ONCOLOGY

Soluble Fas Antigen in Serum of Cancer Patients

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Blood concentration of soluble Fas antigen is higher in patients with benign and malignant tumors in comparison with healthy subjects, which probably suggests its involvement into tumorigenesis.

Key Words: *tumors; apoptosis; soluble Fas antigen*

Apoptosis, programmed cell death, is an important mechanism maintaining homeostasis in multicellular organisms. Disturbances in cell elimination underlie various pathological states such as malignant tumors, Alzheimer disease, multiple sclerosis, amyotrophic lateral sclerosis, thyroiditis, hepatitis, and autoimmune diseases.

Fas/APO-1/CD95 is a type I transmembrane glycoprotein belonging to the TNF/NDF receptor family [5]. Fas is expressed in various tissues, in particular in the thymus, liver, heart, lungs; it is present on activated lymphocytes, natural killers, virus-infected and tumor cells.

Similarly to tumor necrosis factor p55, Fas not only induces cell apoptosis upon interaction with specific ligand FasL or agonistic monoclonal antibodies (MCA) to Fas, but also activate transcription factor NfkB [14]. The mechanism triggering cell death via Fas is studied in detail. The interaction of Fas with FasL or agonistic MCA induces activation of caspase-8 or caspase-2 proteases via adapter proteins FADD/MORT-1 or RIP and RAIDD, respectively [11,14]. The activated proteases hydrolyze vital cell substrates such as poly(ADP-ribose) polymerase, lamin, *etc.*, and mediate apoptosis-specific functional and morphological changes in the cytoplasm and nucleus.

Molecular cloning studies and nucleotide-sequence analysis have demonstrated that apart from transmembrane Fas receptor, cells produce also soluble Fas (sFas), a product of alternative spicing of full-length Fas mRNA, which inhibits cytotoxicity induced by anti-Fas MCA *in vitro* [2,3]. Thus, enhanced production of sFas can underlie cell resistance to apoptosis.

Elevated blood concentration of sFas was observed in some autoimmune disease: systemic lupus erythematosus [15], cutaneous tuberculosis [4], Graves' disease [13], as well as in patients with myocarditis, heart failure [12], T and B cell leukemia [8], and hepatocellular carcinoma [6].

The aim of the present study was to determine serum concentration of sFas in patients with malignant tumors of various morphology and localization and to explore the relationship between sFas and these diseases.

MATERIALS AND METHODS

Hybrid cells producing MCA to recombinant full-length Fas (Coultronics) were obtained according to a standard protocol [9]. Splenocytes from immunized BALB/c mice (3 intraperitoneal injections with a 2-week interval) were fused with mouse myeloma cells NSO/1

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(provided by Institute of Cytology, Russian Academy of Sciences) using 50% polyethylene glycol [9]. Positive clones were selected by immunoenzyme assay. Single-cell clones were prepared by the method of limiting dilutions.

MCA were isolated from mouse ascitic fluid by ammonium sulfate precipitation (pH 7.2). Antibodies were purified by HPLC on a Mono Q column (Pharmacia) and their characteristics (types of light and heavy chains, antigen-antibody binding constant) were determined by immunoenzyme assay [1].

These MCA were used for the development of a sandwich test-system for quantitative analysis of sFas in biological fluids. The sensitivity limit of this test-system was 0.52 ng/ml.

For evaluation of a relationship between sFas and malignant tumors, serum concentration of sFas was measured in patients aged 17-61 years with various tumors at different stages (Table 1). Clinical diagnosis was confirmed by morphological analysis of resected tumor tissues. Before sFas assay the patients received no treatment. Blood samples from 15 healthy donors aged 18-57 were used as the control. Blood was drawn from the cubital vein at 8:00-9:00 a.m.; routinely prepared serum was stored at -20°C before analysis.

MCA against Fas SA-8 [IgG1(κ); $4\pm0.6\times10^7$] in 0.05 M carbonate buffer pH 9.6 (5 mg/ml) were sorbed overnight to multiwell plates (Linbro) at 4°C.

Free binding sites were inactivated by overnight incubation with 1% BSA in phosphate buffer saline (pH 7.2) at 37°C (1 h). Aliquots of patient and donor serum were added to the wells. Full-length recombinant Fas (serial 1:2 dilutions from 20 to 0.15 ng/ml) was added to each plate as the positive control. The plates were incubated for 2 h at 37°C and washed 6 times with phosphate buffer containing 0.1% Tween-20 (Sigma). The washout procedure was repeated after each stage of the test.

The plates were incubated for 2 h with biotinylated MCA against Fas SA-7 [IgG1(κ); $5.8\pm0.7\times10^8$] in a concentration of 12 μg/ml in a buffer containing 0.1% Tween-20 and 0.1% BSA (37°C) and then with streptavidin peroxidase (Amersham) in a working dilution in washout buffer (1 h, 37°C).

o-Phenylenediamine (0.04%) in 50 mM citrate-phosphate buffer (pH 5.0) containing 0.03 hydrogen peroxide was added to each well and incubated at room temperature for 15-20 min for color development. The reaction was stopped with 10% sulfuric acid, optical density was measured on an MR 700 Microplate Reader (Dynatech Labs) at 492 nm.

The concentration of sFas in individual samples was calculated by the calibration curve constructed for each plate.

RESULTS

There are two forms of Fas/APO-1/CD95: membrane-bound FasR and soluble sFas. These molecules have opposite functions. FasR induces apoptosis upon binding with its ligand FasL or agonistic MCA against Fas, while sFas inhibits Fas-dependent apoptosis. FasR is abundantly expressed in tumors of hemopoietic and nonhemopoietic origin. However, many tumors are resistant to Fas-dependent apoptosis. This resistance can be associated, first, with mutations in FasR and proteins involved in signal transmission from activated FasR, and second, with enhanced expression of in-

TABLE 1. Serum Content of sFas in Cancer Patients

Tumors	Number of observations	Mean age, years	sFas-positive, %	Concentration of sFas, ng/ml (range)
Control	15	39	36	0.86 (0.7-1.2)
Osteosarcoma	7	17	57.1	9.46 (1.95-24.6)
Chondrosarcoma	3	24	33	2.24
Ewing tumor	3	18	100	1.2; 8.1; 12.3 (1.28-12.3)
Giant cell tumor	5	32	100	4.91 (3.37-6.3)
Benign bone tumors	10	31	70	6.13 (1.28-11.5)
Soft tissue sarcomas	6	25	66.6	5.87 (0.91-12.9)
Ovarian cancer	15	35	80	5.37 (1.4-20)
Marginal ovarian tumors	2	27	100	0.7; 10 (0.7-10)
Benign ovarian tumors	5	28	80	10.82 (6.5-20)
Vulvar cancer	1	34	100	2.12
Colorectal cancer	13	57	61.5	2.25 (0.6-4.7)
Pancreatic cancer	1	46	100	22
Stomach cancer	1	61	100	4.9

hibitors of Fas-dependent apoptosis, in particular sFas, which offer tumor cell great advantage in survival and proliferation.

The mean serum concentration of sFas in all cancer patients surpassed the control level (Table 1). The highest sFas concentrations were found in patients with osteosarcoma and ovarian cancer. The percentage of Fas-positive sera was higher in cancer patients than in the control group (39%), being maximum in patients with ovarian and colorectal cancer, osteosarcoma, and benign bone and ovarian tumors. This suggests that the high incidence and high concentrations of sFas can be associated with the development of malignant tumors.

However, cells responsible for high serum concentrations of sFas in tumor patients are not yet identified. On the one hand, sFas can be synthesized by tumor cells. Elevated serum content of sFas was observed in patients with hepatocellular carcinoma. Moreover, the concentration of sFas was higher in patients with multiple hepatocellular carcinoma than in those with single tumor node in the liver [6] and decreased within 1 month after resection of the tumor. These observations confirm our assumption.

sFas produced by tumor cells inhibits the effect of not only FasL secreted by or associated with effector cells of the immune system, but also FasL produced by tumor itself. Tumor cells express FasL, which allows them to destroy immune cells. Tumor cells can produce FasL in both membrane-bound and soluble forms. Soluble FasL possesses antiinflammatory activity [7] and promotes migration of immune cell to the tumor. sFas interacting with soluble FasL not only protects the tumor from cytolytic effect of immunocompetent cells, but also inhibits their migration.

On the other hand, sFas can be produced by other cells. It was found that the high concentrations of sFas during acute graft-versus-host reaction correlated with elevated bilirubin content [10]. It can be hypothesized that in this case sFas was produced by damaged hepatocytes.

Moreover, oncological diseases are associated with compromised immune function, therefore sFas can be produced by immunocompetemt cells. In patients with systemic lupus erythematosus the increased serum concentration of sFas correlated with the content of soluble CD4 [15]. A possible explanation of this phenomenon is the production of sFas by $CD4^+$ T cells. Further investigation are required for precise identification of sFas-producing cells.

Patients of all groups should be followed-up for elucidation of the relationships between sFas and the dynamics of the disease, treatment efficiency, incidence of local tumor recurrences and metastases, and for evaluation of the prognostic value of sFas concentration.

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