ONCOLOGY

Comparative Analysis of Secretion of S100A4 Metastatic Marker by Immune and Tumor Cells

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S100A4 protein is present in low concentrations (2.1-15.7 ng/10^6 cells) in lymphocyte and neutrophil culture medium. Addition of stimulants to the cells did not lead to an appreciable increase in the content of this protein. The initial content of S100A4 is significantly higher (92-447 ng/10^6 cells) in culture media of highly metastatic KSML-100 adenocarcinoma and M3 and B16 melanoma cells. The release of S100A4 by these cells significantly increased after addition of lymphocytes and Tag7/Hsp70 cytotoxic complex. Repeated injection of antibodies to S100A4 to mice with transplanted M3 melanoma inhibited tumor growth.

Key Words: *S100A4 protein; lymphocyte-activated killer cells; natural killer cells*

Protein S100A4 (metastasin) is a representative of a family of small (101 amino acids) calcium-binding S100 proteins. All proteins of this family are characterized by high similarity, most pronounced in the EF fragment region [8]. They are involved in the regulation of cell growth, modulation of cell migration and morphology [3,7]. It was found that S100A4 (metastasin) is involved in the development of resistance to the cytolytic effect of dexamethasone [1]. The S100 family proteins sometimes act as biochemical markers of mucoviscidosis and acute rheumatoid polyarthritis and serve as a poor prognostic sign in rectal [9] and urinary bladder cancer [4]. The s100a4 gene is specifically expressed in metastatic tumor cells, in lymphoid tissues, bone marrow, and blood cells (T-lymphocytes, macrophages, and neutrophils) [8]. Destruction of cells in the blood obtained from healthy donors leads to an increase in the content of S100A4 protein in serum samples, which makes doubtful the possibility of cancer prediction by serum S100A4 level [6].

For evaluation of the contribution of normal and tumor cells to the maintenance of high serum level of S100A4 we compared S100A4 production by blood lymphocytes and neutrophils, M3 and B16 melanoma cells, and KSML-100 adenocarcinoma cells.

MATERIALS AND METHODS

The content of S100A4 in the samples was measured by competitive EIA [10]. Recombinant S100A4 in a concentration of 200 ng/ml was adsorbed in wells of a 96-well plate [5]. The reaction was carried out by adding 50 μl polyclonal rabbit antibodies to S100A4 (1:1000, Neo Markers) to 50 μl analyzed fluid. Recombinant S100A4 in concentrations of 1-1000 ng/ml was used for plotting the calibration curve. Lymphocytes were isolated from donor blood by centrifugation in Ficoll-paque den-

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sity gradient (Amersham Biosciences) [11]; neutrophil-enriched fraction was prepared by removing erythrocytes by hemolysis. The donors gave voluntary informed consent to participation in the study. Murine M3 and B16 melanoma, murine KSML-100 adenocarcinoma, and human K562 erythroblastoma cells were used in the study. The cells were cultured in Costar flasks at 5% CO_2 and 37°C in DMEM with 10% FCS, penicillin (100 U/ml), and streptomycin (100 μg/ml; Gibco). Samples for measurements were collected after 24-h incubation. Protein secretion by activated lymphocytes was stimulated by adding lymphocytes isolated from mouse spleen after 4-day incubation with IL-2 in 100:1 proportion to transformed cells [2]. Lipopolysaccharide (LPS; Gibco) in a concentration of 10 μg/ml was used for stimulation. The cytotoxic Tag7/Hsp70 complex was prepared as described previously [11]. The final concentration of Tag7/Hsp70 complex in the incubation medium was 10^{-10} M.

Melanoma M3 cells were subcutaneously transplanted (2×10^6) to DBA mice (n=20) into the inner surface of the thigh. On day 5, when the tumor reached 4 mm in size, the animals were randomly divided into 2 groups (10 per group). Group 1 animals were subcutaneously injected with 100 μl antibodies to S100A4 in a concentration of 10 ng/ml (experimental group). Group 2 animals (controls) were injected with 100 μl rabbit immunoglobulins (10 ng/ml). Repeated subcutaneous injections of antibodies were made every third day to the site of tumor cell injection and tumor diameter was measured. The animals were sacrificed by cervical dislocation after the tumor reached 20 mm in diameter.

TABLE 1. Effect of Stimulation on the Content of S100A4 Protein in Culture Medium of Normal and Tumor Cell ($M\pm m$)

Cells	Content of S100A4, ng/10^6 cells	
	initial	after stimulation
Lymphocytes		
day 1	9.2±1.1	—
day 4	14.3±1.4	14.4±1.2
day 6	7.8±0.5	7.6±0.7
Neutrophils	3.0±0.9	1.7±0.4
M3 melanoma	250±16	375±23*
B16 melanoma	420±27	2200±130*
KSML-100	100±8	140±11*

Note. Data of 3-5 independent experiments are presented. *p<0.05 compared to control values (without stimulation).

The data were statistically processed using Student's parametric test.

RESULTS

During incubation of lymphocytes with IL-2, natural killers predominated on day 4 and lymphocyte-activated killers (LAK) predominated on day 6. Basal content of S100A4 in the culture medium increased by day 4 of culturing and decreased almost 2-fold on day 6 (Table 1). The changes were significant, but the maximum content of S100A4 did not surpass 15.7 ng/10^6 cells.

Addition of the stimulant (LPS) to lymphocytes on day 4 or of target cells (transformed K562 cells) for cytolytic lymphocytes on day 6 did not increased S100A4 content in the conditioned medium. Neutrophil stimulation with LPS also caused no appreciable changes in S100A4 level.

The release of S100A4 protein into culture medium during culturing of transformed highly metastatic cell strains (M3 and B16 mouse melanoma and KSML-100 mouse adenocarcinoma cells) was 100-400 ng/10^6 cells. Addition of LAK or natural killer-rich lymphocyte mass in 100:1 proportion to M3 melanoma culture medium increased the content of S100A4. Accumulation of S100A4 in the culture medium was most pronounced after 4-h incubation of transformed cells with activated lymphocytes (Fig. 1). The cytotoxic Tag7/Hsp70 complex was also used for stimulation of S100A4 protein secretion; the complex was released by LAK reacting with K562 target cells [2]. Addition of the complex to B16 cell incubation medium increased

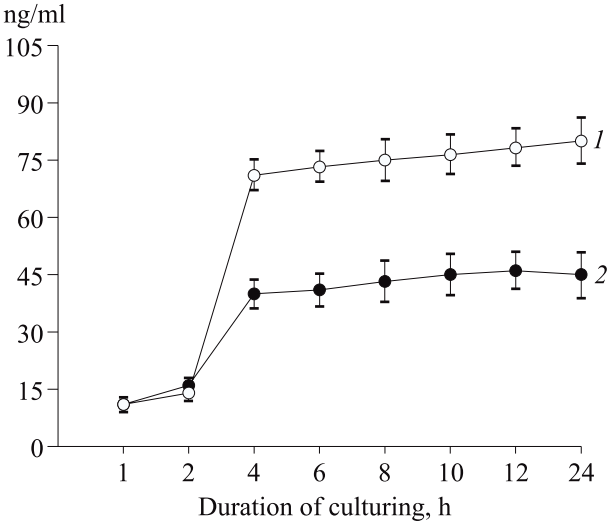

Fig. 1. Dynamics of S100A4 protein accumulation in conditioned medium during culturing of M3 mouse melanoma cells in the absence (*1*) and presence of activated lymphocytes (*2*).

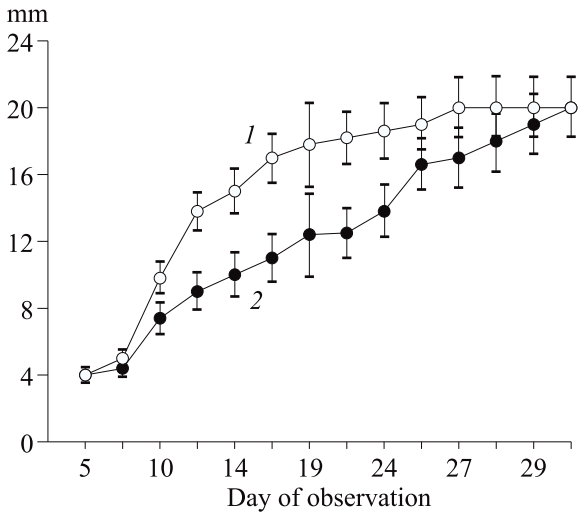

Fig. 2. Antibodies to S100A4 protein inhibit M3 melanoma growth in DBA mice. *1*) control (*n*=10); *2*) injection of antibodies to S100A4 (*n*=10).

the concentration of S100A4 by 5.5 times after 12 h. These data suggest that S100A4 secretion by cancer cells is induced by lymphocytes.

Hence, tumor cells stimulated by immunocompetent cells are responsible for the maintenance of high level of S100A4.

Metastasizing of cancer cell suggests that these cells can resist the attack of the immune system. A possible mechanism of the realization of this capacity is secretion of S100A4 protein; production of this protein correlates with the metastatic phenotype [8].

Injection of antibodies to S100A4 to DBA mice inhibited tumor growth in comparison with the control (Fig. 2). This result suggests that S100A4 production protects tumor cells from the cytotoxic effect of immunocompetent cells.

REFERENCES

1. A. S. Dukhanin, E. A. Romanova, and E. A. Dukhanina, *Byull. Eksp. Biol. Med.*, **131**, No. 1, 88-92 (2001).
2. E. A. Dukhanina, D. A. Yashin, T. I. Lukyanova, *et al.*, *Dokl. Rossiisk. Akad. Nauk*, **414**, No. 2, 1-3 (2007).
3. B. R. Davies, M. P. Davies, F. E. Gibbs, *et al.*, *Oncogene*, **8**, No. 4, 999-1008 (1993).
4. B. R. Davis, M. O'Donnell, G. C. Durkan, *et al.*, *J. Pathol.*, **196**, No. 3, 292-299 (2002).
5. E. A. Dukhanina, A. S. Dukhanin, M. Y. Lomonosov, *et al.*, *FEBS Lett.*, **410**, Nos. 2-3, 403-406 (1997).
6. K. Flatmark, G. M. Maelandsmo, S. O. Mikalsen, *et al.*, *Tumor Biol.*, **25**, Nos. 1-2, 31-40 (2004).
7. H. L. Ford, M. M. Salim, R. Chakravarty, *et al.*, *Oncogene*, **11**, No. 10, 2067-2075 (1995).
8. S. C. Garrett, K. M. Varney, D. J. Weber, and A. R. Bresnick, *J. Biol. Chem.*, **281**, No. 2, 677-680 (2004).
9. S. Gongoll, G. Peters, M. Mengel, *et al.*, *Gastroenterology*, **123**, No. 5, 1478-1484 (2002).
10. M. Grigorian, E. Tulchinsky, O. Burrone, *et al.*, *Electrophoresis*, **15**, Nos. 3-4, 463-468 (1994).
11. L. P. Sashchenko, E. A. Dukhanina, D. V. Yashin, *et al.*, *J. Biol. Chem.*, **279**, No. 3, 2117-2124 (2004).