed and contribute to chemoresistance of non-proliferating leukaemic CD34+ cells. Br J Haematol 2002;118:521–34.
[153] Madjd Z, Mehrjerdi AZ, Sharifi AM, Molanaei S, Shahzadi SZ, Asadi-Lari M. CD44+ cancer cells express higher levels of the anti-apoptotic protein Bcl-2 in breast tumours. Cancer Immun 2009;9:4.
[154] Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 2006;5:67, http://dx.doi.org/10.1186/1476-4598-5-67.
[155] Santer FR, Erb HH, Oh SJ, Handle F, Feiersinger GE, Luef B, et al. Mechanistic rationale for MCL1 inhibition during androgen deprivation therapy. Oncotarget 2015;6:6105–22.
[156] Shervington A, Lu C. Expression of multidrug resistance genes in normal and cancer stem cells. Cancer Invest 2008;26:535–42, http://dx.doi.org/10.1080/07357900801904140.
[157] Brown MD, Gilmore PE, Hart CA, Samuel JD, Ramani VA, George NJ, et al. Characterization of benign and malignant prostate epithelial Hoechst 33342 side populations. Prostate 2007;67:1384–96, http://dx.doi.org/10.1002/pros.20620.
[158] Drake JM, Graham NA, Lee JK, Stoyanova T, Faltermeier CM, Sud S, et al. Metastatic castration-resistant prostate cancer reveals intrapatient similarity and interpatient heterogeneity of therapeutic kinase targets. Proc Natl Acad Sci U S A 2013;110:E4762–9, http://dx.doi.org/10.1073/pnas.1319948110.
[159] Aggarwal RR, Ryan CJ, Chan JM. Insulin-like growth factor pathway: A link between androgen deprivation therapy (ADT), insulin resistance, and disease progression in patients with prostate cancer? Urol Oncol 2013;31:522–30, http://dx.doi.org/10.1016/j.urolonc.2011.05.001.

[160] Harle LK, Maggio M, Shahani S, Braga-Basaria M, Basaria S. Endocrine complications of androgen-deprivation therapy in men with prostate cancer. Clin Adv Hematol Oncol 2006;4:687–96.
[161] Feilotter HE, Nagai MA, Boag AH, Eng C, Mulligan LM. Analysis of PTEN and the 10q23 region in primary prostate carcinomas. Oncogene 1998;16:1743–8, http://dx.doi.org/10.1038/sj.onc.1200205.
[162] Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, et al. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell 2003;4:209–21, http://dx.doi.org/10.1016/S1535-6108(03)00215-0.
[163] Mulholland DJ, Tran LM, Li Y, Cai H, Morim A, Wang S, et al. Cell autonomous role of PTEN in regulating castration-resistant prostate cancer growth. Cancer Cell 2011;19:792–804, http://dx.doi.org/10.1016/j.ccr.2011.05.006.
[164] Ferraldeschi R, Nava Rodrigues D, Riisnaes R, Miranda S, Figueiredo I, Rescigno P, et al. PTEN protein loss and clinical outcome from castration-resistant prostate cancer treated with abiraterone acetate. Eur Urol 2015;67:795–802, http://dx.doi.org/10.1016/j.eururo.2014.10.027.
[165] Jia S, Gao X, Lee SH, Maira S-M, Wu X, Stack EC, et al. Opposing effects of androgen deprivation and targeted therapy on prostate cancer prevention. Cancer Discov 2013;3:44–51, http://dx.doi.org/10.1158/2159-8290.CD-12-0262.
[166] Kolenko V, Teper E, Kutikov A, Uzzo R. Zinc and zinc transporters in prostate carcinogenesis. Nat Rev Urol 2013;10:219–26, http://dx.doi.org/10.1038/nrurol.2013.43.
[167] Smith DJ, Jaggi M, Zhang W, Galich A, Du C, Sterrett SP, et al. Metallothioneins and resistance to cisplatin and radiation in prostate cancer. Urology 2006;67:1341–7, http://dx.doi.org/10.1016/j.urology.2005.12.032.
[168] Yamasaki M, Mimata H, Nomura T, Sato F. Metallothionein is up-regulated under hypoxia and promotes the survival of human prostate cancer cells. Oncol Rep 2007, http://dx.doi.org/10.3892/or.18.5.1145.
[169] Sato M, Bremner I. Oxygen free radicals and metallothionein. Free Radical Biol Med 1993;14:325–37, http://dx.doi.org/10.1016/0891-5849(93)90029-T.
[170] Otsuka T, Hamada A, Iguchi K, Usui S, Hirano K. Suppression of metallothionein 3 gene expression by androgen in LNCaP prostate cancer cells. Biomed Rep 2013;1:614–8, http://dx.doi.org/10.3892/br.2013.107.
[171] Cherian MG, Apostolova MD. Nuclear localization of metallothionein during cell proliferation and differentiation. Cell Mol Biol (Noisy-Le-Grand) 2000;46:347–56.
[172] Abdel-Mageed A, Agrawal KC. Antisense down-regulation of metallothionein induces growth arrest and apoptosis in human breast carcinoma cells. Cancer Gene Ther 1997;4:199–207.
[173] Notta F, Koropatnick DJ. Metallothioneins in drug resistance. In: Teicher BA, editor. Cancer Drug Resistance. Totowa, NJ: Humana Press; 2006. p. 223–39.
[174] Mercader M, Bodner BK, Moser MT, Kwon PS, Park ESY, Manecke RG, et al. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. PNAS 2001;98:14565–70, http://dx.doi.org/10.1073/pnas.251140998.
[175] Drachenberg DE, Elgamal AA, Rowbotham R, Peterson M, Murphy GP. Circulating levels of interleukin-6 in patients with hormone refractory prostate cancer. Prostate 1999;41:127–33.
[176] Cavarretta IT, Neuwirt H, Zaki MH, Steiner H, Hobisch A, Nemeth JA, et al. Mcl-1 is regulated by IL-6 and mediates the survival activity of the cytokine in a model of late stage prostate carcinoma. In: Li JJ, Li SA, Mohla S MD/PhD HR, MD/PhD TM, editors. Hormonal Carcinogenesis V. New York: Springer; 2008. p. 547–55.
[177] Fizazi K, Faivre L, Lesaunier F, Delva R, Gravis G, Rolland F, et al. Androgen deprivation therapy plus docetaxel and estramustine versus androgen deprivation therapy alone for high-risk localised prostate cancer (GETUG 12): A phase 3 randomised controlled trial. Lancet Oncol 2015, http://dx.doi.org/10.1016/S1470-2045(15)00011-X.