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Review article

The ubiquitin-proteasome system in prostate cancer and its transition to castration resistance

Ioannis A. Voutsadakis, M.D., Ph.D.*, Christos N. Papandreou, M.D., Ph.D.

Department of Medical Oncology, University Hospital of Larissa, Larissa, Greece

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Abstract

Prostate cancer is the most common carcinoma in the male population. In its initial stage, the disease is androgen-dependent and responds therapeutically to androgen deprivation treatment but it usually progresses after a few years to an androgen-independent phase that is refractory to hormonal manipulations. The proteasome is a multi-unit protease system that regulates the abundance and function of a significant number of cell proteins, and its inhibition results in cancer cell growth inhibition and apoptosis and is already exploited in the clinic with the use of proteasome inhibitor bortezomib in multiple myeloma. In order to be recognized by the proteasome, a target protein needs to be linked to a chain of the small protein ubiquitin. In this paper, we review the role of ubiquitin-proteasome system (UPS) in androgen receptor-dependent transcription as well as in the castration resistant stage of the disease, and we discuss therapeutic opportunities that UPS inhibition offers in prostate cancer.

Keywords: Prostate cancer; Ubiquitin; Proteasome; Androgens; Transcription factors; Castration resistance

1. Introduction

Prostate cancer is one of the most common malignancies mainly of older men, and a significant number of men are found to have foci of occult prostate carcinoma at autopsy studies [1,2]. The disease has 2 phases and is initially androgen-dependent, a term meaning that cancer cells need androgens for their survival and proliferation in this initial phase. The disease invariably progresses to an androgen-independent phase during which cancer cells have no need of androgens for their survival and proliferation and thus the disease displays resistance to continued androgen deprivation, a fact that is manifested with PSA elevation and clinical or radiographic progression. A role of therapeutic androgen deprivation during the androgen-dependent phase in selecting for androgen-independent tumor cell clones has been proposed and, together with a reduction of therapy-induced side effects, forms the basis of therapeutic strategies of intermittent androgen deprivation [3–6]. Nevertheless, prostate cancer may progress to its castration resistant phase even if left untreated while still castration sensitive. The transition of prostate cancer from the castration sensitive to the castration resistant state involves several mechanisms that have been revealed through studies in different models. These mechanisms include the development of hypersensitivity of androgen receptors (ARs) to small amounts of circulating androgens, the broadening of ARs ligands specificity to include, beyond androgens, androgen antagonists and corticosteroids, the activation of ARs without the need for ligand binding through AR mutations or through phosphorylation by inappropriate activation of up-stream kinases, the bypassing of ARs through activation of alternative survival pathways, and finally the selection of malignant stem cells, which are endogenously androgen-independent for their survival and thus survive after androgen-dependent progeny has succumbed to androgen deprivation [7–12].

The ubiquitin-proteasome system (UPS) is a major regulatory system of the cell and plays a role in most cellular physiologic and pathologic functions. A discussion of the role of UPS in the transition of prostate cancer to androgen-independence will be the subject of this paper.

The ubiquitin-proteasome system

The 26S proteasome is a cylinder-shaped multi-protein cellular structure located in cytoplasm and cell nucleus,

* Corresponding author. Tel.: +30-2413502028; fax: +30-2413502027.
E-mail address: ivoutsadakis@yahoo.com (I.A. Voutsadakis).

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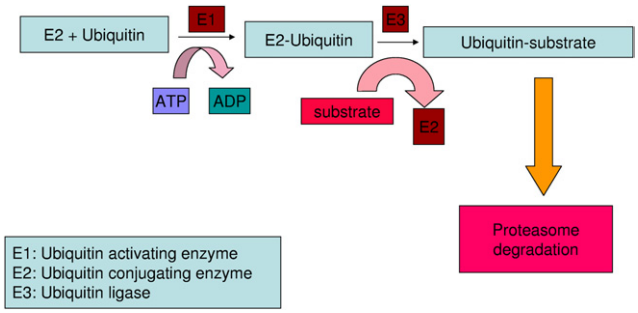

Fig. 1. Ubiquitination process that tags a substrate protein for proteasome degradation. Ubiquitin-activating enzyme binds ubiquitin in an ATP-dependent manner. E1-bound ubiquitin is then transferred to ubiquitin-conjugating enzyme or E2. In the last step of the process, a ubiquitin ligase or E3 transfers ubiquitin from E2 to a target protein. (Color version of figure is available online.)

which cleaves and destroys or modulates various proteins. To be recognized by the proteasome, proteins that are destined targets need to become ubiquitinated, that is, to become covalently ligated to a chain of the small protein ubiquitin [13,14]. Ubiquitination takes place in 3 steps, each catalyzed by a different class of enzymes (Fig. 1). First, ubiquitin-activating enzyme, also called E1, using energy from the conversion of ATP to ADP, binds ubiquitin. Then, in a second step, E1-bound ubiquitin is transferred to a second enzyme called ubiquitin-conjugating enzyme or E2. Finally, a third enzyme, a ubiquitin ligase or E3, transfers ubiquitin from E2 to a target protein [15]. Ubiquitinated proteins are recognized by specific sub-units of the outer part of the proteasome cylinder called 19S cap or regulatory particle (RP). This particle dissociates the target protein from ubiquitin and transfers it to the central part of the proteasome cylinder, which forms the core particle called 20S proteasome and possesses the 3 different proteolytic activities of the complex [16]. The 20S proteasome consists of 4 rings formed by 7 sub-units each. Centrally located are 2 identical β rings formed by subunits β1 to β7 each. The sub-units β1 possess the post-glutamyl proteolytic activity of the proteasome, sub-units β2 the trypsin activity, and sub-units β5 the chymotrypsin activity. The two α rings are located peripherally in the 2 sides of β rings and have the function of compartmentalizing the proteolytic activity of the proteasome by allowing only a narrow passage of 1.3 nm to the centrally located β rings [17].

Virtually all cellular functions are regulated by the proteasome. For example, during progression of cell cycle from G2 phase to mitosis, mitosis-promoting factor (MPF) comprised of cyclin B and cdc2 phosphorylates several mitosis regulating proteins. When mitosis is completed, anaphase promoting complex/cyclosome (APC/C), a E3 ligase, ubiquitinates cyclin B, which is then degraded by the proteasome in order for the cell to exit to G1 phase [18,19]. In addition to cell cycle, another cellular process that is regulated by the UPS is apoptosis. A functional UPS is needed for cell survival and UPS inhibition is a prerequisite for apoptosis to occur [20–22]. Several component proteins of the core apoptosis machinery such as bcl2 family members, caspases, and Smac/Diablo are proteasome substrates [23].

Other cellular functions involved in physiologic homeostasis but also in carcinogenesis, such as DNA transcription, DNA repair, and angiogenesis are regulated by the UPS.

Androgen receptor and physiologic regulation of its transcription activity by the ubiquitin-proteasome system

AR is a nuclear receptor type transcription factor. Its gene is located at Xq11-12 chromosome locus in humans. When ligated by its main ligand dihydrotestosterone (DHT) in the cytoplasm, AR dissociates from a complex consisting of heat-shock protein 90 (Hsp90) and co-chaperones such as small glutamine-rich tetratricopeptide repeat-containing protein and Fkbp52 (FK506-binding protein 52) that keeps it in the cytoplasm and is escorted to the nucleus by Hsp27 where it binds androgen receptor elements (AREs) sequences in the promoters of target genes [24–26]. Hsp27 may promote or inhibit AR transcription depending on the experimental system and cellular context [24,27]. AREs are 15 nucleotides long and are comprised of the consensus sequence 5′-(A/G)G(A/T)ACAnnnTGTTCT-3′ where n is any nucleotide [28]. Divergent sequence AREs have lower affinity for AR but confer enhanced synergistic transcription activity [29,30]. Transcription co-factors and the basic transcription machinery are recruited and transcription begins. Among AR target genes, the AR itself is included as well as many genes involved in steroidogenesis (Table 1), [31,32]. AR is a protein of 110 kDa and 919 amino-acids and has 4 functional domains. The amino-terminal domain contains 2 activation sub-domains called AF-1 (activation function-1) and AF-5 that bind transcription co-factors. In the aminoterminal domain of AR, at amino acids 23 to 27, there is a FQNLF sequence that has been described as a sequence recognized by ubiquitination machinery [33]. In the middle of the AR molecule, the DNA-binding domain with 2 zinc fingers is found, followed by the hinge region, which includes a nuclear localization signal (NLS). The carboxy-terminal part of AR is occupied by the ligand binding domain [34]. This domain organization is similar in all nuclear receptor types both of steroid hormones and of non-steroid nuclear receptors [35].

The UPS is recognized to play an important role in transcription in general and in nuclear receptors transcription in particular [36,37] (Fig. 2). After DHT binding, AR obtains a major conformation change that creates a new binding surface and allows the binding of co-activators such as steroid receptor co-activator 1 (SRC1), SRC2, and nuclear receptor interacting protein (NRIP) [38–40]. At the same time, AR conformational change allows the NLS in hinge region to be uncovered to the surface and the complex enters cell nucleus. Coactivators serve as adapters for re-

Table 1
Examples of androgen receptor (AR) target genes

Target gene	Function
PSA (hK3)	Prostate specific antigen, serine protease of kallikrein family
hK2	human kallikrein 2, serine protease
AR	Nuclear receptor transcription factor ligated by androgens
PPARα	Nuclear receptor transcription factor
HMG-CoA synthase	Steroid biosynthesis enzyme
HMG-CoA reductase	Steroid biosynthesis enzyme
Squalene synthetase	Steroid biosynthesis enzyme
Squalene monoxygenase	Steroid biosynthesis enzyme
Lanosterole synthase	Steroid biosynthesis enzyme
Stearoyl-CoA desaturase	Enzyme of unsaturated fatty acids synthesis
CD10 (NEP)	Cell surface protease inactivating neuropeptides
TMPRSS2	Transmembrane serine protease
TGFBR2	Transforming growth factor-β surface receptor
ϒ-Catenin	Cell adhesion molecule involved in adherens junction
α-Tubulin	Cytoskeleton protein
HSP40	Heat shock protein involved in protein stabilization
Nucleoside diphosphate kinase A/nm23	Metastasis suppressor kinase
p21	Cyclin-dependent kinases inhibitor
Nuclear receptor interaction protein	Co-activator of AR
FK-506 binding protein	Immunophilin protein family member
ANKH	Transmembrane protein involved in pyrophosphate transport
Maf	bZip transcription factor interacting protein
Id2	Helix-loop-helix transcription factor
MMP2	Matrix metalloproteinase

cruiting histone acetyltransferases, such as CREB binding protein (CBP)/p300 and p300/CBP-associated factor (p/CAF) and histone arginine methyltransferases, such as CARM1 coactivator-associated arginine methyltransferase-1 (CARM1) and protein arginine methyltransferase-1 (PRMT-1) [41]. Histone acetylation and methylation open nucleosomes in order for the AR complex to obtain access to ARE elements in target promoters. Sequential histone mono-ubiquitination and de-ubiquitination provides the signal for histone methylation [42–44], a process in which the 19S regulatory part of the proteasome is also involved [45,46]. This process is important in transcription elongation and defines an additional point of regulation of transcription by the UPS. RING domain containing E3 ligase h human p53-induced ring-containing H2 (hPIRH2) binds AR and promotes suppression of histone deacetylase 1 (HDAC1) stabilizing histones in the acetylated state [47]. Histone modifications are an intermediary state that promotes nucleosomal histone octamer dissociation from the promoter transcription initiation site and leaves DNA naked for transcription machinery binding [48,49]. In addition, ubiquitination of co-repressors CtBP1/2 and NCoR/SMRT lead to their proteasome degradation releasing transcriptional repression in order for the transcription complex to bind DNA [50]. ARs recognize their cognate AREs in dimeric form. Concomitantly with or after DNA binding, other ubiquitin E3 ligases, such as Papillomavirus E6 associated protein (E6-AP) and mdm2 are recruited to the complex and promote ubiquitination of ARs [51,52]. In parallel, a molecular complex called mediator is recruited and helps recruit, in its turn, RNA polymerase II to begin transcription [53]. After a few rounds of transcription, ubiquitin ligases have attached 4 ubiquitin molecules to each of the AR molecules, which is now recognized by the proteasome for degradation. Although the specifics of this process are not entirely clear, it appears that components of the general transcription machinery that possess E3 ligase activity collaborate in ubiquitination of AR [54]. A transient mono-ubiquitinated form of AR is stabilized by protein tumor susceptibility gene 101 (TSG101) before this protein is displaced from the complex in order for poly-ubiquitination of AR to take place [55]. The nature of association of AR co-activators glycocorticoid receptor-interacting protein 1 (GRIP1) and CREB-binding protein (CBP) with PSA promoter is transient and cyclic and parallels the association of 19S proteasome regulatory sub-complex to the AR transcription complex in this promoter [56], further supporting a role of proteasome signaling and degradation of transcription machinery components during AR-dependent transcription. Proteasomal degradation is a prerequisite for the transcription process to continue because it frees the way for new AR molecules ligated by androgens to occupy the ARE. In an experimental system visualizing living cells in vitro by fluorescence imaging, a role of nuclear AR exclusion to the cytoplasm after androgen withdrawal has been reported to play an additional significant role for transcription regulation [57]. AR cycling ensures the tight control of

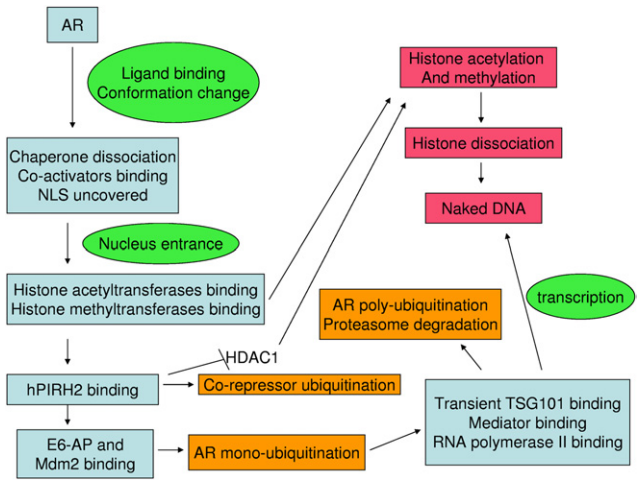

Fig. 2. Ligand binding to androgen receptor (AR) initiates recruitment of various factors (in blue blocks) leading to regulated changes in chromatin (in pink) and AR transcription. Ubiquitin-proteasome system actions in the process are in yellow. (Color version of figure is available online.)

transcription, as there is a need for continuous binding of transcription factors to the promoter for the process to continue, while it stops if new transcription factor molecules are no longer bound. AR-dependent transcription of AR itself ensures the availability of new AR molecules as long as there is continued signal of transcription by androgens. AR transcription program also promotes the production of androgens, given that many enzymes of steroid synthesis are among AR target genes (Table 1). As a result, levels of intracellular androgens in the prostate do not correlate completely with circulating androgen levels. After experimental medical castration in healthy middle-aged men, it was found that intraprostatic testosterone and DHT remained at a level of about 20% to 30% of control values, while serum testosterone dropped by 94% compared with controls [58]. In another study, prostatic tissue testosterone levels were similar in men with prostate cancer on androgen deprivation therapy compared with men with benign prostate disease not receiving therapy, while prostatic DHT levels were reduced by about 80% but remained detectable [59]. Intracellular androgens may create an intracellular feed-forward mechanism of cellular proliferation in prostate cancer cells despite castrate androgen blood levels with androgen deprivation therapies.

The UPS in the transition of prostate cancer to castration resistance

In its initial clinical phase, prostate cancer is castration sensitive. This gives the therapeutic opportunity of controlling the disease by hormonal manipulations that produce castrate levels of circulating androgens. In the castration sensitive phase of prostate cancer, the AR system of transcription is subject to the physiologic regulation by androgen ligation, a fact also underlined by the therapeutic efficacy of androgen ablation [60]. In this phase of the disease, in which at least the bulk of neoplastic cells remain quiescent and may be induced to undergo apoptosis, AR transcription is inactive due to androgen suppression and thus the proteasome system is not recruited in AR transcription sites. Nevertheless, there is a subset of cancer cells that either possesses from the beginning a castration resistant phenotype or acquires such a phenotype during androgen suppression and give rise to the second advanced phase of the disease, which is exactly characterized clinically by insensitivity to androgen ablation. These cells may still depend on AR transcription for their survival and proliferation or may be able to survive in the absence of active AR-initiated transcription [7,61,62]. AR-dependent cells have an AR that can initiate transcription in the absence of androgens ligation or in the presence of very low androgens levels. They possess a mutated AR that has increased affinity for androgens and can be activated even by low residual circulating androgen levels, which remain after therapeutic ablation or by low intracellularly produced androgens that are the product of AR-dependent activation of androgen producing enzymes in the prostate cancer cell. Alternatively, AR mutations may lead to a broadening of activating agonists to include, in addition to androgens, anti-androgens, estrogens, and other steroids, or AR gene may not be mutated but may be amplified, leading to increased levels of the receptor protein, able to maintain an increased level of AR-dependent transcription even in the presence of low androgen levels or androgenic activity of anti-androgens [63]. Expression of high levels of co-activators SRC-1 and transcription intermediary factor-2 (TIF-2) is an additional mechanism through which AR transcription can be maintained in the presence of low levels of androgens [64]. In all these cases in which castration resistance is produced by a mechanism that enables the cancer cell to maintain AR transcription in the absence of circulating androgens (androgen-independent, AR-dependent), AR transcription machinery must be in place and functional, as in the normal physiologic state. The UPS is part of this machinery and interacts with various proteins in order to facilitate AR nuclear localization, clearance of co-repressors in order for the initiation complex to form and, subsequently, clearance of the initiation complex for new rounds of transcription to proceed, as described. The importance of AR even in the androgen-independent state is further confirmed by autopsy studies that document the expression of AR, although heterogeneous, in metastatic foci of castration resistant prostate carcinoma [65]. A role of UPS dysfunction is possible as AR protein stabilization in androgen-independent prostate cancer cell lines is a proposed mechanism that mediates response to low androgen levels [66].

A mechanism that leads to castration resistance of prostate cancer and that may involve both AR-dependent and AR-independent pathways is through activation of signal transduction pathways that start from cell membrane receptors and transduce a survival signal that either activates AR without the need for its ligand or bypasses AR altogether. Neuropeptides such as endothelin and bombesin and their membrane receptors are among these signal transduction pathway initiators and are associated with neuro-endocrine features and with emergence of castration resistance in prostate cancer [67,68]. Loss of the cell membrane-associated enzyme neutral endopeptidase [NEP, also known with the alternative names neprilysin, enkephalinase, common acute lymphoblastic leukemia antigen (CALLA), and CD10] that cleaves and inactivates extra-cellular neuropeptides produces an autocrine and paracrine increase of these peptides (endothelin-1, bombesin, gastrin-releasing peptide, neurotensin) which, remaining intact, can ligate their receptors and initiate signal cascades culminating in cell survival promotion and cancer cell migration [69–71]. NEP gene possesses 2 AREs in its promoter [72] and is regulated by androgens (Fig. 3). Thus, androgen treatment results in NEP induction, which may explain the paradoxical inhibition of androgen-independent cell lines after androgen treatment [73] and may in physiologic conditions be a mechanism for

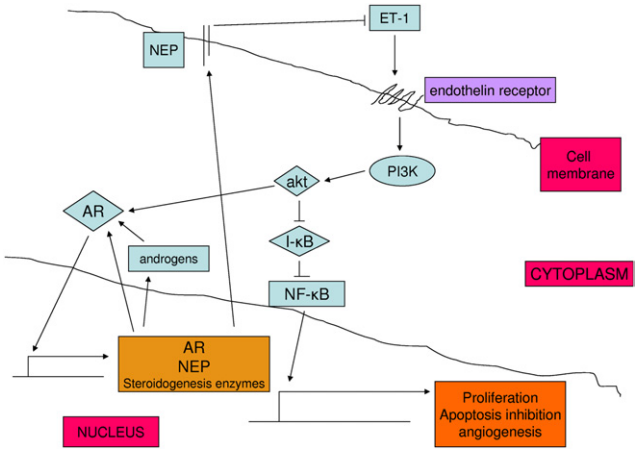

Fig. 3. Ligation of endothelin-1 receptor by endothelin-1 (ET-1) results in activation of transcription factor NF-κB leading to cell proliferation, inhibition of apoptosis, and promotion of angiogenesis. In parallel activation of kinase akt leads to androgen-independent activation of AR. AR transcription may lead to steroidogenesis as well as NEP production, which would prevent further activation of the neuropeptide axis in an attempt of physiologic mechanisms to prevent the initiation of a feed-forward loop. Proteasome inhibition would prevent NF-κB activation and favor AR stability. In diamond, proteasome protein substrates are depicted. (Color version of figure is available online.)

prevention of accidental activation of feed-forward loops between neuropeptides and androgens. Pro-survival signaling initiated by neuropeptides involves activation of the kinases PI3K and akt, which then activate the pro-survival transcription factor NF-κB by phosphorylating its inhibitor I-κB [71,74] (Fig. 3). Phosphorylated I-κB is then ubiquitinated and degraded by the proteasome, an event that allows NF-κB to enter the nucleus and exert its transcription activity. PI3K kinase also activates the kinase focal adhesion-dependent kinase (FAK), which plays a role in metastasis promotion [75]. NEP action inhibits this pathway by interfering with kinase Lyn interaction with PI3K [75]. Direct interaction of NEP with tumor suppressor PTEN has also been documented in androgen-independent prostate cancer cells. This interaction brings PTEN in proximity with the cytoplasmic membrane, where it can inhibit its target kinase akt [76]. An additional action of NEP in PC3 androgen-independent prostate cancer cells is the increase of the PKC isoform PKCδ [77], which promotes apoptosis in these cells [75] and increases their sensitivity to the chemotherapeutic drug etoposide. PC3 cells have been found to possess a PKCδ protein with reduced stability in pulse-chase assay [77]. This may be due to increased proteasome activity that is observed in PC3 cells compared with LnCaP androgen-dependent prostate cancer cells [78]. PKCδ is a ubiquitination target after phosphorylation induced by various stimuli, an event leading to its proteasome degradation [79]. Akt kinase, also a proteasome substrate [80], is further able to phosphorylate AR which, as a result, is becoming transcriptionally active without the need for androgen ligation (Fig. 3). In addition, phosphorylated AR is more prone to ubiquitination and proteasome degradation which, counter-intuitively, may promote AR-dependent transcriptional activity given the need for transcription factor degradation after transcription initiation for the binding of renewed transcription machinery molecules to the promoters in order for the transcription to continue. As a result, neuropeptide-initiated signaling promotes androgen-independent prostate cancer cell survival, which may be both AR-independent and AR-dependent. The important and pathophysiologically relevant role of neuropeptides in the pathogenesis of castration resistant prostate cancer is underlined by the fact that low levels of DHT consistent with castrate levels obtained clinically combined with low levels of the neuropeptide bombesin are able to activate androgen-dependent transcription [81]. Neither DHT nor bombesin at the same levels alone were sufficient to activate this transcription. Moreover loss of NEP expression examined by immunohistochemistry in prostatectomy specimens performed for prostate cancer was associated with PSA relapse [82].

Other transduction pathways starting from receptors such as EGFR family members and IGF-1R and culminating to PI3K/akt activation downstream promote androgen-independent prostate cancer in a manner pathophysiologically similar to neuropeptides. Many component proteins of these cascades are proteasome substrates. For example, insulin-like growth factor binding protein 3 (IGFBP-3) is a protein regulating the abundance of IGF-I in order to bind its cell surface receptor and is, in its turn, regulated by the UPS [83]. EGFR is also a proteasome substrate [84]. In addition to neuropeptides, PI3K kinase is activated in PC3 prostate cancer cells by β1 integrin-initiated signaling [85]. This activation up-regulates ubiquitin ligase sub-unit Skp2, which in turn promotes ubiquitination and proteasome degradation of BRCA2 as well as CDK inhibitor p27 [86]. BRCA2 is a protein participating in a complex that effectuates transcription-coupled nucleotide excision repair of DNA [87] and its presence results in inhibition of cell proliferation. In contrast, BRCA2 down-regulation through proteasome degradation in PC3 cells after β1 integrin-initiated signals promotes cell proliferation [85]. p27 degradation contributes further to proliferation by cell cycle promotion even in cells that are not adhering to the extracellular matrix [86]. PI3K activation leads also to phosphorylation of FOXO1, a transcription factor inducing growth arrest [88]. Phosphorylated FOXO1 is then prone to ubiquitination by Skp2 to be degraded by the proteasome, an event that finally promotes cell growth. Akt can also become activated in prostate cancer due to inactivating mutations of its inhibitor phosphatase PTEN, which is rather common in this cancer. Skp2 expression is elevated in human prostate cancer biopsy samples and is negatively correlated with both p27 and PTEN [89]. Furthermore, transgenic mice expressing skp2 in their prostate gland display decreased p27 expression and develop dysplasia and carcinomas [90].

Additional transcription programs such as those initiated by the transcription factors of the Ets family as well as

β-catenin are playing a role in androgen-independent prostate cancer. E26 transformation-specific sequence (Ets) family transcription factors such as erythroblastosis virus E26 related gene (ERG), Ets Variant Gene 1 (ETV1) also called (ER81), and ETV4 are important for cell proliferation [91,92] and are up-regulated in the majority of human prostate cancers through recurrent chromosome translocations, which juxtapose their genes to the promoter of AR regulated gene transmembrane protease serine 2 (TMPRSS2) [93,94]. In addition, Ets1 co-localizes with AR in a subset of AR sequence-containing promoters and is required for these AR target genes expression in LnCaP prostate cancer cells [95]. Conceivably, translocations of promoters of AR-dependent genes to genes of the Ets family of transcription factors are particularly favoring prostate carcinogenesis because they induce a vicious cycle of cell proliferation by feeding forward to promoting AR transcription in several promoters where AR cooperates with Ets members.

Wnt-1 signaling pathway that results in the activation of the transcription factor β-catenin, is important in embryogenesis and is de-regulated in several instances in carcinogenesis [96]. Wnt signal transduction is regulated by the UPS as, in the absence of wnt-1 initiated signals β-catenin remains in the cytoplasm and is either bound to the cell membrane protein E-cadherin, taking part in inter-cellular adhesions, or is phosphorylated and ubiquitinated in a process mediated by protein kinase glycogen synthase kinase-3β (GSK-3β), ubiquitin ligase β transducin-repeat containing protein (βTrCP), and protein adenomatous polyposis coli (APC), and finally degraded by the proteasome. In the presence of Wnt-1 signals phosphorylation and ubiquitination of β-catenin is inhibited and β-catenin is freed to enter the nucleus and begin its transcription activity in cooperation with transcription factor T Cell Factor 4 (TCF-4). β-Catenin/TCF4 transcription has been found to play a role in prostate cancer [97], especially when AR signaling is inactivated [98]. Unliganded AR activates transcription from β-catenin/TCF4 sites in collaboration with this transcription complex. In contrast, androgenic agonists-ligated AR has an inhibiting effect in β-catenin/TCF4 initiated transcription [98]. Thus, it appears that there is a role of β-catenin/ TCF4 signaling in the castrate state and in androgen-independent phase of prostate cancer, a fact that is further underlined by the observed up-regulation of β-catenin transcription activity in luciferase experiments in prostate cancer cell lines in the absence of AR ligands compared with the same cell lines in the presence of AR ligands.

An additional mechanism mediating transition to castration resistance is the activation of stress response. This response elicited in the androgen-suppressed environment causes an up-regulation of chaperones such as hsp27 and secretory clusterin, which have a protective effect against apoptosis in cancer cells [99–101]. Mechanisms involved in this anti-apoptotic effect include promotion of I-κB degradation by the proteasome and, thus, NF-κB activation, as well as refolding of denatured proteins and protection from proteasome destruction [102,103].

It is evident from this discussion that the UPS is important in the transition of prostate cancer to castration resistance in both the case where AR transcription continues to be important for cancer cell survival and the case where the activation and function of alternative transcription programs such as that of NF-κB take over.

Inhibition of UPS in prostate cancer: Preclinical studies

Given the important role of UPS in cellular homeostasis and the above discussed involvement in several carcinogenesis-related processes in the prostate, as well as the effectiveness of proteasome inhibition in other malignant diseases such as multiple myeloma and sub-types of non-Hodgkin lymphoma, it comes as no surprise that UPS inhibition has anti-carcinogenic effects in the prostate and has been proposed as prostate cancer treatment [104].

Bortezomib is currently the only proteasome inhibitor approved for clinical use and is probably the most extensively studied proteasome inhibitor in vitro [105]. Other proteasome inhibitors such as MG132, MG115, and PSI have been used in preclinical studies but have not been developed for clinical use. Bortezomib directly inhibits the chymotrypsin-like enzymatic activity of the proteasome and, through this action, stabilizes various proteins important for apoptosis and favors apoptotic pathways [106]. For example, after exposure of prostate cancer cell lines and primary prostatic epithelial cells to bortezomib, the half life of the active form of caspase 8 p18 increases from 22 minutes at base line to over 2 hours [107]. Active caspase 8 stabilization sensitizes cells to death receptor-mediated induction of apoptosis. Proteasome inhibition by MG132 also induces caspase-dependent apoptosis in prostate cancer cell line LnCaP and PC3 [108].

Transcription factor NF-κB inhibition is one of the primary mechanisms proposed for the anti-neoplastic effects of proteasome inhibitors. Proteasome function is required for inhibitor I-κB degradation in order for NF-κB to be released and perform its transcription function that results in cell proliferation and inhibition of apoptosis through induction of several genes. Treatment of androgen-independent prostate cancer cell line PC3 with proteasome inhibitor MG132 stabilizes I-κB and promotes its translocation into the nucleus, where it associates with NF-κB to inhibit it [109]. The importance of NF-κB inhibition in PC3 cell apoptosis is further underlined by the fact that the indazole compound YC-1, which inhibits I-κB phosphorylation and proteasome degradation, inhibiting, as a result, NF-κB, is able to induce PC3 apoptosis in vitro and reduce tumor xenograft volume in SCID mice in vivo [110].

An additional mechanism of action of MG132-mediated proteasome inhibition has been revealed in LnCaP and

PC3-TR prostate cancer cells and involves induction of AP-1 transcription factor family members c-fos and c-jun [111]. These transcription factors, then, repress transcription of cellular FLICE inhibitory protein [c-FLIP(L)], sensitizing cells to the apoptotic action of TNF-α related apoptosis inducing ligand (TRAIL). Apoptotic action of TRAIL is also enhanced when prostate cancer cells are exposed to bortezomib through stabilization of cdk inhibitor p21, a known proteasome substrate [112]. Up-regulation of pro-apoptotic bcl-2 family member Bik has also been found to contribute to bortezomib-induced sensitization to TRAIL [113].

Another death receptor, DR5, is also up-regulated after treatment of LnCaP prostate cancer cells with bortezomib through stabilization of its mRNA [114]. DR5 mRNA stabilization is mediated through binding of the protein HuR, a proteasome substrate, to its 3′-untranslated region. Furthermore, DR5 was up-regulated in the transcriptional level after treatment of DU145 prostate cancer cells with proteasome inhibitor MG132 [115]. This up-regulation was mediated through induction of transcription co-factor CCAAT/enhancer-binding protein homologous protein (CHOP) and resulted in enhanced TRAIL-induced apoptosis in DU145 cells.

Xenografts established in the flanks of nude mice from prostate cancer cell line PC-3M previously selected from PC3 cell line for its aggressive growth, displayed decreased growth and increased apoptosis after treatment of the animals with bortezomib compared with control tumors [116].

Collectively these data confirm that proteasome inhibition induces cell death in prostate cancer through a general deregulation of important cellular regulatory pathways.

UPS as a clinical therapeutics target in androgen-independent prostate cancer

With the results of in vitro and preclinical in vivo models confirming the inhibitory effects of proteasome inhibition in prostate cancer cells and prostate cancer xenografts as a solid basis, investigators have moved to testing proteasome inhibition in clinical trials. Bortezomib, the only proteasome inhibitor currently approved for clinical use (in multiple myeloma), has been tested in prostate cancer both as monotherapy and combined with other agents.

In a phase I trial of bortezomib in solid tumors, 48 patients with androgen-independent prostate cancer were included [117]. A weekly dose of 1.6 mg/m^2 for 4 out of 5 weeks was determined as the maximal tolerated dose. Dose limited toxicities included diarrhea and hypotension. A proteasome activity inhibition of about 70% was observed in patients receiving the dose of 1.6 mg/m^2, and 2 of the 47 androgen-independent prostate cancer patients (4%) had a decline of more than 50% in their pretreatment PSA. In addition, 9 patients (19%) had stable PSA. Among 21 patients with measurable disease, 2 (9.5%) had partial response in retroperitoneal lymphadenopathy. Interestingly, from these 2 patients only 1 had a PSA decline, while the other had stable PSA, a fact possibly implying that his partial response concerned an androgen-independent non-PSA producing sub-clone of his cancer.

In a phase I/II trial of bortezomib in combination with docetaxel in 83 patients with androgen-independent prostate cancer, the maximal tolerated dose had not been reached with bortezomib 1.6 mg/m^2 and docetaxel 40 mg/m^2, 2 out of 3 weeks [118]. Most common toxicities of the combination included fatigue, diarrhea, nausea, and flashing. A PSA decline $\geq$ 50% had been achieved in 28% of evaluable patients and, among patients with measurable disease, 78% had a partial response or stable disease. PSA response was seen in 33% of taxane-naïve patients and in 10% of taxane-pretreated patients. In contrast, patients with measurable disease had a similar response percentage (7% and 9%) irrespective of whether they were taxane-pretreated or naïve [118].

In a phase II study of docetaxel 30 mg/m^2 and bortezomib 1.6 mg/m^2 weekly, 3 out of 4 weeks, in 30 patients with androgen-independent prostate cancer, there were 5 partial PSA responses (23%) and 12 patients with stable PSA (54%) among 22 evaluable patients [119]. With a median follow-up of 4 months, 73% of patients had remained progression-free. Grades 3 and 4 fatigue and nausea were seen in 1 patient and grade 2 retinopathy was seen in 2 patients.

Combination of bortezomib 1.4 mg/m^2 and mitoxantrone 2 or 3 mg/m^2 weekly, 4 out of 5 weeks, was used in another study of 21 mostly chemotherapy pretreated androgen-independent prostate cancer patients [120]. Three patients of the 18 completing at least 2 cycles (17%) had a partial response and 2 other patients (11%) had stable disease. Fatigue and neutropenia were the main grade 3 toxicities seen.

These results suggest that bortezomib has clinical activity in androgen-independent prostate cancer but cannot discern the role of the drug in combination with chemotherapy drugs with known activity in prostate cancer. A clear answer would only be provided by randomized trials comparing a control chemotherapy arm with an experimental arm of chemotherapy combined with bortezomib. Another road to explore would be the combination of a proteasome inhibitor with current hormonal treatments such as estrogens. In this respect, it is worth mentioning that the proteasome is playing a role in estrogen-induced down-regulation of AR [121], an event that may promote AR-independence, whereas its reversal may favor a re-establishment of the androgen-dependent state.

An opposite way to exploit the UPS in prostate cancer is to promote UPS-mediated degradation of cancer-favoring proteins. An experimental technology that has yet to be developed for clinical purposes is through the use of (proteolysis targeting chimeric molecules (protacs) [122]. A protac is a chimeric molecule specifically synthesized to target a substrate molecule for proteasome degradation. A specific protac synthesized to target AR for degradation consists of DHT covalently linked with a phosphorylated form of I-κB. This synthetic molecule is recognized by the ubiquitinating machinery through the phosphorylated I-κB

part while DHT binds AR so that AR comes in close proximity with the ubiquitinating enzymes and its ubiquitination is facilitated. This protac has been confirmed to down-regulate AR [122]. Development of other protacs targeting key molecules in prostate carcinogenesis could be envisioned as a means to combat this difficult cancer when castration resistant.

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