From Pathogenesis to Prevention of Castration Resistant Prostate Cancer

Helmut Bonkhoff* and Richard Berges

Pathology Laboratories, Berlin, Germany

BACKGROUND. Significant progress in understanding the molecular basis of castration resistant prostate cancer (CRPCa) has been achieved in recent years. Despite this progress, CRPCa still remains a lethal disease. Early detection and prevention of CRPCa may provide a new strategy to improve survival of patients diagnosed with PCa at risk to fail standard androgen deprivation therapy (ADT).

METHODS. Herein, we review pathogenetic mechanisms implicated in PCa progression toward castration resistant disease that are detectable in hormone naive PCa to define relevant therapeutic targets for prevention.

RESULTS. Upregulation of androgen receptor (AR) expression has been recognized a major determinant for the development of CRPCa. This hypersensitive pathway is further boosted by the increase of intratumoral androgen synthesis. AR mutants bind promiscuous steroids, and may convert AR antagonists to agonists. Various non-hormonal growth factor receptors transactivate the AR, even in absence of androgens (outlaw pathway). Finally, PCa cells can bypass the AR through various mechanisms, including BCL-2, COX-2, neuroendocrine differentiation. Most of these pathogenetic factors involved in the development of CRPCa are detectable in hormone naive PCa tissue even at the time of initial diagnosis, and could be targeted by drugs currently available.

CONCLUSIONS. CRPCa is the end-stage of a multifactorial and heterogeneous disease process. Pathogenetic factors responsible for the development of the CRPCa phenotype are detectable in the patient's PCa tissue long before the clinical onset of the disease. This approach provides opportunity for early detection and prevention by targeting pathways relevant for the individual disease process. *Prostate 70: 100–112, 2010.*

KEY WORDS: **castration resistant prostate cancer; pathogenesis; predictive markers; targeted therapy; prevention**

INTRODUCTION

Despite the recent progress in understanding the pathogenesis of castration resistant prostate cancer (CRPCa), we still face an end-stage disease with poor clinical outcome. Current pathogenetic concepts implicate CRPCa as a multifactorial and heterogeneous disease process involving several pathways [1–9] including:

- Upregulation of androgen receptor (AR) expression in PCa cells maintaining AR signaling under standard androgen deprivation therapy (ADT) (hypersensitive pathway).
- Enhanced ligand-dependent activation of the AR by increase of intratumoral de novo synthesis of testosterone and dihydrotestosterone (DHT).
- Ligand independent activation of the AR by non-hormonal growth factor receptors (HER-1, HER-2, etc.) (outlaw pathway).
- Broadened ligand specificity of AR mutants binding non-androgen steroids (estrogens, progestins, etc.) (promiscuous pathway).
- AR independent mechanisms (BCL-2, neuroendocrine (NE) differentiation, estrogen receptor alpha (ERα), progesterone receptor (PR), etc.) maintaining

*Correspondence to: Helmut Bonkhoff, MD, Pathology Laboratories, Tietzenweg 129, 12203 Berlin, Germany. E-mail: info@prostapath.de
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survival and growth by bypassing the AR (bypass pathway).
- Continuous resupply of tumor cell populations under ADT by prostate cancer stem cell regeneration (stem cell pathway).

In short, the pathogenesis of CRPCa involves a mixture of multiple pathways that increase and broaden the function of the AR, and others that bypass the AR.

It is noteworthy that pathogenetic factors implicated in progression toward the CRPCa phenotype are also required for survival and growth of the normal prostatic epithelium exposed to ADT and other cytotoxic conditions. The cellular integrity of the prostatic epithelium is maintained by basal cells which are particularly resistant to ADT, chemotherapy and radiation (Fig. 1). There are several ways to explain why basal cells, in apparent contrast to secretory luminal cells, are endowed with multidrug resistance properties. The basal cell layer of the prostatic epithelium is androgen-independent and harbors the proliferation and the stem cell compartment of the prostatic epithelium [10]. Basal cells selectively express ERα and the PR, and can use estradiol and progestins for their growth. Both receptors (ERα and PR) have been implicated in bypass pathways used by CRPCa. BCL-2, a major antiapoptotic protein protecting basal cells from programmed cell death, is also involved in bypass pathways of CRPCa. Non-hormonal growth factor receptors (HER-1, HER-2, etc.) responsible for outlaw pathways implicated in CRPCa are selectively expressed in the basal cell layer [10]. Hence, basal cells and CRPCa cells share common multidrug resistance pathways. The progressive emergence of basal cell specific pathways (including ERα, PR, BCL-2, HER-1, HER-2) during progression toward the CRPCa phenotype suggests that these tumors recapitulate biological properties of basal cells and stem cells to acquire multidrug resistance. It has been shown recently that most lethal metastatic PCa's arise from a single precursor cancer cell, and that PCa stem cells are able to generate highly tumorigenic cell populations [11].

Translation of current pathogenetic concepts into potential therapeutic applications, however, remains highly challenging. An essential prerequisite for successful targeted therapy is the presence of the target in the patient's PCa tissue. Multiple targets with potential applications in PCa have been described (reviewed by Taichman et al. [8]). The current status of clinical trials with new treatment targets in patients with CRPCa has been reported recently [9]. Some of them are ubiquitous, and present in most of PCa at advanced stage (e.g., targets related to angiogenesis, tumor–bone microenvironment, and immune response). Other targets are expressed much more heterogeneously and are relevant for the individual disease process only in part of the patients (e.g., BCL-2, HER-1, HER-2, NE pathways). In this case, it is mandatory to ascertain that the target is actually present in the patient's PCa tissue for successful targeted therapy. Given the multifactorial and heterogeneous nature of CRPCa, and the difficulty to obtain tumor tissue from these patients, it is never clear whether a therapeutic target under clinical investigation is relevant for a sufficient number of patients to obtain reliable results about its clinical activity. In addition, it is most unlikely that new drugs which significantly improve the outcome of end-stage CRPCa will be available in the near future. Early detection and prevention of CRPCa may provide another strategy to improve survival of patients diagnosed with PCa at risk to fail standard ADT.

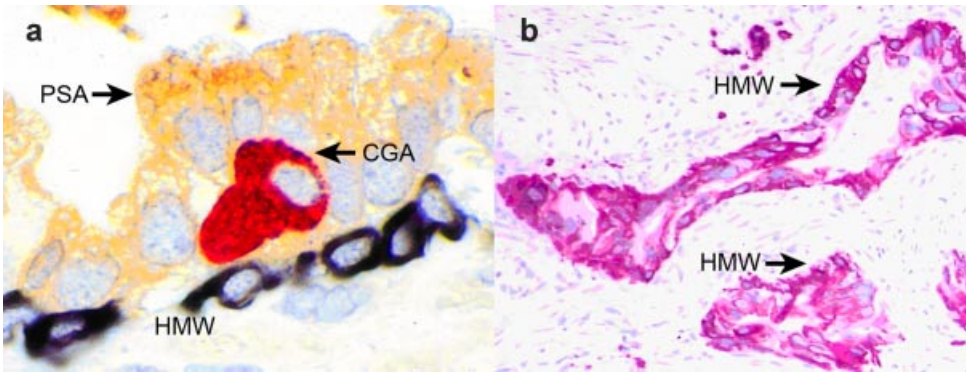

Fig. 1. The normal prostatic epithelium (**a**) is mainly composed of PSA producing secretory luminal cells which are highly androgen dependent. The basal cell layer (characterized by high molecular weight (HMW) cytokeratins) harboring the stem cell and proliferation compartment is androgen-independent. Neuroendocrine cells (characterized by chromogranin A (CGA)) lack the androgen receptor (AR) and are androgen-insensitive. Normal prostatic epithelium after ADT and radiotherapy (**b**). Only basal cells and neuroendocrine cells survive these cytotoxic conditions, and are multidrug resistant phenotypes of the prostatic epithelium. Original magnifications: a (400×), b (300×).

In the present review we focus on pathways implicated in the development of CRPCa which are detectable in hormone naive PCa tissue by routine immunohistochemistry and could be targeted by drugs currently available.

PATHWAYS IMPLICATED IN CRPCa DEVELOPMENT

Hypersensitive Pathway

Experimental data have convincingly demonstrated that upregulation of the AR at the mRNA and protein level is sufficient to convert castration sensitive to CR growth [3]. Hypersensitive AR's require 80% lower concentrations of androgens to maintain AR signaling pathways than castration sensitive PCa cells [3]. AR gene amplification may be involved in upregulation of the AR at the mRNA and protein level, but this phenomenon is detectable in only 20–30% of CRPCa and is generally not encountered in hormone-naive PCa [12].

High levels of AR expression also convert AR antagonists (bicalutamide, flutamide) to agonists, and confer responsiveness to promiscuous ligands like estrogens [3]. Thus, upregulation of the AR in PCa cells has been recognized as a major determinant for the development of CRPCa. The question arises whether hypersensitive AR's are also detectable in clinical specimens before ADT. High level of AR expression has been documented by immunohistochemistry not only in CRPCa, but also in hormone naive PCa. Several clinical studies performed in hormone naive PCa obtained from prostatectomy specimens have shown that high levels of AR expression correlate with the Gleason grade, pathological stage, lymph node status, and PSA recurrence, suggesting that PCa's expressing the AR at high levels in tumor cells behave clinically more aggressively than tumors without AR upregulation [13–16]. Furthermore, recent data indicate that increased AR expression in PCa detected in biopsy specimens significantly predicts resistance to therapy, that is, androgen ablation with or without salvage radiotherapy, and clinical failure [17]. Another important prognostic factor refers to the AR status in the host tissue. Loss of AR in the normal prostatic stroma and tumor stroma has been reported to be significantly associated with metastatic disease, response to ADT and cancer specific survival [18]. Hence, determination of the AR status in clinical specimens by immunohistochemistry is an important parameter for identification of patients at high risk of therapy failure under standard ADT (Fig. 2).

The hypersensitive pathway is further boosted by the increase of intratumoral testosterone and DHT synthesis. In fact, PCa uses cholesterol for intratumoral de novo testosterone synthesis, which is markedly increased in CR disease [19]. The conversion of cholesterol to testosterone requires activity of the P450 aromatase enzyme (CYP17 gene) which can be blocked by abiraterone acetate [9,19]. Furthermore, expression and activity of 5α reductase increase during tumor progression [20,21]. Under ADT, serum testosterone levels decrease by 95%, while tissue DHT is reduced by only 60% [7]. Cumulatively, these data

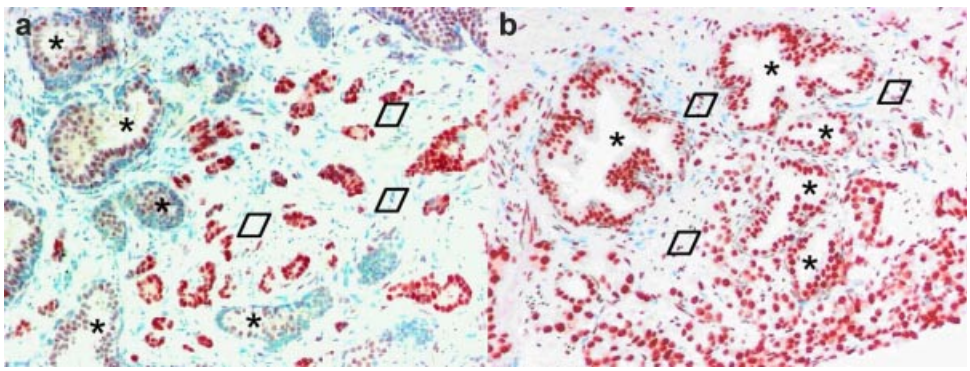

Fig. 2. Androgen receptor (AR) status in hormone naive PCa specimens. When assessing the AR in PCa cells by immunohistochemistry, it is important to refer to the AR status in adjacent benign acini (*). PCa cells and adjacent benign acini are exposed to the same hormonal and stromal microenvironment, which has a major impact on the level of AR expression in benign and malignant cells [3]. In PCa shown in (**a**) (Gleason 4 + 3), AR is expressed at high levels when compared with adjacent benign acini, indicating a hypersensitive pathway. No difference is noted in the AR status between benign and malignant cells in PCa shown in (**b**) (Gleason 4 + 5). Hence, there is no evidence for a hypersensitive pathway in this specimen. Another prognostic factor predicting response to ADT refers to the AR status in the host tissue [18]. In PCa shown in a (Gleason 4 + 3), the normal (androgen-sensitive) prostatic stroma is replaced by an androgen-insensitive tumor stroma with severe loss of stromal AR (▱). In this unfavorable hormonal and stromal microenvironment, expression of the AR in benign acini is typically low, and response to ADT is poor [18]. In apparent contrast, PCa shown in (b) (Gleason 4 + 5) retains a normal androgen-sensitive stroma (▱) and a normal AR status in adjacent benign acini (*). Original magnifications: a (100×), b (200×).

indicate that CRPCa remains androgen-depending, because of hypersensitive AR's using androgens at very low levels, de novo synthesis of androgens, and increase of 5α reductase activity. Familiar idioms like androgen-independent, -refractory, and -insensitive PCa do no longer reflect the biology of CRPCa and should be avoided.

Unopposed tumor cells proliferation under ADT is one of the hallmarks of CRPCa. The tumor suppressor p27 prevents PCa cells from entry into the cell cycle. Loss of p27 in PCa demonstrated by immunohistochemistry has prognostic implications (reviewed by Quinn et al. [22]). In castration-sensitive PCa, ADT increases p27 expression which, in turn, prevents PCa cells from proliferation. In CRPCa p27 is lost, implicating that tumor cells continue to proliferate under ADT. Hence, severe loss of p27 demonstrated by immunohistochemistry in hormone naive PCa is a risk factor for the development of CR disease. PTEN (phosphatase and tensin homolog) is another relevant tumor suppressor implicated in PCa progression. When PTEN is lost on chromosome 10 (which occurs in more than 20% of PCa with Gleason $\geq$7), the kinase pathway PI3K/AKT is activated leading to phosphorylation of the AR ($\rightarrow$ hypersensitive AR), and increase of tumor cell proliferation by downregulating p27 [1]. The PI3K/AKT kinase pathway can be targeted by mammalian target of rapamycin (mTOR) analog (RAD001) which is currently under clinical investigation [8].

Promiscuous Pathway

The ligand specificity of the AR can be broadened by mutations typically clustered in the ligand-binding domains of the AR gene (reviewed by Heinlein and Chang [1]) AR mutants bind other steroids like estrogens, progestins, and may also convert AR antagonists (bicalutamide, flutamide) to agonists, which is the molecular basis of the antiandrogen withdrawal syndrome. AR mutations predominantly occur in CRPCa, but also in about 20–40% of hormone naive metastatic PCa. Its detection, however, requires molecular techniques (RT-PCR, etc.) which are currently not available for routine diagnostic evaluation of PCa patients.

Outlaw Pathway

Ligand-independent activation of the AR may occur in the presence of deregulated growth factors and their receptors which maintain AR signaling even in the absence of androgens [1,2,4–6]. A number of growth factors (vascular endothelial growth factor, insulin-like growth factor, keratinocyte growth factor, epidermal growth factor, transforming growth factor β, and interleukin-6) and their receptors (VEGF-R, IGF1-R, KGF-R, EGF-R (HER-1), HER-2/neu, TGF β-R, IL-6-R) have been implicated in this process. Phosphorylation of the AR by growth factors requires MAPK and PI3K/AKT downstream kinase pathways. As mentioned above, the PI3K/AKT signal transduction pathway is negatively regulated by PTEN and can be targeted by mTOR analog (RAD001).

Activation of growth factors occurs in various non-neoplastic conditions, like inflammation and osteoporosis, but also in the tumor stroma and in bone metastasis. Hence, osteoporotic bone, and tumor stroma offer a favorable microenvironment (fertile soil) for PCa growth [7]. The outlaw pathway also requires the presence of related growth factor receptors on the surface of PCa cells. Some of them are detectable by routine immunohistochemistry in hormone naive PCa and have prognostic implications, including EGF-R, HER-2/neu and VEGF-R [22]. For example, clinical studies have shown that HER-2/neu and AR expressed at high levels in hormone naive PCa is a significant risk factor for metastatic disease at the time of initial diagnosis [23] (Fig. 3).

Src is the prototypical member of the Src family of non-receptor tyrosine kinases involved in PCa progression (recently reviewed by Fizazi [24]). In fact,

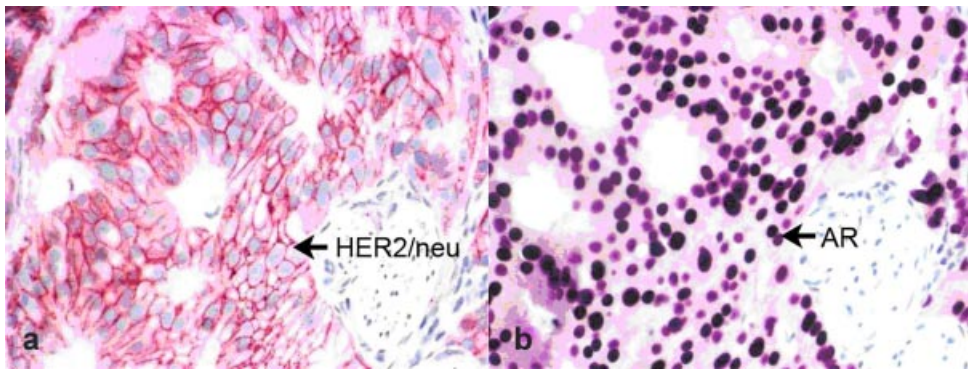

Fig. 3. Hormone naive PCa (Gleason 4 + 4). HER2/neu (**a**) and AR (**b**) are expressed at high levels, indicating that both (hypersensitive and outlaw) pathways are relevant pathogenetic factors for tumor progression under standard ADT. Original magnifications: a (300×), b (300×).

Src modulates transduction downstream of multiple cell surface receptors, including EGF-R, PDGF-R, VEGF-R, IGF1-R, integrins, as well as MAPK and PI3K/AKT downstream kinase signaling implicated in the outlaw pathway. Frequently overexpressed in PCa, Src is involved in multiple disease processes, including proliferation, adhesion, and migration. Src inhibitors have been shown effective in reducing PCa growth and metastasis in mouse xenograft models [24]. In addition, Src signaling is a key pathway during healthy bone turnover by regulating osteoclast and osteoblast activities. Hence, Src inhibitors such as dasatinib may have a therapeutic potential in PCa for decreasing morbidity associated with bone metastases and ADT induced bone loss [24].

Emerging evidence supports a role for endothelin-1 (ET-1), and endothelin A and B receptors (ET (A), ET (B)) in the progression of prostate cancer [25]. ET-1, via Src/PI3k signaling, augments c-myc expression leading to enhanced AR expression (→ hypersensitive pathway) [26]. ET-1 signaling has been associated with proliferation, angiogenesis, and antiapoptotic effects by interfering with EGF-R, VEGF-R, and BCL-2-related pathways, respectively. Finally, ET-1 produced by PCa cells stimulates osteoblasts and thus contribute to osteoblastic bone metastases. The various biological activities of ET-1 are mediated by the ET (A) receptor, while ET (B) functions as a decoy receptor and clearance mechanism for ET-1, thus mitigating its effects. ET (A) receptor antagonists (such as ZD4054) have shown clinical activity in patients with CRPCa [9]. It is noteworthy that the ET (A) receptor is not only expressed in CRPCa, but also in hormone naive PCa. High level of ET (A) receptor expression correlates with the Gleason grade, pathological stage, and PSA recurrence after prostatectomy [27].

Another outlaw pathway recently described refers to prolactin-stat 5 (signal transducer and activator of transcription 5) signal transduction events [28,29]. Stat 5 is a transcription factor which confers resistance to programmed cell death and increases AR expression (→ hypersensitive AR). The presence of stat 5 in PCa tissue correlates with high Gleason grade, early PSA recurrence, and is detectable in about 90% of CRPCa. Interestingly, stat 5 is regulated by the pituitary hormone prolactin which is ectopically produced in about 60–70% of hormone naive metastatic PCa and CRPCa [28]. Thus, targeting the prolactin-stat 5 pathway by inhibition of intratumoral prolactin synthesis (e.g., with cabergoline) may provide a new therapeutic strategy for PCa in which the prolactin-stat 5 pathway is relevant.

Bypass Pathway

A number of mechanisms have been recognized of how PCa cells survive ADT by bypassing the AR.

BCL-2 is a mitochondrial protein conferring resistance to programmed cell death. Uniformly expressed in the basal cell layer of the prostatic epithelium, overexpression of BCL-2 in PCa mainly occurs in poorly differentiated tumors and advanced stages, and is considered a prognostic marker for poor outcome and resistance to ADT and radiotherapy (reviewed by Quinn et al. [22]). BCL-2 expression may also be induced or increased by ADT and radiotherapy (Fig. 4b). A number of substances have been recognized that decrease BCL-2 or inhibit its antiapoptotic function, including pomegranate fruit extract, selenium, and docetaxel [7].

Cyclooxygenase-2 (*COX-2*) is a proinflammatory enzyme necessary for the synthesis of prostaglandins,

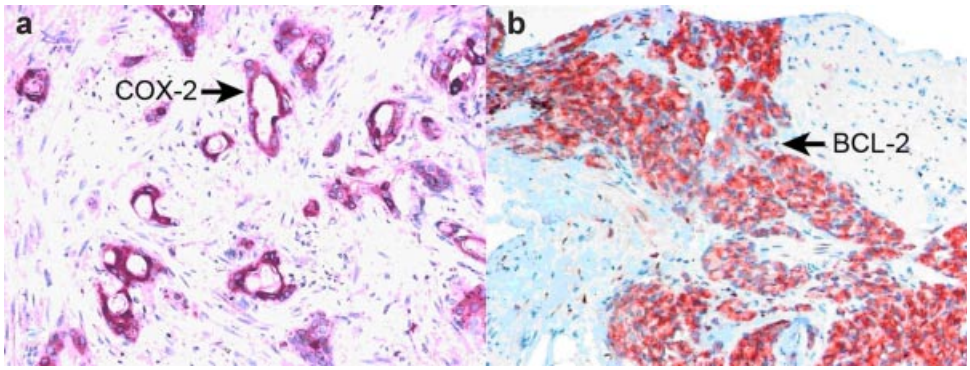

Fig. 4. COX-2 status in a hormone naive PCa (Gleason 3 + 4) (**a**). COX-2 expressed at high levels predicts tumor progression after radical prostatectomy or radiation therapy. Targeting COX-2-related pathways by COX-2 inhibitors may slow disease progression. Local PCa recurrence after salvage radiotherapy (**b**). High levels of BCL-2 expression indicate that standard ADT may be insufficient for local tumor control. Docetaxel inhibits the antiapoptotic function of BCL-2. Thus, ADT combined with low dose docetaxel may be more successful for local tumor control than standard ADT alone in this case. Original magnifications: a (200×), b (100×).

particularly PGE2. Inflammatory processes are implicated in PCa development and tumor progression. During tumor progression, the androgen-sensitive prostatic stroma is replaced by an androgen-insensitive tumor stroma, in which factors such as hypoxia and inflammation promote the release of growth factors implicated in outlaw pathways and angiogenesis [7]. In these inflammatory tumor–microenvironment interactions, COX-2 and PGE2 play a key role. Inhibition of the COX-2 pathway downregulates a number of important targets of CRPCa, including AR, EGF-R, AKT, and cyclin D [30]. Clinical studies have shown that COX-2 expression is an independent predictor of PCa progression following radical prostatectomy. At 62-month follow-up, COX-2 staining predicted progression with 82.4% sensitivity and 81.3% specificity [31]. COX-2 is also an independent marker for therapy failure after external radiation. Increased COX-2 expression was significantly associated with biochemical failure, distant metastasis, and any failure [32]. Hence, COX-2 offers a relevant therapeutic target in patients with PCa, provided that COX-2 is present in the patient's PCa tissue (Fig. 4a).

NE differentiation frequently occurs in common PCa but usually escapes pathological and clinical detection [33–35]. NE tumor cells do not secrete PSA and become detectable in PCa tissue only by immunohistochemistry. About 10% of PCa (mainly poorly differentiated tumors) show significant (extensive and multifocal) NE differentiation upon immunohistochemical analysis with the NE marker chromogranin A (CGA) [33] (Fig. 5a). It is well established that NE PCa cells are androgen-insensitive, because they consistently lack the AR (Fig. 5b). NE differentiation exclusively occurs in the G0 phase of the cell cycle, in which tumor cells are particularly resistant toward cytotoxic drugs and radiation therapy [34,35]. Although NE PCa cells do not proliferate, they produce a number of NE growth factors, including serotonin and bombesin that trigger cell proliferation of adjacent exocrine tumor cells through a paracrine mechanism [34,35]. They also regulate angiogenesis by secreting VEGF. Most strikingly, NE PCa cells escape programmed cell death and represent a potential immortal tumor cell population in PCa [36]. The stem cell marker CD44 is expressed in PCa selectively in NE tumor cells, indicating that these cells have stem cell properties [37]. Given its multidrug resistant nature, it is not surprising that NE differentiation may significantly increase under ADT and radiotherapy. NE differentiation could be targeted by somatostatin analogs, because NE PCa cells may express somatostatin receptors (Fig. 5c). In short, NE differentiation characterizes a multidrug resistant phenotype in common PCa, and may be significant in at least 10% of PCa patients, but usually escapes clinical detection and attention.

Estrogens and their receptors: Although estrogens were proven effective in the hormonal treatment of advanced PCa >60 years ago, there is increasing evidence that estrogens and their receptors are involved in PCa development and progression (recently reviewed by Bonkhoff and Berges [38]). Briefly, the ERα is considered an oncogene which is unregulated in high grade prostatic intraepithelial neoplasia (HGPIN) and most likely mediates carcinogenic effects of estradiol as demonstrated in animal models. The ERα antagonist toremifene have been identified as a promising agent for prostate cancer prevention. The progressive emergence of the ERα and the estrogen-regulated PR during PCa progression suggests that these tumors can bypass ADT by using estrogens and progestins for their growth. Alternatively, the ERβ which predominantly binds phytoestrogens is considered a functional tumor suppressor which is partially lost in HGPIN and CRPCa. Moreover, ER dependent signaling has been involved to regulate a

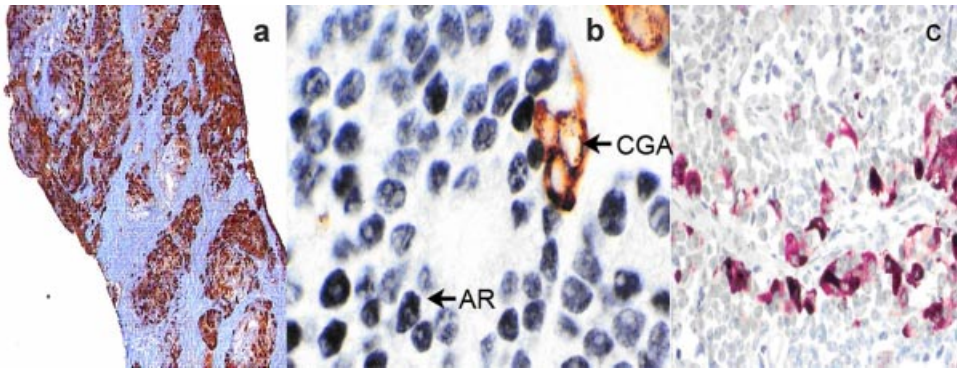

Fig. 5. Conventional PCa (Gleason 4 + 4) detected in a needle biopsy. Chromogranin A (CGA) immunohistochemistry reveals extensive neuroendocrine (NE) differentiation (**a**). NE PCa cells characterized by CGA consistently lack AR expression and are androgen-insensitive in all stages of the disease (**b**). Immunohistochemical detection of somatostatin receptors in NE PCa cells (**c**) provides a therapeutic target for somatostatin analogs. Original magnifications: a (50×), b (400×), c (300×).

potentially aggressive and lethal molecular subtype of PCa, the TMPRSS2-ERG fusion. Its expression is increased by ERα agonists (estradiol) and decreased by ERβ agonists, and could be targeted accordingly [39,40].

RANK ligand pathway: Receptor activator of nuclear factor kappa B ligand (RANKL), a member of the tumor necrosis factor (TNF) superfamily, and its receptor (RANK) are essential for age-, ADT-, and metastasis-related bone loss by increasing osteoclastogenesis, and are implicated in the PCa progression [9]. RANKL is secreated by host stromal cells and osteoclasts, but also by PCa cells, while RANK is expressed on osteoclasts and PCa cells [41]. RANK expression on PCa cells promotes invasion and growth in a RANKL-dependent manner [41]. Increased expression of RANK and RANKL in clinical specimens documented by immunohistochemistry has been associated with more aggressive, advanced, and metastatic PCa [42]. A recent study reported that RANKL expression was a significant risk factor for PSA recurrence after radical prostatectomy [43]. The RANKL pathway can be targeted by denosumab, a monoclonal antibody specifically directed against RANKL, which is under current clinical investigation in CRPCa [9].

Stem Cell Pathway

The basal cell layer of the prostatic epithelium harbors a small stem cell population (<1%) which expresses CD44, α2β1 integrin and CD133 [44]. The same marker profile has been identified in about 0.1% of tumor cells of any PCa. These very rare tumor cells possess a significant capacity for self-renewal and most likely represent prostate cancer stem cells [44]. PCa stem cells are considered multidrug resistant tumor cells that continually resupply tumor cells under ADT and others cytotoxic conditions, and may be responsible for therapy failure. CD117 (c-kit, stem cell factor receptor) has been reported recently as another important marker of a rare adult mouse stem cell population that can generate a prostate after transplantation in vivo [45]. Detection of putative stem cell markers in PCa specimens by immunohistochemistry may become important in the near future to select patients for targeting the stem cell pathway.

POTENTIAL THERAPEUTIC TARGETS FOR PREVENTION OF CRPCa DEVELOPMENT

Clinical diagnosis of CRPCa is based predominantly on serum PSA profiles under ADT. The underlying disease process, however, predates its clinical detection. Relevant pathogenetic factors implicated in the development of CRPCa are detectable in hormone naive PCa long before the clinical onset of the disease. In addition, ADT can induce or enhance relevant pathogenetic pathways in a relative short period of time, including upregulation of AR, BCL-2, CGA, EGF-R, and HER2/neu. For example, significant EGF-R and HER2/neu expression can be induced by neoadjuvant bicalutamide monotherapy within 4 months [46]. It is unlikely that selection of preexisting tumor cells with aberrant signaling accounts for this short time ADT induced changes, although clonal selection and expansion of preexisting PCa cells may occur over time. Irrespective of possible explanations, it is currently not predictable, which of these pathways may be induced in the patient's PCa tissue under ADT. In this respect, neoadjuvant ADT before prostatectomy may become a valuable tool to determine pathogenetic factors relevant for the individual disease process and to define targets for an individualized therapy of patients diagnosed with high risk PCa.

The following predictive markers have the promise for identification of PCa with high risk of therapy failure under standard ADT and may serve as therapeutic targets for prevention to slow disease progression (Table I).

Targeting the Hypersensitive Pathway

Several clinical studies have convincingly demonstrated that determination of the AR status by immunohistochemistry provides a powerful tool for predicting therapy failure after prostatectomy and response to ADT [13–18]. Detection of high-level expression of AR in hormone naive PCa cells implicates the hypersensitive pathway as relevant for the individual disease process (Fig. 2a). In this case, a standard ADT is not sufficient, because hypersensitive AR's use androgens at castration levels. There are currently several therapeutic strategies available to slow or prevent disease progression in the presence of the hypersensitive pathway:

- Decrease of tissue DHT by 5α reductase inhibitors [21].
- Decrease of adrenal and intratumoral androgen synthesis by aromatase inhibitors (abiraterone acetate) [9].
- Decrease of tissue cholesterol by statins.

AR silencing by downregulating AR expression is another approach for targeting the hypersensitive pathway [1,6,7]. Experimental data using synthetic small interference RNA, AR antisense oligonucleotide, and geldanamycin analog have shown that downregulation of AR expression is sufficient to slow tumor growth and to induce apoptosis. HSP-90 is a heat

TABLE I. Selection of Potential Targets and Pathways Implicated in the Development of Castration Resistant Prostate Cancer Detectable by Routine Immunohistochemistry in Hormone Naive Prostate Cancer, Their Prognostic Significance Reported in Untreated Prostate Cancer, and Potential Therapeutic Agents

Target	Pathway	Prognostic significance	Therapeutic agents
AR	Hypersensitive	‡ [13–18]	Abiraterone [9] MDV3100 [47] 5α reductase inhibitors [21] Statins [19]
EGF-R (HER-1)	Outlaw	‡ [22]	Gefitinib [8] mTOR analog RAD001 [8] Dasatinib [9,24] ZD4054 [9,25]
HER-2	Outlaw	‡ [22,23]	Pertuzumab [48] mTOR analog RAD001 [8]
VEGF-R	Outlaw	‡ [22]	Bevacizumab, aflibercept Sunitinib [9]
Endothelin R (A)	Outlaw	‡ [27]	ZD4054 [9]
Src kinase	Outlaw	± [24]	Dasatinib [9]
BCL-2	Bypass	‡ [22]	Docetaxel [49], AT-101 [9]
COX-2	Bypass	‡ [31–32]	Celecoxib, etoricoxib [56–58]
CGA	Bypass	‡ [33,47]	Lanreotide [50]
Somatostatin-R	Bypass	±	Lanreotide [53–55]
RANKL	Bypass	‡ [42,43]	Denosumab [9]
CD117	Stem cell	±	Imatimib [60]
MUC-1	Tumor antigen	‡ [61,62]	MVA-MUC1-IL2 vaccine [63]
Clusterin	Bypass	±	OGX-001 [9]

AR, androgen receptor; EGF-R, epidermal growth factor receptor; VEGF-R, vascular endothelial growth factor receptor; endothelin R (A), endothelin receptor A; COX-2, cyclooxygenase 2; CGA, chromogranin A; somatostatin-R, somatostatin receptor; mTOR, mammalian target of rapamycin; RANKL, receptor activator of nuclear factor kappa B ligand; MUC-1, mucin 1.
‡ = Prognostic significance has been reported in untreated prostate cancer.
± = Prognostic significance in untreated prostate cancer is less well established.

shock protein which stabilizes and prevents a number of relevant targets of CRPCa from degradation, including AR, HER-1, HER-2, and AKT. Inhibition of HSP-90 function by geldanamycin analog results in degradation of these client molecules and impairs their biological functions [1,6,7]. The geldanamycin analog 17-AAC was tested in a phase II trial in patients with CRPCa, but minimal clinical activity was recorded [9].

A new class of selective AR modulators (SARM's) targeting the hypersensitive pathway has been described recently. Non-steroidal antiandrogens diarylthiohydantoins RD162 and MDV3100 bind to AR with greater affinity than the classical antiandrogen bicalutamide, reduce nuclear translocation of the AR (AR silencing) and impair both DNA binding to androgen response elements and recruitment of coactivators [47]. Both SARM's (RD162 and MDV3100) are orally available, and induce tumor regression in mouse CRPCa models. A preliminary clinical phase I/II trial with MDV3100 enrolling 30 patients with CRPCa has shown sustained declines (by >50%) in serum PSA in 43% of patients [47]. It is conceivable that these SARM's (RD162 and MDV3100) targeting the hypersensitive pathway may also be effective to slow disease progression in hormone-naive PCa with established upregulation of AR documented by immunohistochemistry. Finally, AR silencing activity of natural agents has been documented in PCa cell lines, including vitamin D3 and E, selenium, phytoestrogens, resveratrol, pomegranate fruit extract, and silymarin [1,5].

Although the AR is the major therapeutic target of PCa, there are currently no clinical studies available, in which the AR status was considered in the study design for patient selection. This is surprising, because the hypersensitive AR is considered a key determinant in progression toward the CRPCa phenotype. It is noteworthy that evaluation of the ERα and PR status by routine immunohistochemistry has been a standard procedure in patients diagnosed with breast cancer for more than 20 years.

Targeting the Outlaw Pathway

Clinical phase II studies enrolling patients with end-stage CRPCa have documented clinical efficacy *of*

tyrosine kinase inhibitors targeting HER-2/neu (pertuzumab) in at least some of these patients [48]. The VEGF pathway is targeted in current phase III trials enrolling patients with CRPCa with various agents, including bevacizumab, aflibercept, and sunitinib [9]. In breast cancer, tyrosine kinase inhibitors are effective only in tumors expressing pertinent growth factor receptors at high level. Thus, determination of the HER-1, HER-2, and VEGF-R status in PCa tissue would be the first step toward a better identification and selection of patients that will experience maximal benefit from each particular tyrosine kinase inhibitor (Fig. 3a). It is noteworthy that ligand independent activation of AR by HER-1 and HER2/neu requires the PI3K/AKT kinase pathway which can be targeted by mTOR analog (RAD001) currently under clinical investigation [7].

Osteoporosis offers a fertile microenvironment for release of growth factors relevant for the outlaw pathway. Hence, early detection, prevention and treatment of osteoporosis may be of paramount importance to prevent bone metastasis in patients with HER-1 and HER2/neu positive PCa. There are currently two therapeutic agents (dasatinb and ZD4054) available for targeting both, the outlaw pathway and the tumor–bone microenvironment [9].

The non-receptor tyrosine kinase Src family is implicated in the outlaw pathway by modulating transduction downstream of EGF-R, PDGF-R, VEGF-R, IGF1-R, and MAPK and PI3K/AKT downstream kinase signaling, but also regulate osteoclast and osteoblast activities [24]. Clinical activity of the Src inhibitor dasatinib is currently tested in a phase III trial enrolling patients with CRPCa [9]. Dasatinb may be also effective to slow disease progression in hormone naive PCa expressing Src kinase, EGF-R, PDGF-R, VEGF-R, and IGF1-R at high level.

ZD4054 is a specific inhibitor of the endothelin receptor ET (A), which is implicated in a number of pathways relevant in bone-metastatic CRPCa. This includes the increase of AR expression (→ hypersentive pathway), modulation of EGF-R, VEGF-R, and BCL-2-related pathways, and the increase of osteoblast activity [9,25,26]. Three current phase 3 trials are enrolling comparing ZD4054 to placebo in patients with CRPCa with and without bone metastases, and in patients receiving docetaxel [9]. When any benefits emerge from these trials, ZD4054 may also be considered in hormone naive PCa expressing ET (A) at high level to slow disease progression.

Targeting the Bypass Pathway

Targeting the BCL-2 molecule–microtubule complex with docetaxel has let to the first shown survival benefit for patients with CRPCa [7]. Clinical studies have documented that BCL-2 positive PCa's respond better to docetaxel than BCL-2 negative tumors [49]. BCL-2 may be expressed not only in CRPCa, but also in hormone naive PCa. Considering that BCL-2 confers resistance to programmed cell death, it is questionable whether standard ADT is sufficient in BCL-2 positive PCa. Combination of ADT with low dose docetaxel may be more effective in BCL-2 positive tumors than standard ADT alone (Fig. 4b). In addition, BCL-2 positive PCa could by targeted by AT-101, which is a small-molecule inhibitor of multiple BCL-2 family members. Based on the promising results obtained in a single-agent phase II trial, AT-101 is currently tested in a phase II trial in patients with metastatic CRPCa treated with docetaxel [9]. When clinical activity is proven in this disease setting, AT-101 may become a promising agent for hormone naive PCa expressing BCL-2 at high level.

Detection of significant NE differentiation in PCa (Fig. 5a) has several implications in the patient's care [50]. It is clear that radical prostatectomy eliminates the multidrug resistant NE phenotype safer than radiation therapy or ADT. Knowing that NE PCa cells do not produce PSA, NE serum markers like CGA and neuron-specific enolase (NSE) may be more informative than PSA. It is well established that NE differentiation can be induced or enhanced by ADT. Clinical studies investigating CGA velocity under ADT indicate that castration therapy is significantly more effective in inducing the NE pathway than bicalutamide monotherapy [51]. Intermittent ADT significantly decreases CGA serum levels when compared to continuous ADT, indicating that intermittent ADT is safer than permanent ADT in PCa with significant NE differentiation [52]. The NE pathway can be targeted by somatostatin analogs, because NE PCa cells may express somatostatin receptors (Fig. 5c). Objective clinical response and marked decrease of serum CGA have been reported in patients with CRPCa treated with lanreotide in combination with dexamethasone or ethinylestradiol [53–55]. Hence, evaluation of the somatostatin receptor status in PCa tissue may be important for targeting the NE phenotype with somatostatin analogs.

Increased COX-2 expression in PCa tissue has been reported to predict shorter PSA-free survival after radical prostatectomy and is considered an independent risk factor for therapy failure after external radiation [31,32]. Preliminary clinical studies have shown that COX-2 inhibitors celecoxib and etoricoxib significantly extend PSA-free survival after radical prostatectomy, external radiation, and intermittent ADT [56–58]. It is noteworthy that the COX-2 status was not considered in these clinical studies. Knowing that targeting the COX-2 pathway requires the presence of COX-2 in the patient's PCa tissue (Fig. 4a), it is likely

that the real benefit of COX-2 inhibitors in patients with COX-2 positive PCa is much higher than reported by these studies. Besides celecoxib and etoricoxib, there is a number of natural COX-2 inhibitors, including vitamin D, curcumin, resveratrol, green tea and omega-3 fatty acids (fish oil) [5].

Increased expression of RANK and its ligand RANKL in hormone naive PCa has been associated with disease progression [42,43]. Denosumab, a monoclonal antibody directed against RANKL, has proven activity in reducing age-, and treatment-related bone loss [9]. Osteonecrosis of the jaw that may occur under biphosphonate treatment has not yet been reported.

Currently, there are three agents available for targeting bone metastasis, that is, dasatinib, ZD4054, and denosumab, which are under clinical investigation in patients with bone-metastatic CRPCa [9]. When any benefit emerge from these studies, these agents may be considered in hormone naive PCa with bone metastases. Detection of the related targets (Src kinase, endothelin receptor A, and RANKL) in PCa tissue by immunohistochemistry may become important to identify patients more likely to respond to one of these agents.

Targeting the Stem Cell Pathway

CD117 (c-kit, stem cell factor receptor) has been reported recently as an important marker of a rare adult mouse stem cell population that can generate a prostate after transplantation in vivo [45]. CD117 positive tumor cells, which can be targeted by imatimib, are rarely detectable in primary PCa, but in 40% of bone metastases [59]. A phase II clinical trial enrolling 20 patients with non-metastatic, recurrent PCa after definitive local therapy showed activity of imatimib in at least one patient with PSA decrease >50%, and in two other patients with PSA decrease <50% [60]. A better response rate may be expected in patients with PCa expressing CD117.

Targeting Other Pathways

Gene expression profiling has identified MUC-1 as a tumor associated antigen highly related to PCa progression [61]. In hormone naive PCa, MUC-1 (together with AZGP1) staining was a strong predictor of PSA recurrence independent of tumor grade, stage, and preoperative PSA levels [61]. Another study reported that the presence of MUC-1 in PCa at the time of diagnosis is an independent risk factor for PCa death in patients followed by watchful waiting [62]. A recent randomized phase II trial showed biological activity of MVA-MUC1-IL2 vaccine immunotherapy in patients with PSA recurrence. Thirteen of 40 patients had a more than twofold improvement in PSA doubling time [63]. It is likely that MVA-MUC1-IL2 vaccine immunotherapy is significantly more effective in patients with PCa expressing MUC-1 than in patients with MUC-1 negative tumors. Hence, determination of the MUC-1 status in hormone naive PCa by immunohistochemistry is not only an important parameter for identification of patients at high risk of progression, but also to identify patients more likely to respond to MVA-MUC1-IL2 vaccine immunotherapy.

Clusterin is a cytoprotective chaperone that inhibits apoptosis and activates the PI3K/ AKT kinase pathway implicated in the outlaw pathway [64]. In contrast to BCL-2, clusterin has no prognostic significance in hormone naive PCa, but is significantly upregulated under ADT [65]. OGX-011 is a clusterin silencing antisense oligonucleotide, which has shown promising clinical activity in patients with CRPCa treated with chemotherapy [9]. Clusterin silencing by OGX-011 may also be effective to slow disease progression in patients under ADT.

Other promising targets currently under investigation are the TMPRSS2-ERG fusion, and related ER signaling pathways, the prolactin-stat 5 pathway, and more ubiquitous targets like tumor associated antigens to illicit a PCa specific immune response (PAP, PSMA, etc), (recently reviewed by Chi et al. [9]).

Limitations of Tissue-Based Biomarkers

There is a great need for standardization of detection and reporting the above discussed predictive biomarkers in PCa tissue to make these tests available for routine diagnostic evaluation of PCa patients. When assessing the AR in PCa cells by immunohistochemistry, it is important to refer to the AR status in adjacent benign acini for two reasons. First, adjacent benign acini offer an excellent internal control for the quality of staining. Secondly, PCa cells and adjacent benign acini are exposed to the same hormonal and stromal microenvironment, which has a major impact on the level of AR expression in benign and malignant cells [4]. Upregulation of the AR in PCa cells (hypersensitive pathway) should be considered when the staining intensity in PCa cells is significantly higher than in adjacent benign acini (Fig. 2a). Suitable internal positive control for a proper evaluation of the BCL-2, HER-1, HER-2, ERα, PR status in PCa tissue is the basal cell layer of the normal prostatic epithelium, because basal cells express these markers at high levels in well fixed prostate tissue. Another limitation of tissue-based biomarkers is the notorious heterogeneity of PCa, implicating sampling errors, especially in prostate biopsies. A complementary approach of assessing biomarker profiles in PCa is its determination in

circulating tumor cells isolated from blood of PCa patients [66].

CONCLUSIONS

Current concepts implicate the development of CRPCa as a multifactorial and heterogeneous disease process. It is questionable whether a standard ADT offers an optimal therapeutic strategy for a multifactorial and heterogeneous disease. CRPCa still remains an end-stage disease with poor outcome. Magic bullets with the potential to improve significantly the outcome of end-stage solid tumors, including CRPCa, are currently not available and are unlikely to be developed in the near future. Clinical studies dealing with CRPCa are usually initiated in patients with rising PSA under ADT. The underlying disease process, however, may predate the clinical onset of CR by years. A number of well-established etiological factors are detectable by routine immunohistochemistry not only in CRPCa, but also in hormone naive PCa tissue at the time of initial diagnosis. This includes upregulation of AR, HER-1, HER-2, BCL-2, COX-2, MUC-1, and CGA, whose prognostic and predictive value has been well documented in several clinical studies. With these markers it should be possible to identify patients with high risk of therapy failure under standard ADT. In addition, this approach provides opportunity not only for early detection, but also for prevention of CR disease by targeting those predictive factors prevalent in PCa tissue at the time of initial diagnosis. A number of drugs are currently available for successful targeting pathways related to AR, COX-2, BCL-2, MUC-1, and somatostatin receptors.

Promising therapeutic agents targeting bone metastases are under current clinical investigation in CRPCa. Determination of the expression status of the related targets (Src kinase, endothelin receptor A, RANKL) in clinical specimens of hormone naive PCa may become helpful to select patients more likely to respond to one of these therapeutic agents for prevention and treatment of ADT- or metastasis-related skeletal morbidity.

Strategies focusing more on early detection and prevention of CR may be more effective to extent survival of PCa patients than attempts to improve the outcome of patients with established end-stage CRPCa.

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