Different concentrations of two small stress proteins, $\alpha$B crystallin and HSP27 in human urological tumor tissues

Abstract
Concentrations of two small stress proteins, $\alpha$B 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 $\alpha$B 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 $\alpha$B 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 $\alpha$B 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 $\alpha$B crystallin and HSP27 could be immunohistochemically localized in the normal kidney and renal cell carcinoma tissues.

Key words  Heat shock protein · $\alpha$B Crystallin · HSP27 · Renal cell carcinomas · Testicular tumors

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 $\alpha$B crystallin, HSP25, HSP27, and HSP28 [21].

$\alpha$B 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 $\alpha$B 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 $\alpha$B 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 $\alpha$B 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 MgSO4. 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 normal 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, IL).

**Antigens and antibodies**

Bovine αB 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].

**Immunooassay 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-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. In the medulla of the normal kidney, αB crystallin was localized in the epithelial cells of Bowman’s capsule.