Androgen receptor and heat shock proteins in progression of prostate cancer cells

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Introduction

The androgen receptor (AR) is a member of the nuclear receptor subfamily acting as a transcription factor for a wide range of AR-dependent genes. In prostatic tissue, AR is the main regulator of androgen-mediated signaling and is responsible for cell proliferation and differentiation. Because of these functions, the receptor is a key factor in the progression of prostate cancer [1, 2]. Premalignant epithelial prostate cells accumulate a number of genetic changes, resulting in the expression of mediators of oncogenic processes which benefit growth. In PCa, two basic events can be defined as key steps in progression: 1) initial formation of anti-apoptotic mechanisms and later 2) the ability for hormone-refractory growth, accompanied by resistance to antihormonal therapy marking the beginning of late-stage cancer.

A variety of heat shock proteins (HSP) are involved in AR signaling. As a first step to identify molecular factors of HSP and AR-dependent PCa progression, we analyzed AR-associated HSP in PCa cells depicting various stages of PCa progression. Therefore, the well-established PCa cell lines LNCaP, 22Rv1 and PC-3, which exhibit various characteristics according to AR-dependent and androgen-stimulated growth were used.

We defined this experimental cell culture system comprising three PCa cell lines as an in vitro PCa progression model depicting hormone-sensitive to hormone-refractory stages of PCa and we analyzed AR-associated HSP using protein analysis.

This study marks the start of the identification and characterization of AR- and HSP-dependent molecular mechanisms in tumor progression, particularly molecular switches in resistance to apoptosis and hormonal growth. Finally, modulating factors of progression are highly potential candidates for diagnostic markers and pharmacological targets in PCa therapy.

Materials and methods

Cell culture

The well known human PCa cell lines LNCaP, 22Rv1 and PC-3 were propagated in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 100 units/ml penicillin (PAA Laboratories, Pasching, Austria), 100 µg/ml streptomycin (PAA Laboratories), and 10% fetal bovine serum (Invitrogen). Cells were incubated at 37 °C in a 5% CO₂ atmosphere and passaged twice weekly.

Western blot analysis

For characterization of the endogenously expressed protein levels in LNCaP, 22Rv1 and PC-3 cells, 6-well cell culture plates of subconfluent cells were lysed in buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, 10 mM K₂HPO₄, 5 mM EDTA, 10% glycerol, 1% Triton® X-100, 0.05% sodium dodecylsulphate, 1 mM Na₃VO₄, 20 mM NaF, 0.1 mM phenylmethylsulfonyl fluoride, 20 mM 2-phosphoglycerate and Complete Protease Inhibitor Cocktail (Roche Applied Science, Mannheim, Germany). The protein concentration was determined by a modified Bradford assay [3]. Subsequently, comparable amounts of total protein were separated by SDS-PAGE and transferred to a nitrocellulose membrane (Whatman, Dassel, Germany). After blocking with RotiBlock (Carl Roth, Karlsruhe, Germany), the transferred proteins were incubated with primary antibodies raised against AR (Millipore, Temecula, CA, USA), HSP27 (Cell Signaling Technology), HSP60 (Cell Signaling Technology), HSP90α (Cell Signaling Technology) and GAPDH (Abfrontier, Seoul, Korea) overnight, followed by incubation with goat anti-mouse (Cell Signaling Technology) and goat anti-rabbit (Cell Signaling Technology) secondary antibodies conjugated to...
horseradish peroxidase for 1 h. Subsequent visualization of the proteins was carried out by chemiluminescence using LumiGLO Reagent (Cell Signaling Technology). Quantification of Western Blotting signals was performed using Kodak 1D v.3.5.4 image analysis software (Kodak, New Haven, CT, USA) to determine the ratio of target protein per GAPDH reference protein. Relative expression rates of proteins were calculated by setting the LNCaP protein level to 100%. All data are mean values for the results of 3 – 5 independent experiments.

**Results**

It is known that various HSP are involved in AR-dependent signaling mechanisms. In addition to having functions in receptor turnover and stability we postulated that HSP are involved in initiation and modulation of prooncogenic events such as tumor resistance to apoptosis and hormonal growth regulation. We then established a cellular system containing 3 PCa cell lines, reflecting 3 putative stages in PCa progression. LNCaP is an androgen-sensitive and AR-positive cell line representing an earlier stage of PCa. In contrast, the cell line PC-3 is AR-negative and proliferates independently of androgens, corresponding to a more advanced stage of PCa development. 22Rv1 cells constitute an intermediate stage of PCa tissue. This prostate cell line does not need androgens for cell growth, but proliferation is enhanced in the presence of the hormone. Interestingly, 22Rv1 cells express an additional, shortened isoform of AR, which is functionally different from the full-length receptor. The complete amino acid sequence of the truncated receptor is still unclear, but mRNA analysis and antibody-binding studies revealed that this isoform lacks the C-terminus [4, 5].

Characterization of the composition of HSP and expression levels in the progression model showed chaperones differentially synthesized under basic, non-stimulated conditions (Figure 1). The chaperone HSP60 showed similar relative expression rates in LNCaP (100.0%) and 22Rv1 cells (107.0%). However, in AR negative PC-3 cells the HSP60 level decreased to 55.2% compared to LNCaP cells. A reciprocal effect was detected in the case of HSP90α. The relative protein level of this chaperone in LNCaP cells (100.0%) was comparable to that in 22Rv1 cells (88.5%), but HSP90α protein increased in PC-3 cells to a relative expression level of 152.7%. A wide difference was found in the case of the endogenous expression levels of the small chaperone HSP27. Compared to LNCaP cells, relative HSP27 expression level in 22Rv1 cells was 73.9%, but analysis of PC-3 cell revealed a basic level of HSP27 of 6.2%, which may imply a strong reduction in the small chaperone during the tumor cell progression processes.

**Conclusion**

Analysis of HSP27, HSP60 and HSP90α in three PCa cell lines showed different protein expression levels corresponding to different stages of PCa progression. Our data also suggest that regulation of these chaperones underlay progression-specific co-regulation with the expression of AR, particularly, as HSP27 and HSP90α are known as essential factors in AR signaling.

We hypothesize that HSP have additional roles over and above the chaperone function in AR stability. Currently, we are investigating the molecular basis and cellular functions of these differently regulated HSP. These findings may have important implications for the understanding of tumor progression and the future treatment of PCa.

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References


