ONCOLOGY

Soluble Fas Antigen in the Serum of Patients with Colon Cancer

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Serum concentration of soluble Fas antigen (sFas) was measured in 60 normal subjects and 33 patients with colon cancer. The incidence of sFas detection and its serum content were higher in patients with colon cancer compared to normal subjects. No relationships between the incidence and level of sFas and patient's sex, age, duration and stage of the disease were found. Serum content of sFas tended to increase in patients with metastases to regional lymph nodes, liver, and lungs. The role of sFas as a marker predicting clinical course and outcome of colon cancer is discussed.

Key Words: *sFas; adenocarcinoma; colon*

Pathogenesis of many diseases, including malignant tumors, is associated with impaired mechanisms of apoptosis, strictly regulated form of programmed cell death with peculiar morphological and biochemical characteristics [10,12]. Damaged or "unwanted" cells are removed by apoptosis without damaging cell microenvironment [9]. The mechanisms of apoptosis are involved in embryogenesis [2] and maintenance of tissue homeostasis [7]. Apoptosis plays a very important role in removal of cells with genetic abnormalities, thus preventing the formation of clones with oncogenic mutations [10]. Cells infected with viruses are also subjected to apoptosis which arrests viral replication [5].

Fas receptor if a key molecule involved in Fas-mediated apoptosis. In 1989 two independent research groups obtained murine monoclonal antibodies with cytolytical activity towards various strains of human cells [14,15]. Membrane protein recognized by these antibodies was called Fas or APO-1. Two forms of Fas/APO-1/CD95 receptor were identified: membrane-bound (FasR) and soluble (sFas). After reacting with the ligand (FasL) or monoclonal antibodies to Fas, FasR induces apoptosis of the cell. Increased expression of sFas (in particular, by tumor cells) can be regarded as a possible mechanism of its resistance to factors regulating its proliferation and differentiation.

Serum content of sFas increased in patients with systemic lupus erythematosus [13], skin tuberculosis [6], osteosarcoma [3], hepatocellular carcinoma [8], and ovarian cancer [4,11]. However, prognostic value of sFas in these diseases is not yet quite clear.

We measured serum sFas content in patients with colon cancer and analyzed the relationship between this parameter and clinical course and prognosis of the disease.

MATERIALS AND METHODS

Thirty-three individuals (16 men and 17 women aged 35-72 years, mean age 58.3±10.4 years) with colon adenocarcinoma (CAC), patients of the surgical de-

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partment of proctology, N. N. Blokhin Cancer Research Center in 1998-2000, were examined. The duration of the disease from first complaints to the start of specific therapy was 7.4±5.6 months. Stage A (according to Dukes classification) was diagnosed in 9, B in 11, C in 27, and D in 6 patients. Histological structure, degree of invasion, and differentiation of the tumors were examined by morphological analysis of removed carcinomas. Twenty of 33 patients were treated surgically and 13 received preoperative radiotherapy.

For control, sFas was measured in the sera of 60 normal subjects aged 19-70 years (mean age 61.3±8.6 years).

Serum levels of sFas were measured by enzyme immunoassay developed at the Institute of Bioorganic Chemistry in cooperation with Laboratory of Clinical Biochemistry of the Cancer Research Center [1].

The results were statistically processed using Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

sFas in a concentration 0.62-4.70 ng/ml was detected in 24 (72.7%) patients with CAC. The mean sFas content was 2.12±1.16 ng/ml, which was higher than in normal subjects (0.51-0.96, mean level 0.76±0.12 ng/ml), but the difference was insignificant ($p>0.05$). In controls, sFas was less frequently detected (36.7% cases).

The incidence of sFas in men (68.7%) and women (76.4%) and the mean serum content of this antigen (2.55±1.26; 0.69-4.7 ng/ml and 1.75±0.97; 0.62-3.25 ng/ml, respectively) were virtually the same ($p>0.05$).

No relationships between serum concentration of sFas and patient age and duration of the disease were detected ($r=0.25$ and $r=0.2$, respectively).

No correlation was detected between sFas content in patients with CAC and tumor size ($r=0.2$). The incidence and the mean level of sFas did not correlate with tumor size ($p>0.05$).

Of 16 patients with small tumors (<5 cm), sFas in a concentration of 0.62-4.40 ng/ml was detected in 11 (68.8%) cases, its mean concentration being 2.17± 1.96 ng/ml. The level of sFas in patients with tumors of 5 cm and larger varied from 0.69 to 4.70 ng/ml (mean 2.08±1.78 ng/ml), the incidence of sFas in this group being 76.5%.

The mean concentration of sFas in the serum increased with dissemination of the tumor process, but the differences are insignificant ($p>0.05$, Table 1). It is noteworthy that sFas was detected in all CAC patients with Dukes stage D and its concentrations in this subgroup were maximum.

The degree of CAC invasion and differentiation are the most important prognostic criteria. Analysis of the data revealed no relationship between sFas concentration and the degree of tumor differentiation (Table 1). sFas was more often detected in patients with high differentiated CAC.

According to International TNMP Classification, all patients were divided into the following groups: P_2, tumor invades the muscle layer; P_3, tumor grow through all layers of the colon wall; and P_4 tumor penetrate the colon wall.

The incidence and the mean level of sFas did not depend on the degree of CAC invasion ($p>0.05$, Table

TABLE 1. Serum Concentrations of sFas in CAC Patients (*M*±*m*)

Criterion		Incidence of sFas, %	sFas concentration, ng/ml	
			mean	range
Dukes stage	A (9)	55.6 (5)	1.46±0.75	0.8-2.9
	B (11)	81.8 (9)	1.74±1.11	0.62-3.00
	C (7)	57.1 (4)	1.90±0.94	1.00-3.25
	D (6)	100.0 (6)	3.38±0.87	2.4-4.7
Differentiation	low (4)	75.0 (3)	2.09±0.82	1.37-3.25
	moderate (23)	69.6 (16)	2.14±1.27	0.62-4.40
	high (6)	83.3 (5)	2.05±1.38	1.02-4.70
Invasion	P_2 (5)	60.0 (3)	2.20±1.56	1.0-4.4
	P_3 (6)	83 (5)	2.16±2.05	0.8-4.7
	P_4 (22)	72.7 (16)	2.09±1.07	0.62-3.25
Metastases to regional lymph nodes				
	none (23)	73.9 (17)	1.92±1.17	0.62-4.40
	present (10)	70 (7)	2.61±1.68	1.0-4.7

Note. Number of patients is shown in parentheses.

1). sFas was most often detected in the P_3 patients. A trend to a decrease of sFas content in the serum with higher tumor invasion was noted.

The mean serum level of sFas was higher in the patients with metastases to regional lymph nodes in comparison with patients without signs of tumor involvement of the lymphatic system, but the difference was statistically negligible ($p>0.05$, Table 1).

Six patients presented with primary tumor and numerous metastases in the liver. sFas was detected in all of them, its level varying from 2.4 to 4.7 ng/ml and the mean level being 3.38±0.93 ng/ml, which is significantly higher ($p<0.05$) than in the control and in patients without metastases (1.67±0.90 ng/ml, the range 0.62-3.25 ng/ml), but the differences from the latter group were insignificant ($p>0.05$).

Thirteen (62%) patients received radiotherapy before surgery. No significant difference in the incidence and mean levels of sFas in patients treated and not by radiotherapy before surgery was detected. sFas was detected in 69.2% patients receiving radiotherapy (0.62-2.70 ng/ml, mean level 1.98±1.43 ng/ml) and in 75% patients not receiving radiotherapy (1.02-4.40 ng/ml, mean level 2.20±1.16 ng/ml).

Patients with generalized tumor process (multiple metastases in the liver) refused further treatment after removal of the primary tumor. Other 28 were observed for 3-24 months. During this period, relapses and metastases were detected in 5 patients: local relapses in 2, multiple metastases in the liver in 2, and multiple metastases in the lungs in 1 patient.

Retrospective analysis showed the following results. sFas was not detected in 2 patients with CAC, who developed local relapses after removal of the tumor. In 3 patients with generalized tumor process (multiple metastases to the liver or lungs) the levels of sFas 6-8 months after treatment were 1.4, 2.9, and 4.7 ng/ml. Of 16 patients without relapses or metastases during 3-12 months after treatment, sFas was detected in 13 (1.93±1.01 ng/ml, range 0.62-3.25 ng/ml). Of 7 patients without relapses or metastases during 24 months, sFas was detected in only 3 (43%) and the content of this antigen in this subgroup was the lowest (0.80-1.04 ng/ml).

Hence, the incidence and serum content of sFas was higher in CAC patients compared to healthy subjects. The concentration of sFas did not depend on patient's sex, age, duration and stage of the disease, or TNMP parameters. sFas tended to increase in patients with disseminated process. The concentration of sFas increased in patients with metastases to regional lymph nodes and liver. These results attest to a relationship between the incidence and level of expression of sFas and pathogenetic mechanisms of CAC.

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