American Journal of Pathology, Vol. 156, No. 3, March 2000

A Novel Association between the Human Heat Shock Transcription Factor 1 (HSF1) and Prostate Adenocarcinoma

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A search for differentially expressed genes in a pair of nonmetastatic (PC-3) *versus* metastatic variant (PC-3M) human prostate carcinoma cell lines led to identification of the human heat shock factor (HSF1) as an overexpressed gene product in PC-3M cells. Analysis of primary prostate cancer specimens indicated that HSF1 is generally up-regulated in most of the malignant prostate epithelial cells relative to the normal prostate cells. Among the known effectors of HSF1 action, constitutive levels of HSP70 and HSP90 are not significantly altered by the naturally elevated expression of HSF1 as in PC-3M cells or by transduced overexpression of HSF1 in PC-3 cells. The basal levels of HSP27 in both cases are, however, consistently increased by two- to threefold. With respect to response to heat shock, high basal concentration of HSP90 is not further enhanced in these cells, and HSP70 is up-regulated irrespective of HSF1 level. Heat shock, however, causes an increase in HSP27 when HSF1 is up-regulated, except when the expression of HSF1 is already too high. These results document for the first time that HSF1 is overexpressed in human prostate cancer cells, at least one consequence of which in the prostate cancer cell lines tested is stimulation of both basal and stress-induced expression of HSP27, an important factor in cell growth, differentiation, or apoptosis. ***(Am J Pathol 2000, 156:857–864)***

The inducible transcriptional response of genes encoding heat shock proteins and molecular chaperones is mediated by heat shock transcription factors (HSFs). The family of vertebrate *HSF* genes consists of at least four members (*HSF*1-*HSF*4), of which HSF1, HSF2, and HSF3 proteins function as positive regulators, and the HSF4 protein appears to act as a negative regulator.[1–5] Comparison of the sequences of the vertebrate HSFs within a single species shows approximately 40% amino acid homology. Interspecies homology of 73 to 89%, on the other hand, reveals a strong conservation of amino acid sequences of a given member.[3,4]

Human HSF1 is an 82-kd transcription factor that is activated in response to heat shock and other forms of environmental and chemical stress. Normally, HSF1 exists as a monomeric protein in the cytoplasm that, on heat induction, undergoes trimerization and nuclear localization.[6–8] Although the trimer can bind to DNA, its transcription activation activity appears to be enhanced by stress-induced serine phosphorylation.[9] The response is mediated by binding of the activated trimer to the heat shock element (HSE), which consists of three inverted repeats of the sequence NGAAN and which is present in the promoter region of *HSP*90, *HSP*70, and *HSP*27 genes.[4,10]

Although elevated levels of heat shock proteins have been reported in cancer, for example, HSP70 in melanoma[11] and breast carcinoma[12] and HSP27 in malignant fibrous histiocytoma,[13] there is very little information in the literature regarding the status of HSF1 in cancer development. While applying the representational difference analysis (RDA)[14] to examine the differential gene expression patterns between prostate carcinoma cell line PC-3 and its isogenic but metastatic variant PC-3M,[15,16] we found that one of the genes overexpressed in PC-3M cells was *HSF*1. In this report we describe a systematic study of HSF1 expression in prostatic adenocarcinoma cell lines and tissues and our attempts to elucidate the consequence of HSF1 expression in relation to the basal and heat-shock-induced levels of some of the major downstream effectors of HSF1.

Supported by United States Public Health Service grant CA59705 from the National Cancer Institute and, in part, by postdoctoral fellowships from the National Cancer Institute training grant T32-CA09320 (to J. Z.), National Institute of Allergy and Infectious Diseases training grant T32-AI07078 (to W. C. P.), and a grant from the T. J. Martell Foundation.

Accepted for publication November 29, 1999.

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Materials and Methods

Cell Culture

The PC-3 and PC-3M cells were cultured in DMEM containing 10% fetal bovine serum, 1× nonessential amino acid solution, 1× essential amino acid solution, and penicillin-streptomycin solution as described.[16] The other prostate carcinoma cell lines, DU-145, ALVA-31, and LNCaP, were cultured as described before.[17] Adult human normal prostate epithelial cell line MLC SV40[18] was maintained in keratinocyte-serum-free medium with 5 ng/ml recombinant epidermal growth factor and 25 ng/ml bovine pituitary extract (Gibco BRL, Grand Island, NY). A primary prostate epithelial cell line (second passage) was purchased from Clonetics (San Diego, CA) and allowed to expand by two doubling cycles to reach a cell density of 10^6 cells/100-mm dish before harvest. The cell line Rat1a was cultured as described.[19] Xenograft-derived human prostate cancer cell line, LAPC-4[20] was maintained in 1× Iscove with 10% fetal bovine serum, 1% penicillin-streptomycin, and 10 nmol/L R1881 (New England Nuclear, Boston, MA).

Neoplastic and Normal Prostate Specimens

Samples of prostatic adenocarcinoma and uninvolved normal tissues were obtained either from the University of Southern California Norris Cancer Center or from the Department of Pathology, University of Colorado (Denver, CO). Fresh tissues were stored in liquid nitrogen until needed. A total of 18 prostate carcinomas and 4 normal prostate tissues from prostate carcinoma patients was analyzed. The study also included normal and carcinoma tissue sections from a single prostate.

RNA Preparation and Representational Difference Analysis (RDA)

Total RNA from 70 to 80% confluently grown PC-3 and PC-3M cells were isolated with TRIzol reagent (Gibco BRL) as described by the manufacturer. Subsequently, poly(A)$^+$ mRNAs were isolated from the total RNAs by the oligo (dT) affinity column chromatography kit purchased from Gibco BRL. The final yield was calculated spectrophotometrically and the quality of mRNA was checked on a 1.4% agarose, 0.7 mol/L formaldehyde gel. Double-stranded cDNAs were generated from the mRNA through procedure I of the cDNA Synthesis System (Gibco BRL). The RDA was performed as described,[14] with PC-3 cells as the designated driver population and PC-3M as the tester population. During the first round of subtraction and amplification, 100-fold excess of PC-3 cDNA was used against PC-3M, whereas in generating the second round of differential products, a 2000:1 ratio of driver to tester cDNA was used.

Cloning and Sequencing

Differential products generated after two rounds of selection and amplification were subcloned into the *Bam*HI site of pBluescript KS (−). Ligated products were transformed into *Escherichia coli* DH5α and screened for blue/white colony formation on X-gal/AmpR Luria-Bertani plates. Cloned cDNAs were screened for differential expression by Northern blot analyses. Positive cDNAs were sequenced by the Sanger's dideoxynucleotide chain termination method. Readable sequences of 150 bp or more were further identified through the BLASTN search program available from the world wide web at http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST.

Northern Hybridization

Ten micrograms of RNA from either PC-3 or PC-3M were loaded onto a 1.4% agarose gel in the presence of formaldehyde, electrophoresed, blotted overnight onto a Micron Separations, Inc., membrane filter, and probed with randomly primed (Prime-It kit, Stratagene, La Jolla, CA) radiolabeled cDNA from the RDA-generated clone or human β-actin.

Western Blotting and Immunohistochemistry

For Western blot analyses, total cell lysates collected from plates of exponentially growing cells at 70% density were boiled in 2× Laemmli buffer.[21] Polypeptides from one-tenth of total cell lysates from each plate (100 × 20 mm) were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% gel) and subjected to immunoblot analysis using rabbit anti-human HSF1 antibody[8] at 1:5000 dilution. Detection was achieved with a secondary goat anti-rabbit horseradish peroxidase-conjugated antibody at 1:5000 dilution and chemiluminescence.

For the analyses of heat shock proteins the following antibodies were used at the indicated dilutions: anti-HSP27, rabbit polyclonal antibodies at 1:1000 (Upstate Biotechnology, Lake Placid, NY); anti-HSP90 mouse monoclonal antibody at 1:1000 (Transduction Laboratories, Lexington, KY); and anti-HSP70 goat polyclonal antibodies at 1:200 (Santa Cruz Biotechnology, Santa Cruz, CA). The intensities of the bands were determined using Scion Image software. The ratio of the intensity of a given band over the intensity of the corresponding actin band was calculated and compared for an increase or decrease in the expression of HSF1 or each of the HSPs.

For immunohistochemistry, exponentially growing PC-3 and PC-3M cells were washed three times in PBS and deposited on slides by cytospin. Frozen sections 5 μm thick from a number of neoplastic and normal prostate tissues were prepared. All slides were air dried at room temperature for 10 minutes and fixed in 4% paraformaldehyde in PBS, pH 7.4, for 10 minutes. Then the slides were treated in 0.3% H_2O_2 in methanol for 30 minutes and blocked with 1.5% normal goat serum for 1 hour. Polyclonal anti-human HSF1 primary antibody at 1:3000 dilution was added and allowed to bind at 37°C for 1 hour. The Vectastain ABC kit (Vector Labs, Burlingame, CA) was used to complete the staining in which

amino ethyl carbazol was added as a peroxidase substrate. Cell nuclei were counterstained with hematoxylin.

Construction of Retrovirus Expression Vectors

The 2.2-kb insert containing full-length cDNA of human *HSF*1[8] was cut out of the plasmid vector by *Eco*RI and cloned into the *Eco*RI site of the pWZLneo retrovirus vector.[22,23] The construct containing HSF1 in the sense orientation was confirmed by sequence analysis. The sense or the vector-only DNA was transfected into PA317 amphotropic retrovirus packaging cell line by Lipofectamine, and after 48 hours the cells were split into 1:10 and selected in DMEM containing 600 μg/ml G418. Because the selection of transfected cells was based on the expression of the downstream Neo resistance gene product from the same bicistronic proviral DNA carrying the *HSF*1 cDNA, the use of the WZLneo vector allowed virtually all selected resistant cells to express the desired sequences. The surviving clones were trypsinized and expanded individually. The viral titer of the PA317 clones was determined by transfer of the G418 resistance to NIH3T3 cells using serial dilutions of viral supernatant. Clones of cells producing viral titers between 3×10^5 and 5×10^5 infectious units per ml were selected for virus collection and for use in the experiments described.

Infection of PC-3 Cells with the Retroviruses

The harvested virus supernatants of the *HSF*1 sense or the vector-alone construct were used to infect PC-3 cells at a multiplicity of infection of approximately 10 in the presence of 8 μg/ml polybrene, and the infection procedure was repeated three times for the same culture. The infected cells were selected in the medium containing 600 μg/ml G418, and individual drug resistant clones were isolated and expanded for further work.

Heat Shock Treatment

Cultures of PC-3, PC-3M, and infected PC-3 cells were incubated at 45°C in the presence of 5% CO_2 for 1 hour and allowed to recover for 4 hours at 37°C. The cells were then collected by trypsin treatment for 5 minutes and pelleted by centrifugation at 2000 rpm for 5 minutes. The pellets were washed with 1× PBS twice and resuspended in RIPA buffer. After three freeze-thaw cycles, cell lysates were harvested by centrifugation at 10,000 rpm for 10 minutes and kept at −80°C until used for Western blotting.

Results

RDA and the Identification of HSF1 as Differentially Expressed Product

Using mRNAs isolated from PC-3 and PC-3M cells, RDA was applied in which PC-3 cDNA was used to drive out common sequences in PC-3M cDNA. After two rounds of driver/tester selections and subsequent amplification of unique sequences, several small fragment clones were obtained and tentatively identified through GenBank BLAST search program. They included human keratin 19, human transcription factor IIICα subunit, human nuclear corepressor KAP-1, and a homolog of *HSF* family. The current work concerned the *HSF* homolog.[1,3,5] Since the 250-bp HSF fragment isolated was found to share more sequence homology to *HSF*1 (178 bp of readable sequence out of 179 bp of published sequence) as opposed to HSF2 (38% amino acid homology), HSF4 (39% amino acid homology), or chicken HSF3 (46% amino acid homology), the data suggested that our fragment was more likely to be derived from *HSF*1 than from any other members of the *HSF* family. Figure 1 shows a Northern blot of total RNAs isolated from PC-3 and PC-3M probed with either the 250-bp fragment or the β-actin cDNA. A single band approximately 2.0 kb in size, consistent with the known 2156-bp cDNA[1] length of *HSF*1 exhibited a much stronger signal in PC-3M relative to PC-3 cells, denoting differential expression of *HSF*1 in these two cell lines.

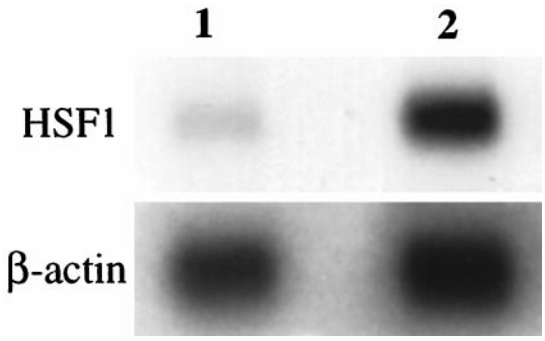

Figure 1. Northern blot showing differential expression of HSF1 in PC-3 and PC-3M cell lines. Total RNA (10 μg) from PC-3 (**lane 1**) or PC-3M (**lane 2**) cells was analyzed using the 250-bp HSF1 fragment as probe. The same blot was subsequently hybridized to β-actin probe as an RNA loading control.

The detection of HSF1 at the protein level is demonstrated in Figure 2. This Western blot illustrated in the upper part of Figure 2A supported the overexpression of HSF1 protein (82 kd) in PC-3M (lane 5) as compared to PC-3 (lane 4). Other prostate tumor cell lines tested, namely, LNCaP (lane 2), LAPC-4 (lane 3), DU-145 (lane 6), and ALVA-31 (not shown) also displayed a detectable 82-kd HSF1 protein band, whereas Rat1a fibroblasts did not express readily detectable level of HSF1 (data not shown). Normal prostatic epithelial cell line MLC SV40$^-$ (lane 1) and primary prostate epithelial cells (not shown) had similar levels of HSF1 expresson. Figure 2B illustrates differential levels of HSF1 protein expression in sections of prostate tissues. Although our test was not sensitive enough to detect a band corresponding to 82-kd HSF1 in three different normal prostate sections (lanes 1–3), two of the three prostate cancer tissues analyzed (lanes 4–6) displayed a reacting band at 82 kd. Interestingly, the intensity of the band appeared to correspond with percentage of tumor cells within the tissue section used, which varied from 50% in lane 4 material to 75% in lane 5 to 90% in lane 6. This estimation of tumor

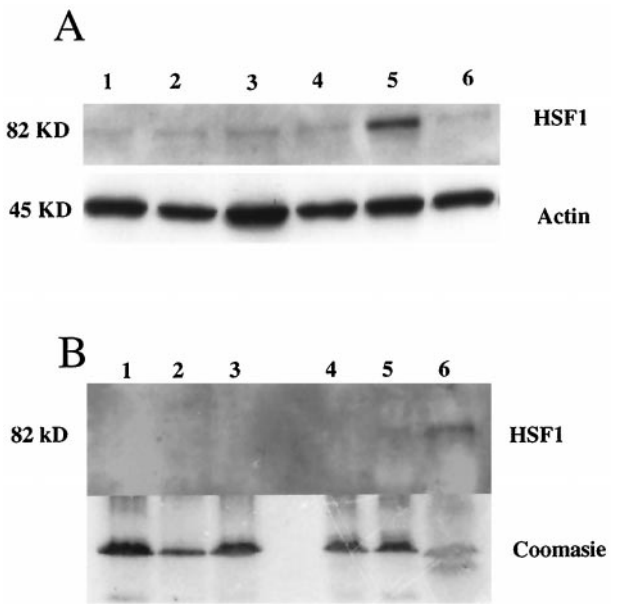

Figure 2. Western blots illustrating the expression of HSF1 protein in prostate cell lines and tissues. **A:** Cells used: **1**, immortalized prostate epithelial (MLC); **2**, LNCaP; **3**, LAPC-4; **4**, PC-3; **5**, PC-3M; **6**, DU-145. **B:** Prostate tissue sections: **1**, normal (S95–1066); **2**, normal (S15113); **3**, normal (S15121); **4**, tumor S96–158; **5**, tumor S15050; and **6**, tumor S15284. The lower part of **A** shows α-actin as loading control; the lower part of **B** depicts Coomassie Blue staining of the gels.

cells was based on nuclear morphology of H&E staining of adjacent tissue sections.[24]

The relative difference in the levels of HSF1 expression between PC-3 and PC-3M cells was also noted by immunohistochemical analysis (Figure 3). Moreover, immunostaining indicated that whereas PC-3 displayed both cytoplasmic and nuclear distribution of the HSF1 protein, the PC-3M cells appeared to have relatively more concentration of HSF1 in the nucleus. Immunohistochemical staining of frozen sections confirmed that prostate carcinoma cells overexpressed HSF1 (Figure 4, B and D) as compared to normal (Figure 4A) or benign hyperplasia sections (Figure 4C). A total of 18 prostate cancer specimens were tested, of which 16 showed positive staining in the malignant epithelium, although the levels varied from specimen to specimen. All four of the normal specimens tested also showed staining but were considerably less intense than the carcinomas.

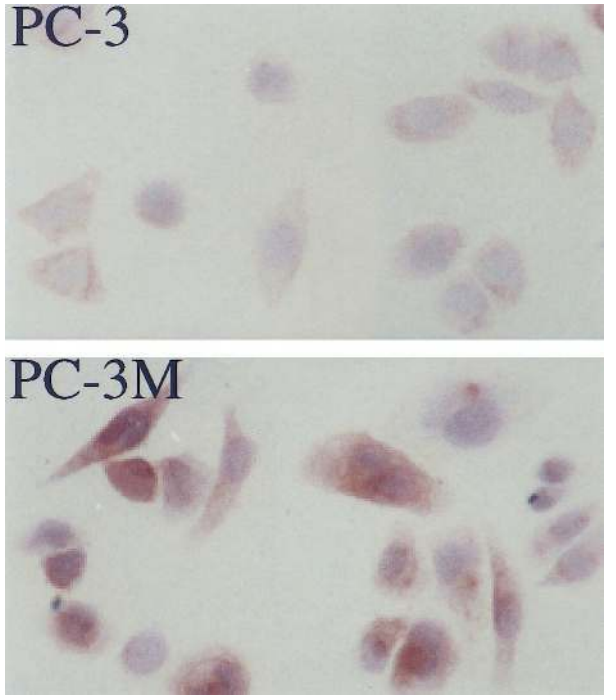

Figure 3. Immunohistochemical staining of HSF1 protein in PC-3 and PC-3M cells. Cytospins of PC-3 and PC-3M cells were stained with both hematoxylin and anti-HSF1 antibodies.

Effect of HSF1 Overexpression on the Expression of Heat Shock Proteins

PC-3 and PC-3M cells that differentially expressed HSF1 were subjected to heat shock treatment. Although heat shock treatment of these cells at 43°C for 1 hour did not induce significant changes in HSP levels, they were affected when the experiments were performed at 45°C. As shown in Figure 5, when the cells were examined immediately after heat shock at 45°C for 1 hour (Figure 5A) the primary species of HSF1 was the phosphorylated form,[25] which, however, was reconverted to the unphosphorylated species after the recovery period of 4 hours at 37°C (Figure 5B). Analysis of the recovered cells for the content of the various heat shock proteins revealed an interesting pattern. In PC-3 cells, the heat shock increased the levels of both HSP27 and HSP70 proteins but not that of HSP90. From each of the three different sets of experiments, HSP27 was determined to be up-regulated approximately two- to threefold, and HSP70 was increased by three- to fourfold. In all three experiments, HSP90 remained unaffected by the heat treatment. Similar analysis of PC-3M cells, however, revealed an altered pattern for HSP27, whereas the effects on HSP70 and HSP90 were quite similar to those in PC-3 cells. In repeated experiments, unlike the effect observed in PC-3 cells, heat treatment did not lead to significant increase in HSP27 levels in PC-3M cells (Figure 7A). This lack of further up-regulation was not surprising, however, because the constitutive levels of both HSF1 and HSP27 were already determined to be severalfold higher in PC-3M cells relative to PC-3 cells (Figure 5).

To examine whether a relationship existed between the level of HSF1 and HSP27 level in the prostate cancer cells, we infected PC-3 cells with either a retrovirus vector expressing HSF1 or vector-alone control retrovirus. Two randomly selected stable transfectant clones for each type of infection, namely, clones PC3V3 and PC3V4 (for vector only), and clones PC3H16/3 and PC3H16/4 (for HSF1 expression), were expanded, and examined for HSF1 and HSP levels by Western blotting. The results were very similar for the independent clones in each case, and for the purpose of illustration, data obtained with one clone of vector-transfected (PC3V3) and one clone of HSF1 transfected PC-3 (PC3H16/3) cells are presented in Figure 6A. When compared to the vector

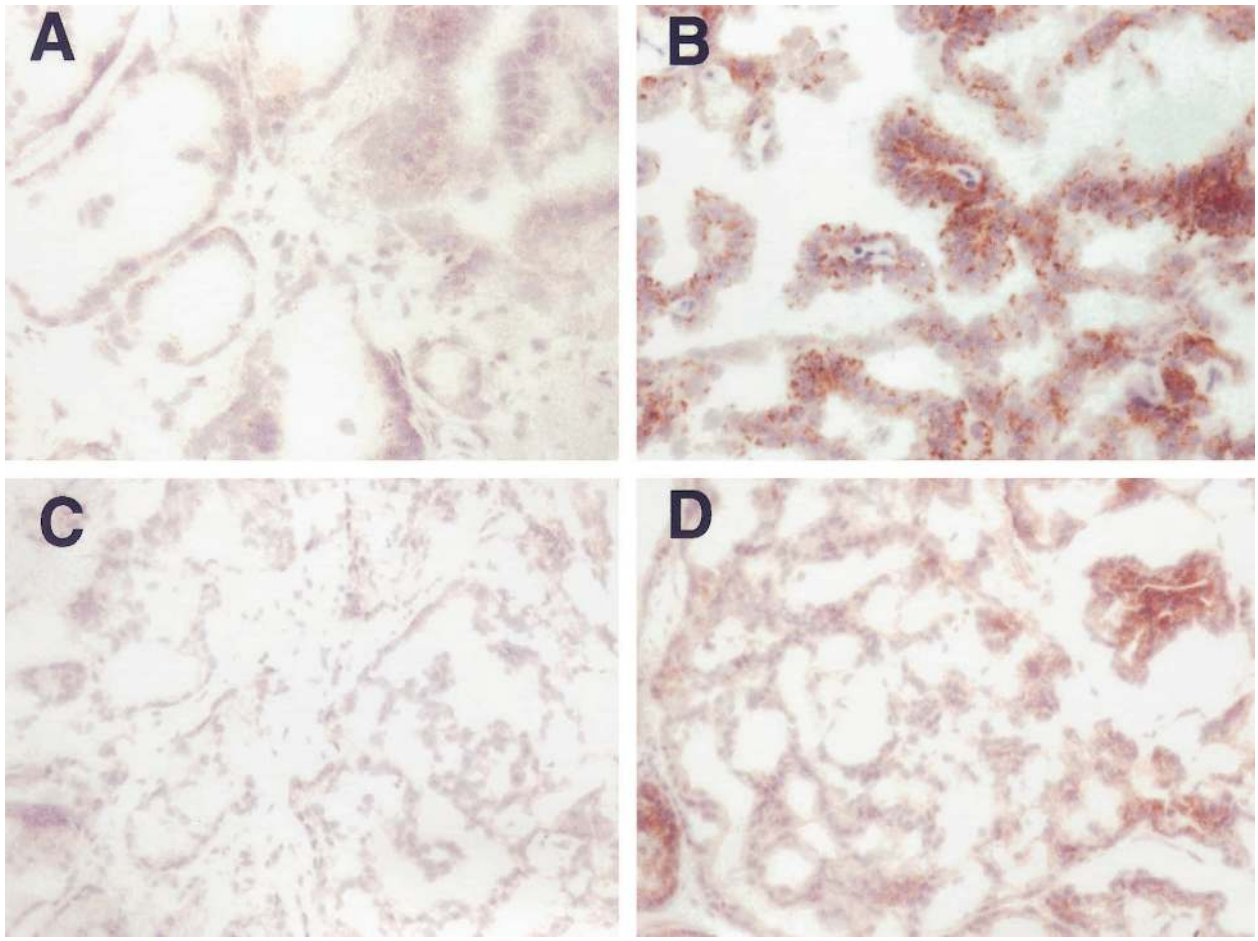

Figure 4. Illustration of HSF1 immunostaining in normal and carcinomatous prostate tissue sections. Formalin-fixed frozen sections were stained with both hematoxylin and anti-HSF1 antibodies. **A:** Normal section (S95–1068) **B:** Tumor section (S15–2484). **C:** Normal section from the specimen P735. **D:** Tumor section from the same specimen, P735.

control, overexpression of HSF1 indeed led to increased expression of HSP27 by about threefold (Figure 7B). There appeared to be some increase also in the constitutive level of HSP70, whereas the HSP90 level remained practically unchanged (Figure 6). On heat shock treatment followed by 4 hours of recovery, the cells responded differentially with respect to HSP protein expression. Although HSP90 was not affected, there was again a considerable increase in HSP70 (Figure 6). HSP27 was further induced by about threefold in vector control cells, whereas in HSF-1-overexpressing clones HSP27 could not be induced to a significant higher level by heat treatment (Figure 7B). This observation was similar to what was found with the parental PC-3 and PC-3M cells.

Discussion

Evidence was obtained that HSF1 expression is up-regulated in a metastatic prostate cancer cell line and in most prostate cancer specimens examined. To determine whether increased HSF1 expression may also involve induction of some of its downstream effectors, we chose two pairs of cell systems for the analysis of HSP27, HSP70, and HSP90 expression levels. The first system was HSF1-overexpressing metastatic PC-3M cells *versus* the nonmetastatic, PC-3 cells expressing low levels of HSF1. The second system involves deliberate overexpression of HSF1 in PC-3 cells via a retrovirus vector for comparison with the corresponding vector-alone transduced PC-3 cells. Results from both of these systems indicate that although constitutive levels of expression of HSP70 or HSP90 may not be significantly influenced by the up-regulation of HSF1, there is a consistent two- to threefold increase in HSP27 levels with overexpression of HSF1 in these prostatic carcinoma cells. It is also noteworthy that HSP27 is unique with respect to response to heat shock. In the cell systems analyzed, the amount of HSP90 expressed constitutively is high which is apparently not further enhanced by heat induced stress. HSP70, on the other hand, is further up-regulated by heat shock irrespective of whether the HSF1 expression is low or high. In contrast, the HSP27 level is increased by heat shock depending, to an extent, on the level of HSF1 expression. When the expression of HSF1 is very high, as seen in PC-3M cells, exposure to heat shock does not

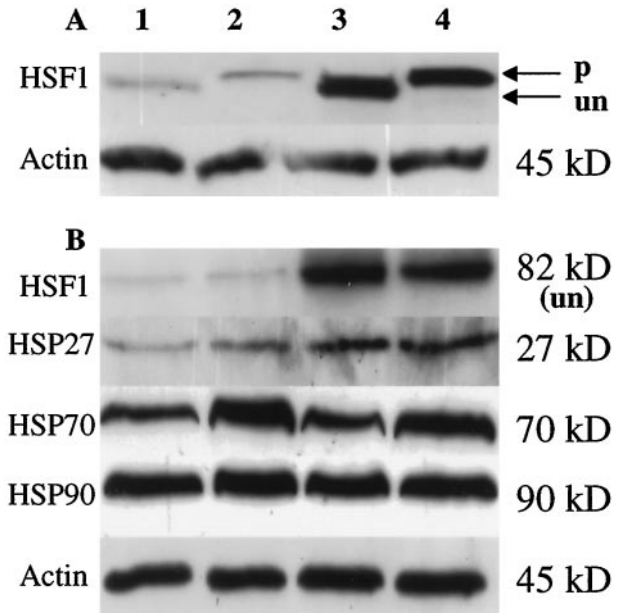

Figure 5. Western blots to determine the levels of expression of heat shock proteins in PC-3 and PC-3M cells before and after heat shock treatment. After heat shock, the cells were either analyzed immediately (**A**) or after 4 hours' recovery time (**B**). **Lane 1:** PC-3; **lane 2:** PC-3 heat-treated; **lane 3:** PC-3M; **lane 4:** PC-3M heat-treated. The blot was first examined for HSF1, and then stripped and reused sequentially for HSP27, HSP70, HSP90, and α-actin. p, phosphorylated; un, unphosphorylated species of HSF1.

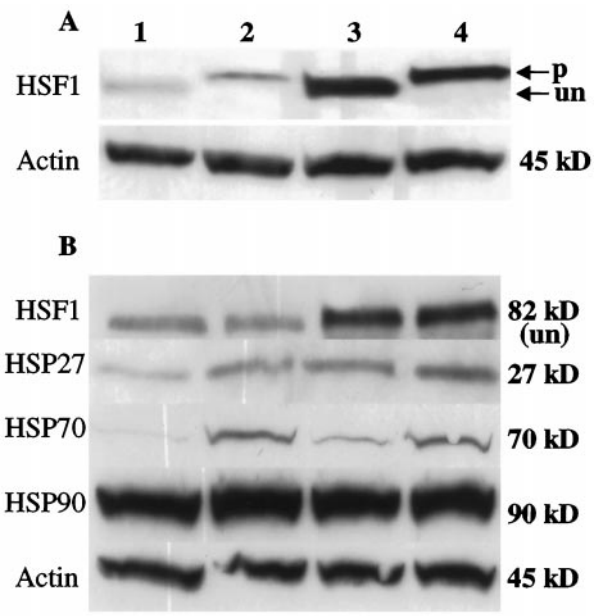

Figure 6. Effect of HSF1 overexpression in PC-3 cells with respect to heat shock protein levels. The results are illustrated from a PC-3 vector clone (PC-3V3) and a PC-3 HSF1-overexpressed clone (PC-3H16/3). Cells were heated at 45°C for 1 hour and cell lysates were harvested immediately (**A**) or after 4 hours' recovery (**B**). Antibodies for HSF1, HSP27, HSP70, HSP90, and α-actin were used as indicated. **Lane 1:** PC-3V3 control; **lane 2:** PC-3V3 heat shock; **lane 3:** PC-3H16/3 control; **lane 4:** PC-3H16/3 heat shock. p, phosphorylated form of HSF1; un, unphosphorylated form of HSF1.

seem to further enhance the already strong level of HSP27, indicating that there may be a maximal level of HSP27 protein that cannot be surpassed.

Correlation of HSF1 up-regulation with increase in the basal level of a member of the family of small heat shock proteins is an interesting issue, particularly because HSP27 could function in multiple aspects of cell growth, differentiation, and apoptosis besides its activity as a stress protein. The protective activity of HSP27 is likely to be related to its action as molecular chaperone[26] or actin capping/decapping protein,[27,28] induction of an increase in glutathione, which may be critical in reduction in the level of intracellular reactive oxygen species,[29] and, in general, as a negative regulator of apoptosis induced by various factors.[30] There is evidence that transient expression of HSP27 may be linked to decrease of cellular proliferation before cell differentiation.[30] As far as a role for HSP27 in tumor growth or progression, there appears to be opposing effects based on the tumor cell lines studied.[13,31] For example, there are reports of increased tumorigenicity or invasiveness of some colon or breast cancer cells by HSP27.[32,33] Increased basal levels of HSP27 are also noted to be associated with growth rates of certain breast carcinoma, promyelocytic leukemia or testicular cell lines.[34–36] Proliferation and tumorigenicity of melanoma and squamous cell carcinoma cell lines are also reported to be inhibited by overexpression of HSP27.[37] A recent study, however, described a positive correlation between HSP27 expression and tumorigenicity and metastasis of mammary and prostate tumor cells.[38]

In this work, besides noting an association of HSP27 with increased HSF1 expression, we have not investigated the pattern of HSP27 expression in human primary prostate cancers. However, our data clearly indicate that HSF1 expression is significantly up-regulated in the primary prostatic cancer relative to the benign epithelium. The up-regulation, at least in the case of PC-3M, was not due to *HSF*1 DNA amplification (data not shown). Because PC-3 and PC-3M cells differ significantly in invasive properties in matrigel assay,[23] we also examined *HSF*1 transduced HSF1-overexpressing PC-3 clones for the *in vitro* invasion and motility in comparison to vector-only transfected PC-3 clones using the same procedure.[23] There was, however, no significant change in these properties of PC-3 cells from HSF1 overexpression (data not shown). Whether such modulation of HSF1 would influence *in vivo* invasiveness remains to be determined. Thus, at this time, it is not clear how HSF1 may directly contribute to prostate cancer cell metastasis. Considering that subcellular localization of HSF1 is functionally critical, it is quite interesting that although HSF1 protein may be occurring both in the cytoplasm and nucleus of PC-3 cells, nuclear staining of HSF1 is relatively more pronounced in PC-3M cells. In this regard, our preliminary study (Shi, Roy-Burman, and Cote, unpublished data) of a series of prostate cancers with high risk *versus* low risk for progression (stage C *versus* B) but matched for Gleason grade suggests distinct patterns of HSF1 subcellular localization: 14/32 of stage C tumors showed strong nuclear localization *versus* 1/23 stage B. Thus, intriguing leads have been obtained with HSF1 and its interesting association with HSP27 up-regulation in the context of the prostate cancer cell lines. In this regard, a report on coexpression of the multidrug-resistant gene

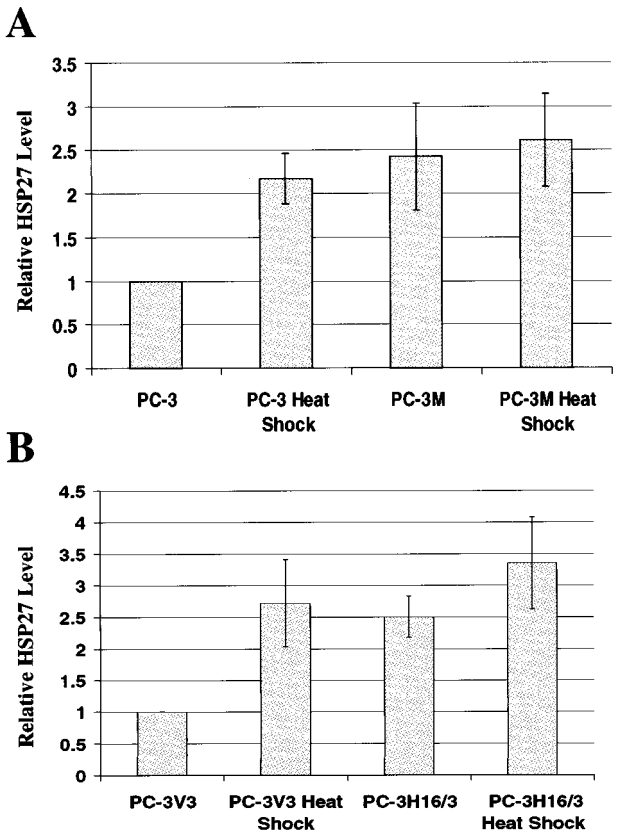

Figure 7. Quantitation of HSP27 levels by densitometry normalized with respect to α-actin loading. HSP27 level in PC-3 (**A**) or PC-3V3 (**B**) was set as 1. Error bars represent SD from three independent experiments in each case.

(*MDR*1) and HSP27 in human ovarian cancer is noteworthy.[39] Like the *HSP* genes, the *MDR*1 promoter has been shown to contain heat shock elements,[40] and it appears that inhibition of HSF DNA-binding activity may decrease the expression level of P-glycoprotein, *MDR*1 gene product.[41] At this time it is unknown whether HSF1 overexpression is linked to P-glycoprotein up-regulation in the prostate cancer cells. Hypothetically, a simultaneous increase in HSP27 and P-glycoprotein expression, will have a significant effect on resistance to anticancer drugs, an issue which remains to be carefully scrutinized. There is, however, evidence that HSP27 is involved in the development of chemoresistance to doxorubicin in human breast cancer cells.[42,43] It appears that in Chinese hamster ovary cells overexpressing HSP27 resistance to doxorubicin may not be related to P-glycoprotein up-regulation.[44] In this case, HSP27 confers increased survival independent of MDR1 mechanism.

In conclusion, a novel association between HSF1, a protein that activates cellular responses to stressful conditions in the environment, and prostate cancer has been uncovered. Of the well known downstream effectors of HSF1 function, namely the heat shock proteins (HSP70, HSP90, and HSP27), we find that HSP27 is particularly further influenced by increased levels of HSF1. How HSP27 up-regulation may contribute to prostate cancer or whether HSF1 may influence metastatic phenotype of prostate tumor cells through interaction with hitherto undefined protein factors are two interesting issues for further investigation.

Acknowledgments

We are grateful to A. Raz for the PC-3 and PC-3M cell lines, G. J. Miller and R. J. Cote for prostate tissue sections, and C. L. Sawyers for the LAPC-4 cell line. We thank Jo Gallo for preparation of this manuscript.

References

1. Rabindran SK, Giorgi J, Clos J, Wu C: Molecular cloning and expression of a human heat shock factor, HSF1. Proc Natl Acad Sci USA 1991, 88:6906–6910
2. Sarge KD, Zimarino V, Holm K, Wu C, Morimoto RI: Cloning and characterization of two mouse heat shock factors with distinct inducible and constitutive DNA-binding ability. Genes Dev 1991, 5:1902–1911
3. Schuetz TJ, Gallo GJ, Sheldon L, Tempst P, Kingston RE: Isolation of a cDNA for HSF2: evidence for two heat shock factor genes in humans. Proc Natl Acad Sci USA 1991, 88:6911–6915
4. Wu C: Heat shock transcription factors: structure and regulation. Ann Rev Cell Dev Biol 1995, 11:441–469
5. Nakai A, Tanabe M, Kawazoe Y, Inazawa J, Morimoto RI, Nagata K: HSF4, a new member of the human heat shock factor family which lacks properties of a transcriptional activator: Mol Cell Biol 1997, 17:469–481
6. Newton EM, Knauf U, Green M, Kingston RE: The regulatory domain of human heat shock factor 1 is sufficient to sense heat stress. Mol Cell Biol 1996, 16:839–846
7. Wisniewski J, Orosz A, Allada R, Wu C: The C-terminal region of Drosophila heat shock factor contains a constitutively functional domain. Nucleic Acids Res 1996, 24:367–374
8. Rabindran SK, Giorgi G, Clos J, Wisniewski J, Wu C: Regulation of heat shock factor trimerization: role of a conserved leucine zipper. Science 1993, 259:230–234
9. Coto JJ, Kline M, Morimoto R: Activation of heat shock factor 1 DNA binding precedes stress induced serine phosphorylation: evidence for a multi-step pathway of regulation. J Biol Chem 1996, 271:3355–3358
10. Morimoto RI, Santoro MG: Stress-inducible responses and heat shock proteins: new pharmacologic targets for cytoprotection. Nat Biotechnol 1998, 16:833–838
11. Protti MP, Heltai S, Bellone M, Ferranini M, Manfredi AA, Rugarli C: Constitutive expression of the heat shock protein 72 kd in human melanoma cells. Cancer Lett 1994, 85:211–216
12. Ciocca DR, Clark GM, Tandon AK, Fuqua SA, Welch WJ, McGuire WL: Heat shock protein hsp70 in patients with auxillary lymph node-negative breast cancer: prognostic implications. J Natl Cancer Inst 1993, 85:570–574
13. Tetu B, Lacasse B, Bouchard HL, Lagace R, Huot J, Landry J: Prognostic influence of hsp27 expression in malignant fibrous histiocytoma: a clinicopathological and immunohistochemical study. Cancer Res 1992, 52:2325–2328
14. Hubank M, Schatz G: Identifying differences in mRNA expression by representational difference analysis of cDNA. Nucleic Acids Res 1994, 22:5640–5648
15. Kaighn ME, Shankar-Narayan K, Ohnuki Y, Lechner JF, Jones LW: Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest Urol 1979, 17:16–23
16. Silleti S, Yao P, Pienta KJ, Raz A: Loss of cell-contact regulation and altered responses to autocrine motility factor correlate with increased malignancy in prostate cancer cells. Int J Cancer 1995, 63:100–105
17. Zheng J, Rudra-Ganguly N, Miller GJ, Moffatt KA, Cote RJ, Roy-Burman P: Steroid hormone induction and expression patterns in normal and carcinomatous prostate tissues. Am J Pathol 1997, 150: 2009–2018

18. Lee M-S, Garkovenko E, Yun JS, Weijerman PC, Peehl DM, Chen L-S, Rhim JS: Characterization of adult human prostatic cells immortalized by polybrene-induced DNA transfection with a plasmid containing an origin-defective SV40 genome. Int J Oncol 1994, 4:821–830
19. Hoang AT, Cohen KJ, Barrett JF, Bergstrom DB, Dang CV: Participation of cyclin A in c-myc induced apoptosis. Proc Natl Acad Sci USA 1994, 91:6875–6879
20. Klein KA, Reiter RE, Redula J, Morad H, Zhu XL, Brothman AR, Lamb DJ, Marcelli M, Belldegrun A, Witte ON, Sawyers CL: Progression of metastatic human prostate cancer to androgen independence in immunodeficient SCID mice. Nat Med 1997, 3:402–408
21. Laemmli K: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970, 227:680–685
22. Rudra-Ganguly N, Zheng J, Hoang AT, Roy-Burman P: Downregulation of human FGF8 activity by antisense constructs in murine fibroblastic and human prostatic carcinoma cell systems. Oncogene 1998, 16:1487–1492
23. Zheng J, Rudra-Ganguly N, Powell WC, Roy-Burman P: Suppression of prostate carcinoma cell invasion by expression of antisense L-plastin gene. Am J Pathol 1999, 155:115–122
24. Salem CA, Tomasic NA, Elmajian DA, Esrig D, Nichols PW, Taylor CR, Skinner DG, Roy-Burman P, Lieskovsky G, Cote RJ: p53 Protein and gene alterations in pathological stage C prostate carcinoma. J Urol 1997, 158:510–514
25. McMillan DR, Xiao X, Shao L, Graves K, Benjamin IJ: Targeted disruption of heat shock transcription factor 1 abolishes thermotolerance and protection against heat-inducible apoptosis. J Biol Chem 1998, 273:7523–7528
26. Jakob U, Gaestel M, Engel K, Buchner J: Small heat shock proteins are molecular chaperones. J Biol Chem 1993, 268:1517–1520
27. Miron T, Vancompernolle K, Vandekerckhove J, Wilchek M, Geiger B: A 25-kD inhibitor of actin polymerization is a low molecular mass heat shock protein. J Cell Biol 1991, 114:255–261
28. Lavoie JN, Gingras-Breton G, Tanguay RM, Landry J: Induction of Chinese hamster HSP27 gene expression in mouse cells confers resistance to heat shock: HSP27 stabilization of the microfilament organization. J Biol Chem 1993, 28:3420–3429
29. Mehlen P, Kretz-Remy C, Preville X, Arrigo AP: Human hsp27, Drosophila hsp27 and human αB-crystallin expression-mediated increase in glutathione is essential for the protective activity of these proteins against TNFα-induced cell death. EMBO J 1996, 15:2695–2706
30. Mehlen P, Mehlen A, Godet J, Arrigo AP: Hsp27 as a switch between differentiation, and apoptosis in murine embryonic stem cells. J Biol Chem 1997, 272:31657–31665
31. Thor A, Benz C, Moore D, Goldman E, Edgerton S, Landry J, Schwartz L, Mayall B, Hickey E, Weber LA: Stress response protein (srp-27) determination in primary human breast carcinomas: clinical, histologic, and prognostic correlations. J Natl Cancer Inst 1991, 83:170–178
32. Garrido C, Fromentin A, Bonnotte B, Favre N, Moutel M, Arrigo P, Mehlen P, Solary E: Heat shock protein 27 enhances the tumorigenicity of immunogenic rat colon carcinoma cell clones. Cancer Res 1998, 58:5495–5499
33. Lemieux P, Oesterreich S, Lawrence JA, Steeg PS, Hilsenbeck SG, Harvey JM, Fuqua SA: The small heat shock protein hsp27 increases invasiveness but decreases motility of breast cancer cells. Invasion Metastasis 1997, 17:113–123
34. Kindas-Mugge I, Micksche M, Trautinger F: Modification of growth in small heat shock (hsp27) gene transfected breast carcinoma. Anticancer Res 1998, 18:413–417
35. Spector NL, Ryan C, Samson W, Levine H, Nadler LM, Arrigo AP: Heat shock protein is a unique marker of growth arrest during macrophage differentiation of HL-60 cells. J Cell Physiol 1993, 156:619–625
36. Richards EH, Hickey E, Weber L, Master JR: Effect of overexpression of the small heat shock protein HSP27 on the heat and drug sensitivities of human testis tumor cells. Cancer Res 1996, 56:2446–2451
37. Kindas-Mugge I, Herbacek I, Jantschitsch C, Micksche M, Trautinger F: Modification of growth and tumorigenicity in epidermal cell lines by DNA-mediated gene transfer of M(r) 27,000 heat shock protein (hsp27). Cell Growth Differ 1996, 7:1167–1174
38. Morino M, Tsuzuki T, Ishikawa Y, Shirakami T, Yoshimura M, Kiyosuke Y, Matsunaga K, Yoshikumi C, Saijo N: Specific expression of HSP27 in human tumor cell lines in vitro. In Vivo 1997, 11:179–184
39. Schneider J, Jimenez E, Marenbach K, Marx D, Meden H: Co-expression of the MDR1 gene and HSP27 in human ovarian cancer. Anticancer Res 1998, 18:2967–2971
40. Kioka N, Yamano Y, Komano T, Ueda K: Heat-shock responsive elements in the induction of the multidrug resistance gene (MDR1). FEBS Lett 1992, 301:37–40
41. Kim SH, Yeo GS, Lim YS, Kang CD, Kim CM, Chung BS: Suppression of multidrug resistance via inhibition of heat shock factor by quercetin in MDR cells. Exp Mol Med 1998, 30:87–92
42. Ciocca DR, Fuqua SAW, Lock-Lim S, Toft DO, Welch WJ, McGuire WL: Response of human breast cancer cells to heat shock and chemotherapeutic drugs. Cancer Res 1992, 52:3648–3654
43. Oesterreich S, Weng C-N, Qiu M, Hilsenbeck SG, Osborne CK, Fuqua SAW: The small heat shock protein is correlated with growth and drug resistance in human breast cancer cells. Cancer Res 1993, 53:4443–4448
44. Huot J, Roy G, Lambert H, Chre-tien P, Landry J: Increased survival after treatments with anticancer agents of Chinese hamster cells expressing the human Mr 27,000 heat shock protein. Cancer Res 1991, 51:5245–5252