Effect of Heat Shock Protein 90 (Hsp90) on Migration and Invasion of Human Cancer Cells in Vitro


We studied the effect of purified native heat shock protein 90 (Hsp90) from bovine and mouse brain on migration and invasion of human glioblastoma (A-172) and fibrosarcoma (HT1080) cells. Hsp90 in concentrations of 0.01-0.10 mg/ml stimulated migration and invasion of tumor cells in vitro by 20-32% (p<0.05). Polyclonal antibodies to Hsp90 blocked the Hsp90-dependent stimulation of cell invasion, which indicates specificity of the stimulating effect of extracellular Hsp90 on tumor cell invasion. Hence, extracellular Hsp90 can be considered as a promising molecular target, because its inhibition can suppress invasion and metastasizing of tumor cells.

Key Words: heat shock protein 90 (Hsp90); cell migration; cell invasion

MATERIALS AND METHODS

We used A-172 (human glioblastoma), HT1080 (human fibrosarcoma), and NIH3T3 (mouse fibroblasts) cells from the culture collection of Institute of Cytology, Russian Academy of Sciences. The cells were grown in DMEM containing 10% FBS and antibiotics penicillin, streptomycin, gentamycin (40 U each) (DMEM-10% FBS).

Purification of Hsp90 and Hsp70 from bovine and mouse brain was performed according to the previously developed method [11]. For generation of polyclonal Hsp90-specific antibodies (a-Hsp90 antibodies), the rabbits were immunized 4 times with Hsp90 purified from bovine brain. Immunopurification of a-Hsp90 antibodies was performed on Hsp90-Sepharose prepared according to the previously described protocol [8]. The titer and specificity of the purified a-Hsp90 antibodies were assessed by indirect ELISA. Cytotoxicity and anti-proliferative activity of Hsp90 and a-Hsp90-antibody preparations were evaluated using A-172, HT1080, and NIH3T3 cells by MTT colorimetry [1].

Cell migration was determined using inserts with polyethyleneterephthalate (PET) membrane (pore size 8 μ; CellSert, Millipore) according to standard protocols. Before the experiment, the cells were maintained...
in DMEM medium containing 0.2% BSA (DMEM-BSA) for 20 h at 37°C (starvation). Cells in DMEM-BSA medium were placed into the inserts, DMEM-5% FBS in the lower reservoir was used as the chemoattractant. The cells that had crossed the PET membrane, were fixed, stained with crystal violet, lysed, and transferred to wells of a 96-well plate. Absorbance was measured at 595 nm and the number of cells passing through the membrane was calculated [9,12]. The effect of Hsp90 preparations on cell migration was assessed by comparing the number of cells migrating through the PET membrane in control wells and in wells containing Hsp90 in concentrations of 0.01 and 0.1 mg/ml. DMEM-BSA medium without Hsp90 and the medium containing control proteins (BSA and purified bovine Hsp70; 0.1 mg/ml) were used as control.

For evaluation of cell invasion, the inserts with PET membrane were treated with basement membrane extract (Trevigen), an analog of extracellular matrix, according to manufacturer’s recommendations. The influence of Hsp90 and control proteins on cell invasion was evaluated in the same way as in the case of cell migration, but incubation of cells in the inserts was carried out for 24 h at 37°C. For assessing the influence of a-Hsp90 antibodies on Hsp90-dependent stimulation of cell invasion, A-172 cells were placed into an insert in DMEM-BSA medium containing Hsp90 (0.1 mg/ml) and a-Hsp90 or control antibodies (0.2 mg/ml).

Each experiment on cell migration and invasion was repeated at least 5 times. Each point represents the arithmetic mean±standard deviation for 3-5 repeats. Statistical processing of the data was performed using nonparametric Mann–Whitney U test (p<0.05).

RESULTS

Purity of the Hsp90 preparations was 95-97%. Purified Hsp90 from mouse and bovine brain in concentrations up to 1.0 mg/ml exhibited no direct cytotoxicity and had no effect on proliferation of A-172 and HT1080 cells (Table 1).

The effect of Hsp90 on migration of A-172 and HT1080 cells was evaluated by penetration through the barrier (PET membrane with pore size 8 μ) stimulated by the chemotactic gradient. In the absence of the chemoattractant (5% FBS) in the lower tank, the tumor cells were almost not migrating (spontaneous migration). In the presence of FBS in the lower reservoir, the cells actively migrated through the membrane. Hsp90 in concentrations of 0.01-0.10 mg/ml stimulated cell migration, this effect was close to the maximum at concentrations of 0.01-0.02 mg/ml (data not shown). Hsp90 from bovine and mouse brain in a concentration of 0.1 mg/ml stimulated migration of A-172 cells by 21±2 and 25±2%, respectively, and

![Fig. 1. Effect of Hsp90 on migration of A-172 (light bars) and HT1080 cells (dark bars). 1) Spontaneous migration (without chemoattractant), 2) control (without Hsp90), 3) bovine Hsp90 (0.01 mg/ml), 4) bovine Hsp90 (0.1 mg/ml), 5) mouse Hsp90 (0.01 mg/ml), 6) mouse Hsp90 (0.1 mg/ml), 7) BSA, 8) Hsp70. *p<0.05 in comparison with the control.](image1)

![Fig. 2. Effect of Hsp90 on invasion of A-172 (light bars) and HT1080 cells (dark bars). 1) Spontaneous invasion of cells (without chemoattractant), 2) control (without Hsp90), 3) bovine Hsp90 (0.1 mg/ml), 4) mouse Hsp90 (0.1 mg/ml), 5) BSA, 6) Hsp70. *p<0.05 in comparison with the control.](image2)

![Fig. 3. Effect of Hsp90-specific polyclonal antibodies on invasion of A-172 cells. 1) Control (without Hsp90), 2) bovine Hsp90 (0.1 mg/ml), 3) bovine Hsp90 (0.1 mg/ml)+antibodies to Hsp90, 4) bovine Hsp90 (0.1 mg/ml)+antibodies from nonimmune rabbit serum.](image3)