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Different concentrations of two small stress proteins, αB crystallin and HSP27 in human urological tumor tissues

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Abstract Concentrations of two small stress proteins, αB crystallin and the 27-kDa heat shock protein (HSP27) were quantitated in tissues of the human normal genitourinary system and their tumors. Levels of HSP27 in renal cell carcinomas (mean ± SE: 1450 ± 262 ng/mg protein, $n = 15$) were significantly higher than in normal kidney (the cortex: 540 ± 99 ng/mg protein, $n = 13$; the medulla: 600 ± 106 ng/mg protein, $n = 13$) while those of αB crystallin tended to be increased without statistical significance. These findings were similar to those previously reported for renal cell tumors chemically induced in rats. Concentrations of αB crystallin in prostatic carcinoma tissues (410 ± 129 ng/mg protein, $n = 10$) were also significantly higher than in benign prostatic hyperplasia (54 ± 12 ng/mg protein, $n = 14$), whereas αB crystallin levels in testicular tumors including seminomas (2.1 ± 0.8 ng/mg protein, $n = 11$) and non-seminomas (5.2 ± 2.3 ng/mg protein, $n = 9$) were significantly lower than in normal testicular tissues (29.7 ± 6.2 ng/mg protein, $n = 5$). Both αB crystallin and HSP27 could be immunohistochemically localized in the normal kidney and renal cell carcinoma tissues.

Key words Heat shock protein · αB Crystallin · HSP27 · Renal cell carcinomas · Testicular tumors

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Introduction

Heat shock proteins (HSP), a group of molecules induced in mammalian cells by heat shock and other stresses, are involved in the acquisition of thermotolerance and in protein folding and degradation [1, 8, 24]. Although low-molecular-weight (molecular masses of 20–30 kDa) and high-molecular-weight (60, 70, 90 or 100 kDa) HSPs have been identified, it remains unclear whether they play different biological roles in cells. Members of the small HSP family include αB crystallin, HSP25, HSP27, and HSP28 [21].

αB Crystallin, a major constituent of vertebrate lens, is a 23-kDa protein which is generally expressed as a polymeric form with a molecular mass of 500–800 kDa [4, 18]. Recent studies have revealed that it is present in various tissues other than the lens, including skeletal muscle, brain, and kidney [7, 15, 17]. Its amino acid sequence features elements in common with those of several small HSPs [10, 14, 23] and it is in fact produced in response to heat shock [13, 20, 23].

Other small HSPs with 27–28 kDa molecular masses are also detected in various normal tissues, including the kidney and urinary bladder [18]. Despite the differences in molecular mass (27 kDa in the rat and 28 kDa in man), the abbreviation used here is HSP27 for this mammalian small stress protein. Several lines of evidence suggest that αB crystallin and HSP27 may be associated in cells [18, 19]. However, while it is known that small HSPs effectively prevent heat-induced aggregation of other proteins, acting as molecular chaperones [11, 16], their precise biological significance, alone or in combination, remains unclear.

Recently we determined concentrations of αB crystallin and HSP27 in normal rat kidney and renal cell tumors and demonstrated significant elevation of HSP27 in the latter [34]. Regarding human genitourinary tissues and their neoplasms only a few immunohistochemical studies of small HSPs have been performed [16, 22, 28], and no quantitative investigations of αB crystallin and

HSP27 have been reported. To clarify the tissue distributions of these two HSPs, a quantitative determination of their concentrations in the normal genitourinary tissues and their tumors was therefore performed in addition to immunohistochemical localization in normal and neoplastic kidney tissues.

Materials and methods

Tissue samples

Neoplastic ($n = 80$) and nonneoplastic tissues ($n = 51$) were obtained at surgery. For immunoassay they were promptly frozen and kept at −80°C until analysis, when they were homogenized at 0°C with 10 volumes (V/W) of 50 mM TRIS-HCl (pH 7.4) containing 5 mM $MgSO_4$. Homogenates were centrifuged at 4°C at 20 000 *g* for 20 min, and the soluble fractions were analyzed. For histological examination and immunohistochemistry, tissues of nomal kidney ($n = 5$) and renal cell carcinomas ($n = 15$) were fixed in periodate-lysine-4% paraformaldehyde for 6 h, washed in phosphate-buffered saline (PBS, pH 7.2) containing increasing concentrations of sucrose, and embedded in OCT compound (Tissue-Tek, Naperville, I11.).

Antigens and antibodies

Bovine α crystallin was purified from fresh lenses obtained at a local slaughterhouse as described by Spector et al. [30] and then αB crystallin was isolated by chromatofocusing column chromatography in the presence of 6 M urea as reported by Bloemendal and Groenewoud [2]. Human pectoral muscles were obtained at surgical resection of breast cancer and HSP27 was purified by the procedures that Kato et al. [18] first developed.

Antibodies to αB crystallin and HSP27 were raised in Japanese rabbits by injecting the respective antigens, purified from bovine lenses and human pectoral muscles, respectively, with Freund's complete adjuvant, as described elsewhere [17, 18]. Antibodies monospecific for the two antigens were purified by immunoaffinity column chromatography using antigen-coupled Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). The specificities of the purified antibodies to αB crystallin and HSP27 thus obtained were confirmed previously [17, 18]. As secondary antibodies for immunohistochemistry, horseradish peroxidase (HRP)-labeled rabbit IgG Fab′ fragments against rabbit IgG were prepared [32].

Immunoassay methods

Concentrations of αB crystallin and HSP27 in the soluble fractions of tissues were determined by the sandwich-type enzyme immunoassay system developed by Kato et al. [17, 18]. In brief, extracts were incubated with polystyrene balls bearing immobilized monospecific rabbit antibodies to the respective antigens, and then the balls were incubated with the same antibodies labeled with β-D-galactosidase from *Escherichia coli*. The bound galactosidase activity was assayed with 4-methylumbelliferyl-β-D-galactoside as a substrate. Purified human αB crystallin and HSP27 were used as standards and the results expressed as antigen amounts equivalent to nanograms per milligram of soluble protein. The assay systems were all highly sensitive, the limit of detection for each antigen being 3 pg per test tube.

Immunohistochemistry

The indirect HRP-labeled antibody method was employed for the immunostaining as described previously [32, 33]. In brief, 5-μm-thick cryostat sections were placed on albumin-coated slides and dried at room temperature. They were treated with 100% methanol and 0.3% hydrogen peroxide solution for 30 min to inactivate endogenous peroxidase, washed in PBS, and then incubated with purified anti-αB crystallin IgG or anti-HSP27 IgG (4 μg/ml) for 12 h at 4°C. For control sections, antibodies absorbed with the purified respective antigen were substituted for the primary antibodies. After being washed in PBS, all sections were incubated with HRP-labeled secondary antibodies for 60 min at room temperature. After further washing in PBS, they were reacted with 0.025% 3,3′-diaminobenzidine solution containing 10 mM hydrogen peroxide, and counterstained with methyl green.

Other methods

Protein concentrations of the tissue extracts were determined with the aid of a Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Richmond, Calif.), utilizing the principle of protein-dye binding [3]. Quantitative data were expressed as mean ± standard error (SE) values and the results compared using the Wilcoxon's rank-sum test.

Results

Concentrations of αB crystallin and HSP27 in normal genitourinary tissues and their tumors

Table 1 summarizes data for concentrations of αB crystallin and HSP27 in normal genitourinary tissues and neoplastic tissues. Levels of HSP27 in renal cell carcinomas were significantly higher than in the cortex and medulla of the normal kidney ($P = 0.01$ and $P = 0.02$, respectively). Those of αB crystallin only showed a tendency for increase. Concentrations of αB crystallin in prostatic carcinoma tissues were significantly higher than in benign prostatic hyperplasia tissues ($P = 0.007$), whereas those in testicular tumors including seminomas and nonseminomas were significantly lower than in normal testicular tissues ($P = 0.0005$ and $P = 0.002$, respectively). Of note were the low concentrations of αB crystallin in testicular tumor tissues as compared with other normal or neoplastic tissues. Concentrations of HSP27 in tissues of superficial and invasive bladder cancers appeared higher than in normal bladder tissues although the differences did not reach statistical significance.

Immunohistochemical localization of αB crystallin and HSP27 in normal kidney and renal cell carcinoma tissues

Figure 1 illustrates immunohistochemical localization of αB crystallin and HSP27 in normal kidney tissues. In the cortex αB crystallin staining was weakly positive in some of the epithelial cells of the proximal tubules and thin limbs of loops of Henle but negative in the distal tubules and glomerular components (Fig. 1A, B). HSP27 was immunohistochemically localized in epithelial cells of proximal and distal tubules as well as in epithelial cells of the Bowman's capsule in the cortex (Fig. 1C), and in

Table 1 Concentrations of αB crystallin and HSP27 in genitourinary tissues and neoplastic lesions

Tissues	No. of samples	Tissue concentration (ng/mg protein)	
		αB crystallin	HSP27
Normal kidney			
Cortex	13	1160 ± 204 (296–2800)	540 ± 99 (140–1400)
Medulla	13	962 ± 176 (235–2250)	600 ± 106 (120–1400)
Renal cell carcinoma	15	2310 ± 603 (293–7160)	1450 ± 262 (113–3340)[a]
Normal testis	5	29.7 ± 6.2 (12.1–50.2)	171 ± 19 (125–227)
Testicular carcinoma			
Seminoma	11	2.1 ± 0.8 (0.08–6.9)[b]	473 ± 143 (42–148)
Nonseminoma	9	5.2 ± 2.3 (0.06–21.6)[c]	196 ± 38 (34–360)
Normal bladder	10	84.3 ± 27.7 (17.9–284)	436 ± 107 (56.7–1060)
Bladder carcinoma			
Superficial	11	80.9 ± 35.4 (5.2–357)	933 ± 273 (43.1–2780)
Invasive	10	123 ± 41 (17.8–431)	987 ± 372 (90.2–3910)
Normal prostate	10	111 ± 34 (10.6–384)	986 ± 200 (488–2230)
BPH	14	54.4 ± 12.3 (11.2–168)	960 ± 260 (93.5–3470)
Prostatic carcinoma	10	410 ± 129 (18.9–1260)[d]	862 ± 141 (224–1460)

Data are mean ± SE values with range limits. *BPH* benign prostatic hypertrophy
[a] Significantly higher than the values for the renal cortex ($P = 0.011$) and medulla ($P = 0.020$)
[b] Significantly lower than the value for the normal testis ($P = 0.0005$)
[c] Significantly lower than the value for the normal testis ($P = 0.002$)
[d] Significantly higher than the value for BPH ($P = 0.0071$)

epithelial cells of the loops of Henle and the collecting ducts in the medulla (Fig. 1D).

Of 15 renal cell carcinomas studied, all (100%) were positively stained for both αB crystallin and HSP27. Fig. 2 illustrates the immunohistochemical localization of both small stress proteins in a typical case of renal cell carcinoma. The cytoplasm was consistently positive but also occasionally the nuclei were stained. The intensity varied from case to case, and from site to site within individual tumors. The staining intensity and number of positive tumor cells were not apparently related with cell type, histological grade, or stage of renal cell carcinomas. Control sections treated with antibodies preabsorbed with the respective antigens were uniformly negative.

Discussion

In the present study we first demonstrated concentrations of αB crystallin and HSP27 in human normal genitourinary tissues and their tumors. Generally increase was associated with neoplasia except in the testis case. The findings for renal cell carcinomas were similar to those we previously reported for chemically induced rat lesions [34]. Various adverse conditions (such as oxidative injury resulting from ischemia) that prevail in neoplastic tissues may bring about induction of small HSPs [6].

Lowe et al. [26] earlier reported αB crystallin to be immunohistochemically positive in proximal tubules and thin limbs of loops of Henle of the human normal kidney. Our results are generally in line with their findings although the staining intensity in epithelial cell proximal tubules was weak and the number of positive cells was much lower in the present material, presumably due to variation in the tissue preparation and antibodies to αB crystallin used. Localization of αB crystallin in the loops of Henle in the medulla might be associated with the hypertonic environment, which is a stress factor for cells in the medulla [5, 9]. The present immunohistochemical and consistent quantitative findings and those of Pinder et al. [28] indicate that renal cell carcinoma tissues have significant amounts of αB crystallin.

The immunohistochemical localization of HSP27 in the present study was not in line with the report of Khan et al. [22] and clearly different from that of αB crystallin in normal renal tissues. The authors localized the HSP27 in proximal and distal tubules and collecting ducts, but not in loops of Henle [22]. At present it remains unknown whether the different localization of the small HSPs is due to their different biological function in cells.

Recently it has become clear that small HSPs are involved in some forms of chemoresistance and may participate in the loss of sensitivity to anticancer drugs [12]. For example, Oesterreich et al. [27] reported HSP27 to be correlated with growth and drug resistance in human breast cancer cell lines. A recent study demonstrated that human testicular cells overexpressing HSP27 are resistant to heat shock, cisplatin, and doxorubicin, suggesting that low constitutive levels may contribute to sensitivity to chemotherapy [29]. The present study demonstrated that renal cell and prostatic carcinomas may contain high concentrations of αB crystallin and HSP27, in contrast to testicular tumors. These findings might imply resistance of renal cell and prostatic carcinomas to chemotherapeutic agents and relative sensitivity of testicular tumors [38]. Although the relationship between small HSPs and the multidrug resistance (*mdr1*) gene remains unclear, this question clearly warrants attention.

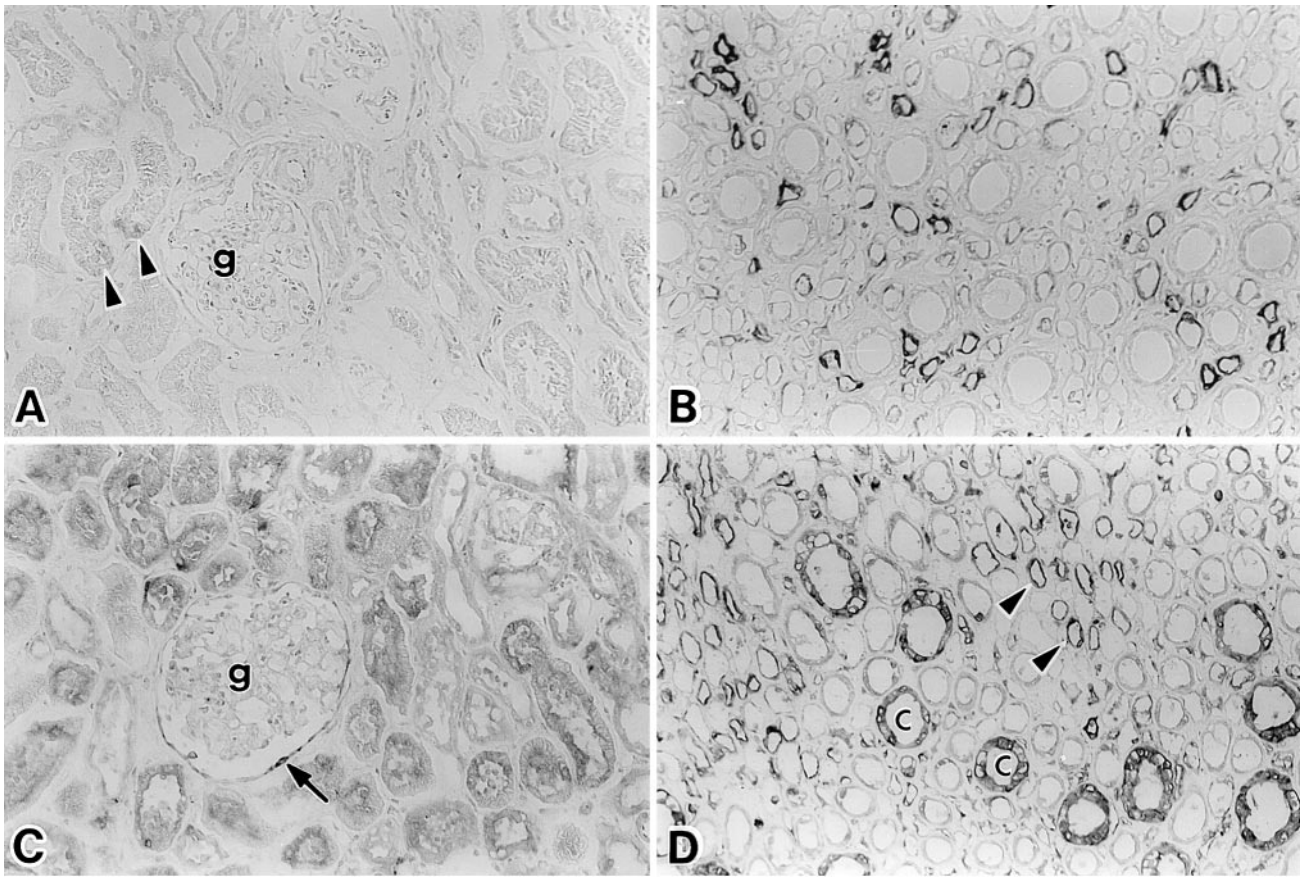
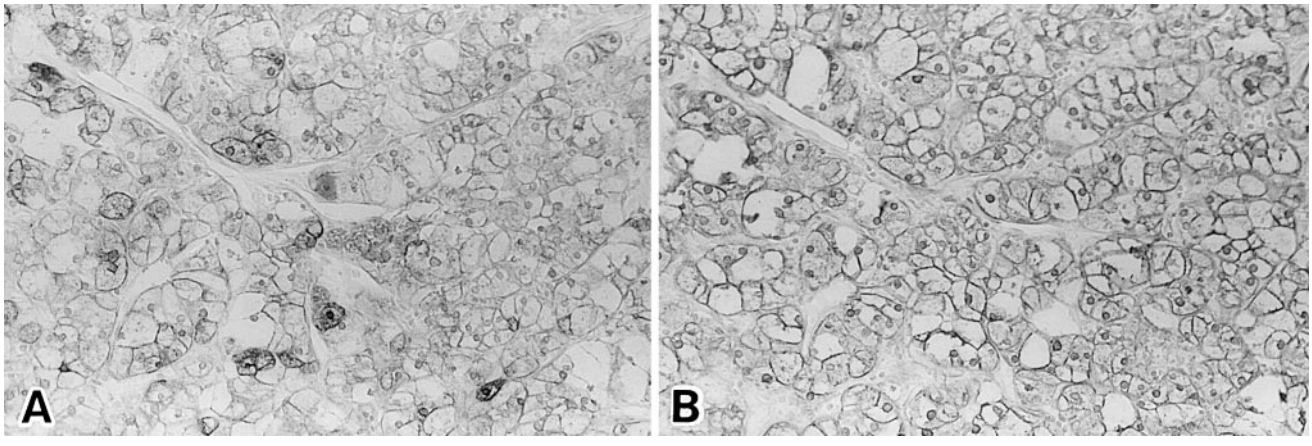


Fig. 1 Immunohistochemical localization of αB crystallin (**A** and **B**) and HSP27 (**C** and **D**) in the renal cortex and medulla. αB Crystallin is weakly positive in some epithelial cells of proximal tubules (*arrowheads*) in the cortex (**A**) and positive in epithelial cells of thin limbs of the loops of Henle in the medulla (**B**). In contrast, HSP27 in the cortex is positively stained in epithelial cells of the proximal and distal tubules and in those of the Bowman's capsule (*arrow*) (**C**) and in the medulla the epithelial cells of collecting ducts (*c*) and loops of Henle (*arrowheads*) are stained (**D**). *g* glomerulus. Indirect immunoperoxidase method, ×130

Several studies have revealed that expression of HSP27 is associated with aggressive behavior and patient survival in several types of malignancy, including breast cancer and the malignant fibrous histiocytoma [25, 35, 37]. However, no diagnostic or prognostic significance has been found in cases of prostate or bladder cancers [31]. The present study revealed no apparent relationship between concentrations of HSP27 and his-

Fig. 2 Immunohistochemical localization of αB crystallin (**A**) and HSP27 (**B**) in a renal cell carcinoma. αB crystallin is positive in the cytoplasm, and occasionally in the nucleus, of some of the tumor cells (**A**). In contrast, HSP27 is immunostained diffusely in the cytoplasm of most of the tumor cells (**B**). Indirect immunoperoxidase method, ×150



tological grade, cell type, or stage of renal cell carcinoma or stage of bladder cancer. Regarding the potential link with malignancy, Thomas et al. [36] reported immunohistochemical localization of HSP27 in prostate neoplastic epithelial cells, with varied staining intensities of individual cases, the staining intensity being reduced along with increasing Gleason score and invasiveness. Since only a small number of cases of prostate cancers were examined in the present study, further studies of αB crystallin and HSP27 are required to confirm our results.

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