Cancer and Soluble FAS

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A test system developed by the authors was used to measure serum concentrations of soluble Fas in patients with malignant and benign tumors of different location and morphology. Relationships between soluble Fas levels and the main clinical and morphological characteristics of cancer were evaluated. It is proven that the concentrations and incidence of detection of soluble Fas in the sera of patients with tumors are significantly higher than in normal subjects. No appreciable differences in the concentrations of soluble Fas were detected in malignant and benign tumors of the mammary gland, bones, ovaries, and adrenals. In thyroid cancer, soluble Fas levels were higher than in benign and hyperplastic processes in this organ. High level of soluble Fas is associated with late stages of the disease (ovarian cancer, cancer of the corpus uteri, adrenocortical and colorectal cancer) and with poor differentiation of the tumor (ovarian cancer and cancer of the corpus uteri), with local metastases (colorectal and adrenocortical cancer), and with tumor invasion into the myometrial tissue, intestinal wall, and adjacent tissues (cancer of the corpus uteri and colorectal cancer). A significantly high level of soluble Fas was detected in colorectal and adrenocortical cancer in the presence of at least 2 local metastases. Soluble Fas levels depended on tumor histogenesis in malignant and benign ovarian tumors. High concentration of soluble Fas was detected in large tumors in patients with ovarian cancer, cancer of the corpus uteri, colorectal cancer, thyroid cancer and adenoma, and in adrenocortical cancer. Initially high levels of soluble Fas are characteristic of patients whose tumors are little sensitive to nonadjuvant radiotherapy. The overall 5-year survival of patients with low levels of soluble Fas is better in osteosarcoma, cancer of the corpus uteri, ovarian and adrenocortical cancer.

Key Words: *soluble Fas; serum; malignant and benign tumors; prognosis*

Fas/APO-1/CD95 is a key receptor triggering apoptosis in cells. Cell resistance to Fas-dependent apoptosis is determined by disorders in the expression, structure, and functioning of proteins mediating Fas-dependent apoptosis and by hyperproduction of soluble Fas (sFas) by these cells. Soluble Fas is a product of alternative splicing of full-length Fas matrix RNA [2]. Soluble Fas distantly inhibits the effects of FasL, allowing escape of sFas producer cells from the host antitumor defense.

Disorders in Fas-dependent apoptosis were detected in melanoma, colorectal and breast cancer, in hepatocellular carcinoma, lung adenocarcinoma, and many other malignant tumors [9]. High concentrations of sFas were detected in patients with non-Hodgkin's

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lymphoma [4], prostatic [3], vesical [6], and breast cancer [8]. It was shown that high level of sFas is an independent factor of unfavorable prognosis in non-Hodgkin's lymphoma [7], melanoma [10], prostatic [3], vesical [6], and ovarian cancer [5].

We compared serum sFas levels in healthy. subjects and patients with malignant and benign tumors of different location using a test system developed and characterized previously [1] and studied the relationship between sFas values and the main clinical and morphological characteristics of tumor diseases and their prognosis irrespective of therapy.

MATERIALS AND METHODS

The study was carried out on 1061 primary patients with malignant and benign tumors of different morphogenesis and location (861 women, 200 men) aging 14-80 years. The patients were examined and treated at N. N. Blokhin Cancer Research Center, M. F. Vladimirskii Research and Clinical Institute, Moscow University for Medicine and Dentistry, Hospital No. 2 for War Veterans, Department of Health of Moscow, from December 1999 to November 2003. Clinical diagnosis was confirmed by the results of histological analysis of the tumor in all patients. Cancer patients were divided into 7 groups.

Group 1 consisted of 141 females: 119 patients with breast cancer aged 28-82 years and 22 patients with benign formations of the mammary gland aged 33-58 years. The following morphological variants of cancer were detected in patients with breast cancer: ductal infiltrative (59.7%), lobular infiltrative (24.3%), and rare variants of breast cancer (16%). The majority (73.9%) of breast cancer patients had stages I and II of tumor process.

Group 2 consisted of 99 patients with bone tumors (61 men and 38 women aged 14-59 years) with primary (n=32), secondary (n=1), parostal (n=1), periosteal (n=1) osteosarcomas, primary (n=17), secondary (n=1), chondrosarcomas, chordoma (n=3), Ewing's tumor (n=10), malignant fibrous osteohistiocytoma (n=9), giant cell tumor of the bone (n=10), plasmocytoma (n=1), hemangioendothelioma (n=1), hemangiopericytoma (n=1), bone lipoma (n=1), benign osteoblastoma (n=2), chondroblastoma (n=3), chrondroma (n=1), osteochondral exocytosis (n=2), aneurysmal bone cyst (n=2).

The ovarian tumor group consisted of 141 patients with ovarian cancer (aged 24-80 years) and 206 patients with benign ovarian tumors (BOT; 18-69 years).

The study included 111 patients with cancer of the corpus uteri (CCU) aged 31-80 years, the majority (62.8%) with stages I and II of tumor process.

The colorectal cancer (CRC) group consisted of 113 patients (57 men and 56 women) aged 35-72 years. The tumors were detected in the patients at different stages of the process (after Duke), the majority (61%) presented with stages A and B.

The total group of patients with thyroid diseases consisted of 141 patients (127 women and 14 men) aged 17-73 years. Thyroid cancer was diagnosed in 32 of these: papillary in 17, follicular in 14, and undifferentiated in 1 patient. The majority of patients with thyroid cancer presented with stages II (37.5%) and III (40.6%) of tumor process. Benign thyroid diseases were diagnosed in 109 patients: adenoma in 16, nodular colloid micromacrofollicular goiter in 68, diffuse toxic goiter in 23, and Hashimoto's autoimmune thyroiditis in 2 patients.

The study included 109 patients (59 men and 50 women) with adrenal tumors aged 45-75 years. The diagnoses were as follows: adrenocortical cancer in 32, adrenocortical adenoma in 52, adrenocortical tumor with indefinite malignancy potential in 6, pheochromocytoma in 18, and myelolipoma in 1 case. Almost half (53%) of patients with adrenocortical cancer presented with stage III tumor process.

Concomitant diseases were detected in 56.0% patients (essential hypertension, chronic coronary disease, uterine myoma, uterine polyp, mastopathy, chronic gastritis, chronic colitis, chronic pyelonephritis, urolithiasis, type 2 diabetes mellitus).

Twenty percent of patients had combinations of several chronic diseases.

Control group consisted of 457 normal subjects (332 women and 125 men) aged 17-76 years.

Blood was collected from the cubital vein after overnight fasting. After clot formation, the serum specimens were centrifuged (10 min) at 2500 rpm, the serum was collected, put into labeled tubes, and stored at -70°C.

The concentrations of sFas were measured in all patients before treatment.

Monoclonal antibodies SA-8 to Fas [(IgG_1 (κ); $(4.0\pm0.6)\times10^7$ M^{-1})] were adsorbed on EIA plates (Costar) in 0.05 M carbonate buffer pH 9.6 in a concentration of 10 μg/ml overnight at 4°C. Free adsorption sites were blocked with 1% BSA in PBS (pH 7.2) for 1 h at ambient temperature. Sera of patients and normal subjects were then pipetted into the wells.

For constructing calibration curve, serial 2-fold dilutions of full-length recombinant baculovirus Fas (40.0-0.08 ng/ml) were pipetted into the wells of each plate. The plates were incubated for 1.5 h at 37°C.

After incubation, the plates were intensely washed several times with PBS with 0.1% Twin-20 (Sigma; washing buffer). The washing procedure was repeated after each stage of the test.

After washing SA-7 biotinilated monoclonal antibodies to Fas [(IgG_1 (κ); $(5.8\pm0.7)\times10^8$ M^{-1})] in a

concentration of 10 μg/ml in washing buffer with 0.1% BSA were pipetted into the plates and incubated for 2 h at 37°C.

Streptavidine peroxidase (Amersham) in working dilution in the washing buffer was then added to the plates. The plates were incubated for 1 h at 37°C.

Fresh solution of 0.04% orthophenylene diamine in 50 mM citrate phosphate buffer (pH 5.0) with 0.03% H_2O_2 served as the substrate. The plates were incubated for 15-20 min at ambient temperature until color development. The reaction was stopped by adding 10% hydrochloric acid. Optical density was measured at λ=492 nm on an MR 700 Microplate Reader spectrophotometer (Dynatech Labs).

Serum sFas concentration was evaluated by the calibration curve plotted for each plate. The sensitivity of the method for sFas detection was 0.3 ng/ml.

The results were processed using Statistica and SPSS software.

RESULTS

The results of comparative analysis of sFas in patients and normal subjects are summed up in Table 1.

Soluble Fas was detected in 78% of all cancer patients, its level varying from 0.3 to 24.6 ng/ml (median concentration 2.9 ng/ml), while in the control sFas was detected in 40% examined subjects, its concentration did not surpass 1.2 ng/ml, the median concentration being 0.8 ng/ml (p=0.0001 between patients and controls).

The levels of sFas did not depend on age, sex, history of concomitant diseases in the overwhelming majority of patients. In women sFas levels did not depend on the reproductive function, duration of the postmenopausal period, menstrual cycle phase, number of previous pregnancies, deliveries, or induced abortions.

The highest sFas medians were detected in bone tumors, CCU, and thyroid cancer, the lowest in breast cancer. No significant differences in sFas levels in patients with malignant and benign tumors of the mammary gland, bones, ovaries, and adrenals were detected. By contrast, in thyroid diseases sFas levels were significantly higher in cancer than in benign and hyperplastic processes in this organ (p=0.041).

A significant relationship between sFas values and tumor stage process was detected in patients with

TABLE 1. Concentrations of sFas in Patients and Normal Subjects

Group		Number of cases	Concentrations of sFas		
			% detection	range of concentrations, ng/ml	median, ng/ml
Control (normal subjects; *n*=457)			40	0.3-1.2	0.8
Total group of patients (*n*=1061)			78	0.3-24.6	2.9
Breast tumors (*n*=141)	malignant	119	59	0.4-5.8	1.7
	benign	22	73	0.4-2.4	1.2
Tumors of skeletal bones (*n*=99)	malignant	67	89	0.3-24.6	3.8
	benign	32	92	0.3-15.6	3.7
Ovarian tumors (*n*=347)	malignant	141	55.3	0.7-20.5	2.5
	benign	206	80.2	0.7-20.0	2.2
CCU (*n*=111)	malignant	111	76	0.6-21.0	3.7
	benign	—			
CRC (*n*=113)	malignant	113	92.5	0.6-14.5	2.3
	benign	—			
Adrenocortical tumors (*n*=109)	malignant	32	100	1.4-8.0	2.0
	benign	77	95	0.5-24.6	1.8
Thyroid tumors (*n*=141)	malignant	32	96.8	0.8-13.9	2.8
	benign	109	75.0	0.4-7.3	1.1

Note. Here and in Tables 2, 3: *n*: number of subjects.

some of malignant tumors (Table 2). For example, the sFas median concentration increased with increasing the stage of the disease in patients with CCU, ovarian and adrenocortical cancer, and CRC. High serum level of sFas was detected in patients with CRC and adrenocortical cancer with 2 and more regional metastases in comparison with patients without regional metastases. The sFas median concentration in CRC patients with regional metastases was 3.6 ng/ml *vs.* 1.6 ng/ml in patients without metastases (p=0.03), in adrenocortical cancer 4.2 and 1.4 ng/ml, respectively (p=0.023).

The levels of sFas did not correlate with disease stage in breast cancer, thyroid cancer, and osteosarcomas.

The concentration of sFas depended on the histological structure of ovarian benign tumors and cancer. In the ovarian cancer group the highest serum sFas levels were detected in the patients with serous (median 5.2 ng/ml) and mucinous (median 3.4 ng/ml) adenocarcinomas.

The concentration of sFas in patients with endometrioid ovarian cyst was significantly higher than in those with cysts of the corpus luteum (p=0.038) or with follicular cysts (p=0.01). The levels of sFas were exclusively high in papillary cystadenoma, Brenner's benign tumor, mucinous cystadenoma, and mucinous borderline ovarian tumor, while in patients with fibroma and in 50% patients with follicular cysts, cystadenofibroma, common serous ovarian cyst this value was at the level of the controls.

A relationship between sFas levels and degree of malignant tumor differentiation was noted. The median sFas concentrations in patients with poorly and moderately differentiated CCU, ovarian, adrenocortical, and thyroid cancer were significantly higher than in patients with well-differentiated tumors of this location (Table 3).

The initial sFas levels in patients with CCU and CRC correlated with the depth of tumor invasion in the wall of these organs. For example, the sFas median in 71 patients with CCU invading the uterine wall by ≥1 cm was significantly higher than in 40 patients in whom the tumor invaded the uterine wall to a depth of <1 cm (6.8 and 1.9 ng/ml, respectively, p<0.01). A similar picture was detected in 69 CRC patients with the tumor invading the colonic wall to a depth of ≥1 cm and in 44 patients with tumor invading the colonic wall to a depth of <1 cm (sFas median concentrations 2.2 and 1.0 ng/ml, respectively; p<0.05).

Low levels of sFas can be a predictive factor of CRC patients' response to nonadjuvant radiotherapy. Radiotherapy was ineffective in patients with serum sFas concentrations ≥4.5 ng/ml, while its level of ≤0.9 ng/ml was associated with the maximally pronounced postradiation pathomorphosis of the primary tumor. Coefficient of correlation between serum sFas levels in CRC patients and intensity of postradiation pathomorphosis was -0.52 (p=0.049).

Irrespective of therapy, overall 5-year survival of cancer patients was analyzed with consideration for high and low sFas levels, which were differentiated arbitrarily (by statistical analysis). Overall 5-year survival of patients with osteosarcoma with high (≥1.2 ng/ml) and low (<1.2 ng/ml) sFas levels was 13.1 and 55.2%, respectively; for CCU these values were 65.3 and 95.7%, respectively. For patients with adrenocortical cancer with high (≥2.0 ng/ml) and low (<2.0 ng/ml) sFas levels the overall 5-year survival was 0 and 62.5%, respectively. For patients with ovarian cancer with high (≥5.0 ng/ml) and low (<5.0 ng/ml) sFas levels overall 5-year survival was 31.1 and 63.3%, respectively. These results indicate that initially high concentrations of sFas in patients with osteosarcoma, CCU, adrenocortical cancer, and ovarian cancer significantly correlate with low overall 5-year survival. No significant correlations between the initial levels of sFas and overall 5-year survival were detected for patients with breast cancer, thyroid cancer, and CRC.

Analysis of modern publications and our results suggest that sFas is involved in the pathogenesis of oncological diseases, correlates with certain clinical

TABLE 2. Median Serum sFas Concentrations in Patients with Malignant Tumors and the Disease Stage

Groups (disease, stage)		Number of cases	Median sFas concentration, ng/ml
CCU (n=111)	I	24	1.0
	II	40	2.4
	III	37	6.8
	IV	10	8.4
Ovarian cancer (n=141)	I	28	0.8
	II	34	1.2
	III	74	1.4
	IV	5	1.6
Adrenocortical cancer (n=32)	I	3	1.4
	II	6	1.8
	III	17	2.1
	IV	6	3.0
CRC (n=113)	I	30	1.2
	II	39	2.1
	III	30	2.7
	IV	14	4.7

TABLE 3. Median sFas Concentrations in Patients with Malignant Tumors of Various Differentiation

Group, differentiation		Number of cases	Median sFas concentration, ng/ml
CCU (*n*=111)	poor and moderate	74	7.1
	high	37	2.6
Ovarian cancer *n*=141)	poor and moderate	62	1.7
	high	79	1.1
Adrenocortical cancer (*n*=32)	poor and moderate	10	2.9
	high	22	1.5
Thyroid cancer (*n*=32)	poor and moderate	7	3.5
	high	25	2.2

morphological characteristics of tumors, and is one of important factors for evaluating the biological behavior of the primary tumor and for its prognosis. Hence, analysis of the initial serum levels of sFas in cancer patients together with clinical and morphological characteristics of these tumors is important not only theoretically, but practically as well.

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