The Role of Stress Proteins in Prostate Cancer

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Abstract: The development of therapeutic resistance, after hormone or chemotherapy for example, is the underlying basis for most cancer deaths. Exposure to anticancer therapies induces expression of many stress related proteins, including small heat shock proteins (HSPs). HSPs interact with various client proteins to assist in their folding and enhance the cellular recovery from stress, thus restoring protein homeostasis and promoting cell survival. The events of cell stress and cell death are linked, as the induction of molecular chaperones appears to function at key regulatory points in the control of apoptosis. On the basis of these observations and on the role of molecular chaperones in the regulation of steroid receptors, kinases, caspases, and other protein remodelling events involved in chromosome replication and changes in cell structure, it is not surprising that molecular chaperones have been implicated in the control of cell growth and in resistance to various anticancer treatments that induce apoptosis. Recently, several molecular chaperones such as Clusterin and HSP27 have been reported to be involved in development and progression of hormone-refractory prostate cancer. In this review, we address some of the molecular and cellular events initiated by treatment induced stress, and discuss the potential role of chaperone proteins as targets for prostate cancer treatment.

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INTRODUCTION

Prostate cancer is the most common cancer and the third most common cause of cancer related mortality in men in the United States [1]. While early detection has increased with the advent of serum prostate specific antigen (PSA) testing, the disease is often advanced when patients present with symptoms. For those with metastatic disease, androgen withdrawal is the most effective form of systemic therapy, producing a symptomatic and/or objective response in 80% of patients. Unfortunately, androgen-independent progression and death occur within a few years in the majority of cases [2]. Historically, chemotherapy was thought to have minimal clinical efficacy in men with metastatic hormone-refractory prostate cancer. However, more recently a role has emerged both in palliation with the use of mitoxantrone, and for docetaxel based chemotherapy a 20% prolongation in survival was demonstrated [3-5]. These improvements are significant but modest, and novel drugs and therapeutic strategies that target the molecular basis of resistance to androgen withdrawal and chemotherapy are urgently required.

The development of therapeutic resistance is the underlying basis for most cancer deaths and therefore of great interest in research. Selective pressures of treatments like hormone or chemotherapy lead to multiple changes in DNA structure and gene expression. Androgen withdrawal, for example, prolongs life in men with advanced prostate cancer, but remissions are temporary because surviving cells progress as hormone-refractory cancer [2]. This complex process involves variable combinations of clonal selection [6], adaptive upregulation of antiapoptotic genes and alternative growth factor pathways [7-11], as well as ligand-independent androgen receptor (AR) transactivation [12-15].

In this review, we will focus on stress-induced increases in antiapoptotic genes in chemoresistance and summarize the role of certain cytoprotective chaperones that may promote progression by inhibiting cell death. To treat this form of chemoresistance in advanced prostate cancer, one rational strategy incorporates agents that target stress-associated changes in gene expression precipitated by androgen or chemotherapy. These agents will hopefully delay progression of recurrent and refractory cancers and sensitize cells to treatment-induced apoptosis.

Heat shock proteins (HSPs) were first discovered in 1962 as a set of highly conserved proteins that were induced by hyperthermia and other kinds of cellular insults [16]. They are ubiquitous proteins and have been characterized as cytoprotective molecular chaperones. The typical function of a chaperone is to assist a protein to attain its functional conformation, to mediate interaction with other proteins and to prevent non-functional side reactions such as the non-specific aggregation of misfolded proteins [17-19]. Mammalian HSPs have been classified into groups according to their electrophoretic characteristics. The 4 principal HSP families are HSP90, HSP70, HSP60 and the small HSPs including HSP27. High molecular weight HSPs are ATP-dependent chaperones while small HSPs act in an ATP-independent manner. They are important for signalling and protein traffic even in the absence of stress and regulated by specific heat shock transcription factors [20]. However, the need of HSPs increases markedly after a cellular assault as a defence mechanism to allow cells to survive otherwise lethal conditions.

HSP27 and HSP70 are the most strongly induced chaperones during cellular stress. Initially ATP-independent chap-

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erones like HSP27 prevent the aggregation and precipitation of damaged proteins. When the cell recovers and ATP levels rise, ATP-dependent chaperones like HSP70 and HSP60 participate in refolding, transport and/or degradation of proteins [21-26]. HSP27 and HSP70 are abundantly expressed in malignant cells and participate in conferring chemoresistance [27]. High levels of HSP27 are commonly detected in prostate [28-31], breast [32, 33], ovarian [34, 35] and gastric tumours [36]. HSP27 expression is induced in response to hormone or chemotherapy and inhibits treatment-induced apoptosis [37, 38]. Moreover, high levels of HSP70 were shown to be a prerequisite for the survival of various human cancer cells and HSP70 depletion in tumour cell lines resulted in massive cell death [39, 40]. Similar to HSP70 HSP90 is indispensable for growth and survival signalling pathways [41]. Inhibition of HSP90 function by the small molecule 17-Allylamino-17-demethoxygeldanamycin (17-AAG) resulted in degradation of the androgen receptor and inhibition of prostate tumour growth in mice [42].

HSPs are not universally of prognostic value, however some have been demonstrated to have clinical utility as prognostic markers, such as HSP27 [7, 43]. Furthermore and beyond the scope of this review, high molecular weight HSPs play a role in many immunological processes and might stimulate immune defence responses against tumour cells [44-47].

Clusterin (CLU) is another important stress protein we focus on in this review. CLU overexpression was shown in various human malignancies including prostate [7, 48], breast [49], lung [7], bladder [7], kidney [50] and colon cancers [51]. In prostate cancer, CLU levels are low in hormone-naïve tissue, but increase significantly after hormone therapy [7]. Consequently, CLU gene silencing is an attractive antitumour therapeutic. At present, especially HSP90, HSP27 and CLU are being pursued as therapeutic targets in prostate and other cancers in late-stage preclinical or clinical research. Since cancer cells are very proficient at adapting to noxious environments, therapy with these novel chaperone inhibitors and their combination with conventional chemotherapeutics seems to be more promising than treatment with highly selective single-target agents.

CLUSTERIN

The Clusterin gene encodes a cytoprotective chaperone protein also known as testosterone-repressed prostate message-2, apolipoprotein J, or sulphated glycoprotein-2. It is a secretory heterodimeric disulphide-linked glycoprotein that is expressed in virtually all tissues, and found in all human fluids at relatively high concentrations [7, 51, 52]. CLU was first described for its ability to cause clustering of a variety of cell types [53]. Since then it has been revealed to be involved in a variety of physiological processes important for carcinogenesis including apoptotic cell death, cell cycle regulation, DNA repair, cell adhesion, tissue remodelling, lipid transportation, membrane recycling, as well as immune system and complement regulation [51-53].

Similar to the small HSPs CLU has been shown to interact with a variety of unrelated proteins that had been subjected to elevated temperature or reduction, establishing the chaperone activity of this molecule [54]. As a chaperone, CLU is upregulated under conditions of cellular stress in order to interact with many different target proteins. It forms a heterogeneous aggregate with these proteins, preventing their stress-induced aggregation and precipitation. CLU has also been shown to be regulated by heat shock factor-1 further suggesting a similar function to the small HSPs [55]. Increased CLU mRNA and protein levels have been consistently detected in various tissues undergoing stress, including heart, brain, liver, kidney, breast, and retinal tissues *in vivo* and *in vitro* [56].

CLU upregulation has been reported in numerous human malignancies [7, 48-51]. Observations have indicated an association of CLU expression with contradictory functions, either cell survival, tumour progression, treatment resistance *in vivo*, or apoptosis [7, 52, 57, 58]. These contradictory functions are likely attributed to two CLU protein isoforms, a secreted glycosylated form (sCLU), and a nonglycosylated nuclear form (nCLU). sCLU is a highly conserved heterodimeric disulphide-linked glycoprotein comprised of 40 and 60 kDa subunits derived from the first ATG codon of the full length Clusterin [59], while the other isoform, which translocates from the cytoplasm to the nucleus following several apoptosis inducing stimuli, starts from the second ATG codon and therefore omits the endoplasmic reticulum-targeting signal. It has been suggested that tumour cell survival is connected with overexpression of the pro-survival form (sCLU) and loss of the proapoptotic form (nCLU) [51, 56]. Overexpression of sCLU has been demonstrated to protect cells from a variety of agents that otherwise induce apoptosis such as androgen withdrawal, radiation and chemotherapy [7]. The mechanism by which CLU exerts these effects is not yet elucidated, however, it has been suggested that extracellular CLU might interact with stressed cell surface proteins (e.g. protein receptors) to inhibit proapoptotic signal transduction [26]. Some reports have also suggested that under stress conditions newly synthesized CLU may be redirected to the cytosol or nucleus exerting an antiapoptotic effect by preventing inappropriate interactions of intracellular proteins during stresses [53].

In the prostate gland, expression levels of CLU increase in response to castration-induced apoptosis in rat prostatic epithelial cells [60, 61], androgen-dependent mouse Shionogi tumours [7, 8] and several human prostate xenograft models [28, 62, 63]. CLU also increases following other apoptotic stimuli including cytotoxic chemotherapy [7], radiation [7], oxidative stress [7], and adenoviral-mediated p53 gene transfer [64].

CLU as a Diagnostic and Prognostic Marker in Prostate Cancer

In prostate cancer, CLU levels have been correlated with pathological grade on both biopsy and radical prostatectomy (RP) specimens [7, 48]. Further, CLU expression in RP specimens has been shown to be significantly associated with the preoperative PSA value and pathological stage [7]. CLU expression has also been used as a possible predictor for biochemical recurrence following RP [7, 65]. Consistent with the preclinical studies, although CLU expression is low or absent in most untreated hormone-naïve tissues, levels increase significantly within weeks after neoadjuvant hor-

mone therapy [7]. The question of whether antiapoptotic sCLU is the only form of CLU expressed in cancer, or whether proapoptotic forms of nCLU are downregulated in more aggressive tumours, has not been answered to date.

CLU as a Therapeutic Target in Prostate Cancer

Preclinical and clinical observations as discussed above strongly suggest that CLU is a cytoprotective gene upregulated by apoptotic triggers and confers resistance to conventional therapeutic modalities used in clinical practice. Downregulating CLU expression is an attractive therapeutic strategy to enhance therapy-induced apoptosis by targeting the CLU gene upregulation following proapoptotic stimuli.

Antisense oligonucleotides (ASOs) offer one approach to target genes involved in cancer progression, especially those that are not amenable to small molecule or antibody inhibition [7]. ASOs are single-stranded, chemically modified DNA-like molecules that are 17-22 nucleotides in length and designed to be complementary to a selected gene's mRNA and thereby specifically inhibit expression of that gene. It is estimated that any sequence of at least 13 bases in RNA and 17 bases in DNA is represented only once within the human genome. Thus, the exquisite specificity implicit in the design of ASOs theoretically leads to decreased toxicity. Translation is inhibited by the formation of RNA/DNA duplexes, thereby suppressing the mRNA and protein levels of the target genes [66-68]. The most commonly accepted mechanism of ASO action implicates RNase H-mediated hydrolysis of the target strand mRNA of RNA/DNA duplexes [69], while RNase-independent mechanisms are also implicated, such as steric blockade of splicing, translational arrest and the prevention of 5'-capping of the mRNA which affects nuclear transport of RNA [70-73]. Recently, better chemical modifications of ASOs have increased resistance to nuclease digestion and prolonged tissue half-lives. Among various modifications, the 2'-O-(2'-methoxy)ethyl (2'-MOE) incorporation was identified as enhancing both binding affinity and further resisting degradation by intracellular nucleases, as illustrated in Fig. (**1**) [73]. This property results in a longer duration of action and allows for a more relaxed dosing regime [74]. Indeed, recent clinical trials confirm the ability of this class of drugs to significantly suppress target gene expression [75].

A number of antisense molecules targeting hormone-refractory prostate cancer are currently in preclinical and clinical stages of development, including OGX-011, a 2nd generation antisense inhibitor incorporating the 2'-MOE backbone and targeting the translation-initiation site of human CLU mRNA. The modified backbone enables a tissue half-life of greater than one week. In preclinical models of prostate cancer, OGX-011 improved the efficacy of androgen withdrawal, chemotherapy and radiation by inhibiting expression of CLU and enhancing the apoptotic response, as illustrated in Fig. (**2**) [7]. In phase I clinical trials, OGX-011 was recently reported to potently suppress CLU expression in prostate cancer tissues in combination with androgen deprivation therapy [75]. This trial had a unique design in that patients with localized prostate cancer were administered OGX-011 prior to RP, and drug tissue level and serum CLU expression was determined for each patient and dose level. Concentrations of OGX-011 associated with preclinical effect were measured in tumour tissue and 90% suppression in CLU was achieved at 640 mg dose level. A second phase I trial combined increasing doses of OGX-011 with docetaxel in patients with metastatic breast, non-small cell lung, and hormone-refractory prostate cancers established a phase II dose for OGX-011 of 640 mg in combination with weekly or q3weekly docetaxel (Chi, K.N. *et al*. (2005). A phase I study of a second generation antisense oligonucleotide to Clusterin (OGX-011) in combination with docetaxel: NCIC IND. 154. ASCO abstract 3085). Four phase II trials of OGX-011 in combination with chemotherapy are now underway in patients with prostate, breast and lung cancers.

The use of OGX-011 in combination treatments with chemotherapy is of increasing interest in research in order to improve second line therapeutic strategies. The antitumour activity of docetaxel and mitoxantrone has previously been shown to be enhanced both *in vivo* and *in vitro* when combined with OGX-011 induced CLU suppression [7]. Of further importance is the recent finding in our lab that identifies CLU to be overexpressed in a chemoresistant PC-3 cell line (PC-3R). We report for the first time that knockdown of CLU using sequence-specific ASOs or siRNA chemosensitizes this PC-3R cell line to taxane and mitoxantrone based chemotherapy both *in vitro* and *in vivo* (unpublished data). Collectively, these findings provide preclinical proof of principle to support the use of OGX-011 in multimodal therapy for chemoresistant disease.

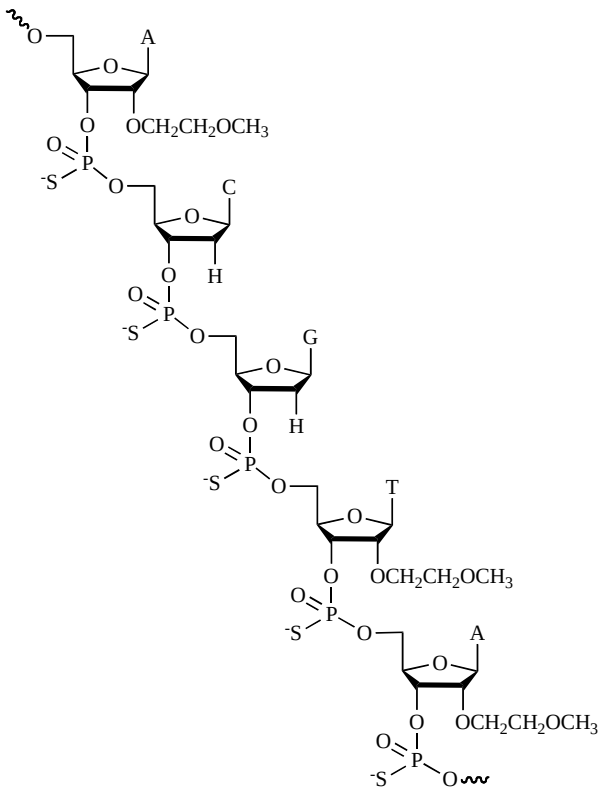

Fig. (1). 2nd generation 21-mer phosphorothioate 2'-O-(2'-methoxy)ethyl (2'-MOE) ASO to Clusterin mRNA. OGX-011, a 2nd generation antisense inhibitor incorporating the 2'-MOE backbone and targeting the translation-initiation site of human CLU mRNA. The modified backbone enables a tissue half-life of greater than one week.

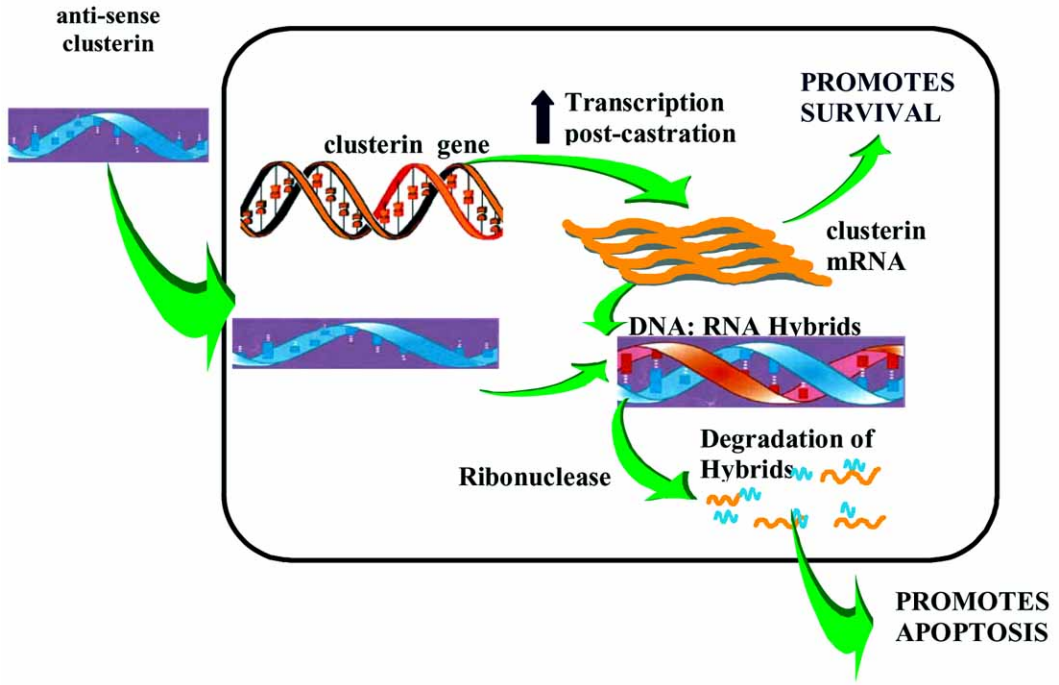

Fig. (2). Inhibiting adaptive increases in Clusterin expression to enhance apoptosis. In preclinical models of prostate cancer, OGX-011 inhibited the expression of CLU and enhanced the apoptotic response, as illustrated in Fig. (**2**).

HSP27

Recent immunohistochemical studies using human prostate tissue have shown an association between HSP27 and prostate cancer aggressiveness, progression, and development of a hormone-refractory phenotype. The earliest immunohistochemical studies of HSP27 in prostate cancer have demonstrated that the intensity of HSP27 expression is correlated with Gleason score [29, 76]. Furthermore, HSP27 expression appears to be highest in hormone-refractory tumours, suggesting an important role in the development of hormone-refractory disease [29, 38]. Lately, Miyake *et al*. have shown that in a cohort of 97 patients undergoing RP the expression level of HSP27 in diagnostic biopsy samples was correlated with preoperative serum PSA levels as well as surgical pathology outcomes [76]. Accordingly, biochemical free survival was inversely correlated with intensity of HSP27 expression in prostatectomy specimens. This corresponds to other studies showing that HSP27 expression is an independent predictor of survival in prostate cancer [30, 77, 78]. Hence, HSP27 appears to play a role in the different phases of tumorigenesis and progression typically found in patients with prostate cancer (Fig. (**3**)).

In different tumour models including prostate cancer, HSPs have been associated with multidrug resistance [79] and apoptosis [80, 81] and are functionally linked to increased tumorigenicity and treatment resistance in breast [82] and colon cancers [83]. The cytoprotective effects of HSP27 may result from its role as molecular chaperone or through direct interference with caspase activation, modulation of oxidative stress, and regulation of the cytoskeleton [84, 85]. Increased HSP27 expression in hormone-refractory prostate cancer suggests that HSP27 may confer resistance to androgen withdrawal by blocking apoptotic signals from androgen ablation [28, 77]. Moreover, HSP27 expression in hormone-naïve prostate cancer may increase resistance to androgen ablation because a higher proportion of nonresponders or early relapses to hormonal therapy occurred in patients strongly expressing HSP27 [29].

Blockage of apoptosis is important in hormone-refractory prostate cancer and is associated with the differential expression of cell survival genes like Bcl-2 and CLU [86]. HSP27 and Bcl-2 act at different levels to regulate apoptosis depending on the type of apoptotic signal [87]. Rocchi *et al*. have previously shown that HSP27 levels are higher in androgen-independent PC-3 cells compared with androgen-sensitive LNCaP cells, supporting earlier reports by Cornford *et al*. [29, 38]. The pattern of change in HSP27 expression in human prostate cancer after androgen withdrawal parallels that seen in LNCaP tumours after castration. Tissue microarray staining of 232 prostate cancer specimens showed an increased HSP27 level after androgen withdrawal, with uniformly high expression in all cases of hormone-refractory prostate cancer. Bubendorf *et al*. [28] also noted that HSP27 was one of the most overexpressed genes in hormone-refractory prostate cancer xenografts. Moreover, HSP27 is overexpressed in several other hormone-sensitive organs and human tumours and correlates with increased resistance to various cytotoxic chemotherapeutic agents [82, 88, 89].

HSP27 as a Therapeutic Target in Prostate Cancer

To specifically silence HSP27 gene expression the use of ASOs is a rational approach. Rocchi *et al*. [38, 90] showed using prostate cancer cell lines, that HSP27 ASOs reduced

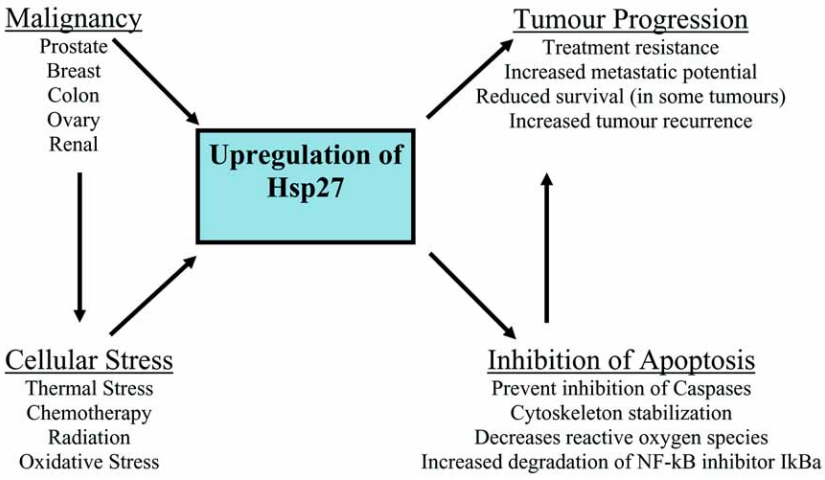

Fig. (3). The Role of Heat Shock Protein 27 in Malignancies.

HSP27 levels by 75% and significantly decreased (90%) cell growth *in vitro*. Pretreatment of PC-3 cells with HSP27 ASOs enhanced apoptosis *via* caspase-3 activation, supporting recent data showing that HSP27 functions as a negative regulator of cytochrome c-dependent activation of procaspase-3. Concannon *et al*. [84, 85] also reported that HSP27 inhibits cytochrome c-mediated caspase activation by sequestering both procaspase-3 and cytochrome c. Consistent with these *in vitro* data, systemic administration of HSP27 ASO monotherapy suppressed PC-3 tumour growth *in vivo* and also significantly enhanced paclitaxel activity *in vitro* and *in vivo*. These results are consistent with earlier reports that HSP27 overexpression confers resistance to doxorubicin in MDA breast cancer cells [82].

Accumulating evidence links rising HSP27 levels with hormone-refractory prostate cancer and development of treatment resistance and therefore identifies HSP27 as a potential therapeutic target. However, the functional significance of changes in HSP27 expression associated with drug resistance and hormone-refractory prostate cancer remains undefined. Plans for phase I/II clinical trials using a 2nd generation antisense called OGX-427 targeting the translation-initiation site of human HSP27 mRNA are being planned and will be underway in late 2006.

HSP10 AND HSP60

HSP60 is constitutively expressed in most mammalian cells [91]. Coregulated by another heat shock protein, HSP10 [92], HSP60 functions as a protein chaperone and also is involved in protein folding [93]. Capello *et al*. showed that the expression of HSP10 and HSP60 is high in early prostate carcinogenesis [94] and Cornford *et al*. demonstrated that HSP60 is highly expressed in both early and advanced prostate cancer [29]. Recently, immunolocalization studies of prostate cancer specimens revealed that normal prostate tissue weakly expressed HSP60 only in the basal cell layer of the prostatic glands, whereas all prostate cancers highly expressed HSP60 throughout the epithelium [95]. Similar to HSP27, expression of HSP60 also appears to increase after androgen ablation [95]. The universal and high expression of HSP60 in prostate cancer has prompted some authors to propose HSP60 expression to be a marker for prostate cancer [95].

Increased HSP60 has been associated with autoimmune disease and various other malignancies including breast carcinoma [96] and myeloid leukemia [97]. The overexpression of HSP60 and its coregulator, HSP10, in early prostate cancer, suggest that these two heat shock proteins may be important in prostate cancer development. However, further investigation of HSP60 is required to validate this protein as a therapeutic target for prostate cancer.

HSP70

HSP70 is a stress-inducible protein that is known for its inhibitory effects on apoptotic cell death induced by TNF-alpha and TNF-beta [98]. Furthermore, HSP70 interacts with and stabilizes mutant p53 [99]. In contrast, wild-type p53 down-regulates HSP70 expression [100]. Although p53 appears to have prognostic significance in prostate cancer [101], the role of HSP70 in prostate cancer is unclear.

Increased HSP70 expression has been found in hepatocellular [102] and cervical [103] cancers as well as acute myeloid leukemia [97]. Immunohistochemical studies do not consistently show a correlation of HSP70 expression with prostate cancer, Gleason score, or prognosis [29]. Interestingly, forced overexpression of HSP70 in TRAMP-C2 mouse prostate cancer cells inhibited tumour growth, and mice with these xenografts had improved survival over mice with TRAMP-C2 control xenografts. However, latest studies using PC-3 and DU-145 prostate cancer cells showed that suppression of HSP70 expression, by antisense oligonucleotide therapy [40], the bioflavinoid drug quercetin [40], or siRNA [104], enhanced apoptosis in combination with various types of cellular stress, including exposure to heat, radiation, chemotherapeutics and inhibitors of HSP90 and proteasome [40, 104]. These studies suggest that HSP70 may be an important inhibitor of apoptosis after stress and a potential target for multi-agent apoptosis-based cancer therapy. Accordingly, plasma levels of HSP70 have been shown to be higher in patients with localized untreated prostate cancer compared to controls [105]. Like HSP60, further investiga-

tion will be essential before validation of this chaperone protein as a target for prostate cancer therapy.

HSP90

HSP90 is among the most abundant proteins of eukaryotic cells, comprising 1-2% of the cell's total proteins [106]. HSP90 interacts with several key proteins in promoting prostate cancer progression, including wild-type and mutated AR, HER2, ErbB2, Src, Abl, Raf and Akt. Hence, the multifaceted role of HSP90 makes this protein an enticing target; one in which multiple different pathways of carcinogenesis are potentially influenced.

One of these roles that may be useful to exploit in prostate cancers, is HSP90's interaction with the AR. HSP90 plays an important role in AR stabilization; prior to ligand binding, the AR is bound to HSP90. This chaperone heterocomplex stabilizes the AR to be in a partially unfolded, high-affinity conformation, which is necessary for efficient response to androgens [107]. In fact, HSP90-receptor heterocomplexes exist of the glucocorticoid receptor, progesterone receptor, mineralocorticoid receptor, and estrogen receptor [108]. The interaction of HSP90 with the AR has provided an exciting target in men with hormone-refractory prostate cancer as one mechanism in the development of hormone resistance is the ligand-independent activation of the AR. Drugs that inhibit the activation of the AR are thus an attractive treatment option for patients with disease progression following medical or surgical castration.

The most studied HSP90 inhibitors include: geldanamycin [109], 17-AAG [110], and radicicol [111-114]. These inhibitors cause the proteasomal degradation of proteins that require this chaperone for stability. Through this mechanism, HSP90 inhibition leads to inhibition of various proliferative signals, including the Akt pathway [115, 116], chemosensitization [117], or sensitivity to hypoxia or complement induced lysis [118]. *In vitro* and *in vivo* experiments demonstrated that treatment with 17-AAG caused the degradation of HER2, Akt, and both mutant and wild-type AR, and the retinoblastoma-dependent G1 growth arrest of prostate cancer cells [42]. At nontoxic doses, 17-AAG resulted in a dose-dependent decline in AR, HER2, and Akt expression in prostate cancer xenografts. Furthermore, 17-AAG inhibited the growth of both hormone-sensitive and hormone-refractory cell lines, *in vitro* and *in vivo*. These data suggest that inhibitors of HSP90 may represent a novel strategy for the treatment of patients with prostate cancer and Phase I/II clinical trials to test this hypothesis are currently ongoing [41, 106].

BAG-1

BAG-1 is a member of an evolutionarily conserved family of proteins that binds to the molecular chaperone HSP70 and that functions as co-chaperone, modulating HSP70 activity [119]. In addition, BAG-1 contains an ubiquitin-like domain, which mediates interactions of BAG-1 with the proteasome, directing misfolded proteins for destruction. The human BAG-1 gene encodes three proteins through alternative translation initiation sites, including the predominantly cytosolic BAG-1 protein of 34 kDa, the cytosolic and nuclear protein BAG-1M of 46 kDa, and the predominantly nuclear BAG-1L protein of 50 kDa. The BAG-1 proteins have diverse biological functions ranging from inhibition of apoptosis to modulation of the action of steroid receptors [120, 121].

BAG-1 expression has been shown to be elevated in prostate cancer. Higher nuclear BAG-1 expression in hormone-refractory compared to localized untreated tumours has been demonstrated suggesting that upregulation of the nuclear isoform may contribute to disease progression. Also, in early stage patients treated with external-beam irradiation, cytosolic BAG-1 expression levels correlated with higher pretreatment levels of serum PSA and shorter time to disease progression [122].

Of importance to prostate cancer pathogenesis, BAG-1L has been demonstrated to enhance the transactivation function of the AR [123]. It has been demonstrated that BAG-1L enhances the transactivation function of the AR by using its NH_2- and COOH-terminal domains to bind to the COOH- and NH_2- terminal sequences of the AR. Binding to and regulation of AR action by BAG-1L is assisted by HSP70. In the benign prostate, BAG-1L is highly expressed in the basal cells but not the secretory epithelium where the AR is expressed. In prostate carcinoma however, BAG-1L is highly expressed in the secretory epithelium, and is found in association with HSP70. These findings collectively indicate that in prostate carcinoma cells, BAG-1L and HSP70 are expressed in secretory epithelial cells where they function together to enhance the action of the AR.

CDC37

Cdc37 is another chaperone protein that has displayed increased expression in prostate cancer. The Cdc37 gene encodes a 50 kDa protein which targets intrinsically unstable oncoprotein kinases such as Cdk4, Raf-1, and Src to the molecular chaperone HSP90. It has been demonstrated that human prostatic tumours and certain premalignant lesions display increased expression of Cdc37. Further, transgenic mice expressing Cdc37 in the prostate displayed a wide range of growth-related abnormalities including prostatic epithelial cell hyperplasia and dysplasia [124]. These data suggest that expression of this chaperone may promote inappropriate proliferation and may be an important early step in the development of prostate cancer.

CONCLUSION

The development of hormone-refractory prostate cancer is the underlying basis for most deaths in prostate cancer patients. Exposure to cytotoxic therapies like hormone withdrawal or chemotherapy leads to the overexpression of many stress-induced proteins. As an adaptive defence mechanism, these molecular chaperones interact with various client proteins in the cancer cell and appear to function at key regulatory points in the control of apoptosis. Recently, several stress proteins such as Clusterin and HSP27 have been reported to be involved in mediating therapeutic resistance in prostate cancer. Inhibition of their upregulation with targeted therapeutics like antisense oligonucleotides can enhance cell death following treatment with androgen ablation, chemotherapy and radiation. In the last decade, ASO technology has quickly moved from preclinical proof of principle to clinical studies. Although recent findings are very promising,

challenges remain to demonstrate effective antitumour activity in phase III trials.

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ABBREVIATIONS

AR = Androgen receptor

ASOs = Antisense oligonucleotides

CLU = Clusterin

sCLU = Secretory Clusterin

nCLU = Nuclear Clusterin

HSPs = Heat shock proteins

PSA = Prostate specific antigen

RP = Radical prostatectomy; 2'-MOE, 2'-O-(2'-methoxy)ethyl; 17-AAG, 17-Allylamino-17-de-methoxygeldanamycin

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