ONCOLOGY

Identification and Characterization of Serum Protein in Patients with Ovarian Cancer

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A 36 kDa protein was isolated from the sera of patients with ovarian cancer and rabbit antisera to this protein were prepared. Precipitation test with these antisera detected an antigen with electrophoretic mobility corresponding to α-1-globulins and molecular weight of 36 kDa. Direct comparison of precipitating test systems showed that this antigen is not identical to the known carcinoembryonic, placental, and reactive proteins. Serum α-1-globulin was not detected in the sera of healthy humans, pregnant women, newborns, and in human adult and fetal visceral tissues at the level of precipitating test system sensitivity 1 mg/liter. It was detected in the sera of patients with ovarian cancer, in ovarian tumor (cancer) tissues, in the contents of ovarian tumor cavities, and in concentrated specimens of amniotic fluid. The antigen was not detected in ascitic fluid of patients with ovarian cancer, but it was present in 75% serum samples from these patients. The antigen was called serum oncoovarian α-1-globulin. SDS—PAAG electrophoresis showed that this antigen is an oligomer consisting of subunits (monomers) with molecular weight of 36 kDa. Under denaturing conditions in the presence of 2-mercaptoethanol these monomers dissociate into polypeptide chains with a molecular weight of 18 kDa. The protein is liable to oligomerization. Comparative characteristics of serum oncoovarian α-1-globulin and CA-125 antigen are presented.

Key Words: *ovarian cancer; serum antigens; serum onco-ovarian α-1-globulin*

Ovarian cancer remains one of the most difficult diseases for diagnosis. About 80% patients (76.8% according to our data) are diagnosed at late (III-IV) stages, which means that about 90% of these patients live for no more than 3 years and all patients die within 5 years after diagnosis irrespective of the treatment and age [12]. More than ten new tumor-associated antigens (including CA-125) were revealed in the last three decades [6,7] and the possibilities of using these antigens for the diagnosis of ovarian cancer were studied. In addition, all known tumor markers, hormones, reactive proteins were tested, and new enzymes and their combinations are investigated [3,8,9,11,13], but the problem of early diagnosis of ovarian cancer, called "killer No. 1 among malignant genital tumors" [12], is still far from being solved. Young age of patients makes this problem particularly important: in our sampling the youngest patient with ovarian adenocarcinoma aged 17 years, and 10 years ago the youngest patient we observed aged 18 years [3].

We investigated the protein isolated from the sera of patients with ovarian cancer and called serum oncoovarian α-1-globulin (SOVA-1).

MATERIALS AND METHODS

Protein fraction was precipitated with 30-50% ammonium sulfate from 500 ml pooled serum of pa-

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tients with ovarian cancer. The precipitate was dissolved in 250 ml 0.05 M sodium acetate buffer (pH 4.4), pH was adjusted to 4.4, and the solution was centrifuged at 6000 rpm for 30 min. Cold (-10°C) 24% ethanol was slowly added to cold supernatant with gentle stirring (0.5:1 v/v), the mixture was incubated for 16-18 h at -10°C, and insoluble protein fraction was precipitated by centrifugation at negative temperature (20 min at 6000 rpm). The precipitate was dissolved in 100 ml 0.01 M K-phosphate buffer (KPB) (pH 7.4), pH was brought to 7.4, and added (with stirring) to 100 ml Toyopearl Blue adsorbent (or Blue Sepharose) equilibrated with the same buffer. The mixture was incubated for 2 h at 18-20°C with constant stirring, the suspension was transferred to Buchner funnel and washed with 2 liters of the initial buffer. Adsorbent-bound proteins were eluted with KPB containing 2 M NaCl (3×100 ml). All three fractions were pooled, crystal ammonium sulfate was dissolved in the total volume (final saturation 70%), and the mixture was incubated at negative temperature for 16-18 h, after which insoluble protein fraction was precipitated by centrifugation (6000 rpm, 30 min). The precipitate was dissolved in 10 ml KPB and subjected to gel filtration on Sephadex G-100 (2.5×100 cm column). Rabbits were immunized with fraction with molecular weight of 36±4 kDa according to the common protocol [3].

Antisera were exhausted with dry plasma (10 mg/ml) and donor serum (0.1 ml/ml). Antiserum exhaustion was controled by agar precipitation with individual plasma and donor serum specimens and extracts of visceral tissues from adult humans. After antibodies to normal serum and tissue antigens in the antiserum were completely adsorbed (negative precipitation test), individual serum samples and extracts from ovarian tumors were tested. The antigen detected in the precipitation test was used for modeling precipitation test systems for evaluation of antigen specificity with individual extracts of adult and fetal visceral tissues, sera of normal subjects, pregnant women, newborns, and erythro- and leukocyte lysates. The detected antigen was identified by comparing the precipitating test systems with known embryonic, tumor-associated, placental, and reactive proteins: α-fetoprotein, trophoblastic β-glycoprotein, pregnancy-associated α-2-globulin, carcinoembryonic antigen, ferritin, lactoferrin, carcinocerebral antigen, embryonic prealbumin, human chorionic gonadotropin, placental proteins 12 and 14, β-2-microglobulin, C-reactive protein, and ovariometastatic antigens 1-20 [3]. Direct comparison of the precipitating test systems with CA-125 was impossible, because no polyclonal antibodies to this antigen were obtained, and therefore only indirect identification and comparison of physicochemical parameters of these antigens were carried out.

Agar precipitation test was carried out using a standard test system. The sensitivity of the test system in different variants was 5, 1, 0.1, 0.05, and 0.01 mg/liter. This latter variant (Parus) was performed in a Petri dish with 7-10-ml sample; it was developed for antigen testing in biological fluids with low protein content (urine, amniotic fluid, *etc*.) and in growing cell cultures.

Preparative isolation of SOVA-1 molecular structures (oligomers, polypeptide chain subunits) from PAAG was carried out by routine blind clipping of protein zones from unstained gel and by a non-traditional method. Purified protein was subjected to SDS—PAAG electrophoresis with or without 2-mercaptoethanol at 100°C (5 min), the gels were stained with 0.25% Coomassie G-250, washed in 7% acetic acid until clear differentiation of stained zones, and then washed for 24 h in distilled water or buffer. Stained zones were cut out, homogenized, the protein was eluted with the buffer and analyzed by SDS—PAAG electrophoresis with or without 2-mercaptoethanol. The gels were stained routinely: polypeptides migrated in the zones corresponding to their molecular weights. All samples were diluted with 4% SDS (1:1 v/v). The buffer and gel contained 0.1% SDS.

Common immunohistochemical methods and methods of protein chemistry were also used: immunoelectrophoresis, SDS—PAAG electrophoresis with 2-mercaptoethanol, isoelectric focusing, gel filtration, affinity and ion-exchange chromatography [1,2,4,5,10].

RESULTS

Precipitation test with adsorbed antiserum No. 502, obtained from rabbits immunized with 36 kDa serum fraction from patients with ovarian cancer, detected an antigen with electrophoretic mobility of α-1-globulins (Fig. 1) and molecular weight 36 kDa (Table 1). This serum antigen was immunochemically not identical to the known tumor-associated, placental, and reactive proteins listed above and completely identical to oncoovarian α-1-globulin detected previously after rabbit immunization with ovarian adenocarcinoma extracts. SOVA-1 isolated from the serum of patients with ovarian cancer at the level of the precipitation test sensitivity 1 mg/liter was not detected in the sera of normal subjects, pregnant women, newborns, and visceral tissues of adults and fetuses, but was present in 75%

TABLE 1. Comparative Characteristics of SOVA-1 and CA-125 Antigen

Characteristics	SOVA-1	CA-125 [6,7,9,10]
Electrophoretic mobility	α-1-globulin	—
Molecular weight, kDa:		
in gel filtration	36	1500, 700, 205
in electrophoresis	36/18*	55/75*
Isoelectric point	6.17	—
Thermodenaturing	65°C in 15 min	100°C in 20 min
Solubility:		
in 0.6 M perchloric acid	Insoluble	Soluble
at 50% saturation with ammonium sulfate	Insoluble	—
in 12% ethanol	Insoluble	—
Resistance to proteases	Inactivates	Inactivates
Ionic interactions with DEAE sepharose	Binds	—
Affinity interactions:		
Toyperl Blue—sepharose	Binds	—
Concanavalin A with sepharose	Does not bind	Binds
Specific reactions to:		
glycoproteins	Negative	24% carbohydrates
lipids	Negative	—
ferroproteins	Negative	—

Note. "—" no data. *Electrophoresis in PAAG—SDS/PAAG—SDS+2-mercaptoethanol.

sera of patients with ovarian cancer (69% cases with stages I-II and 76% cases with stages III-IV). It was detected in 25% serum samples from patients with other tumors (uterus, stomach, intestine). Blood content of this antigen in patients can reach 10-15 mg/liter. It was not detected in ascitic fluid of patients, and its incidence in the blood did not correlate with the presence of ascites [3]. At the sensitivity level of 0.05 mg/liter SOVA-1 was not detected in pooled serum of 250 healthy women and 200 healthy men.

Apart from monomeric SOVA-1 (a-1-globulin with molecular weight of 36 kDa) electrophoresis in 7.5% PAAG revealed immunochemically identical isoforms corresponding to α-1-2- and γ-globulins. Under denaturing conditions (SDS—PAAG+2-mercaptoethanol) (Fig. 2) all three protein isoforms were represented by one protomer (polypeptide chain) with molecular weight of 18 kDa. Analysis of purified SOVA-1 polypeptide chains revealed, apart from protomers, oligomers with molecular weights of 54, 72, and 90 kDa , *i. e.* tri-, tetra-, and pentamers,

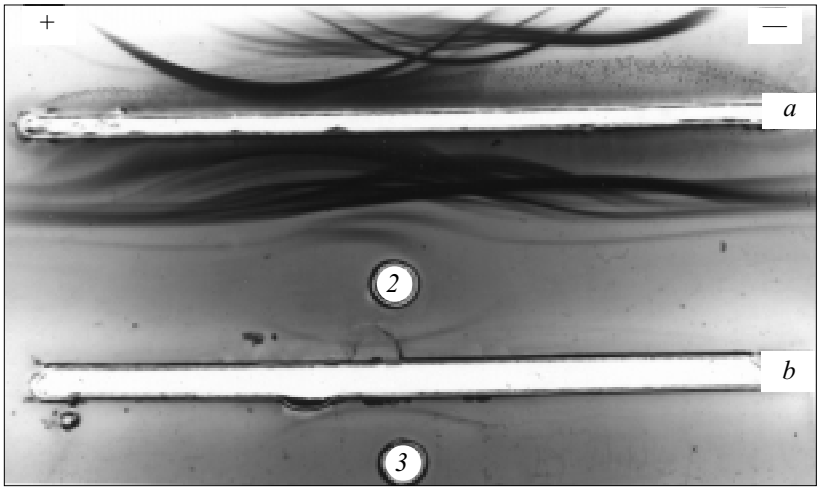

Fig. 1. Immunoelectrophoresis of serum onco-ovarian α-1-globulin. *a*) antiserum to normal human plasma protein; *b*) antiserum to serum fraction from patients with ovarian cancer. *1*) donor serum; *2*) tumor (ovarian adenocarcinoma) cavity contents; *3*) extract from ovarian adenocarcinoma tissue.

which attested to easy protein oligomerization and explained its electrophoretic polymorphism.

Nonidentity to the known proteins and comparison of physicochemical properties of SOVA-1 and CA-125 antigen (Table 1) suggest that the detected serum antigen is a protein unknown to oncologists. Comparison of SOVA-1 and CA-125 persuasively demonstrated the difference between these proteins. However, one of the most persuasive arguments (although inferior to evaluation of the amino acid sequence) is immunochemical identification, *i. e.* direct comparison of the precipitating test systems, which is now impossible because of the absence of CA-125 precipitating test system. Therefore ultimate conclusion on identity of these proteins will be done only after obtaining polyclonal antibodies to CA-125 and classical immunochemical identification, which will be the object of our further research.

On the basis of our findings and published reports we concluded that SOVA-1 is a new and the most perspective marker of ovarian cancer. However, objective evaluation of its specificity requires more extensive clinical investigation of tumors of other organs.

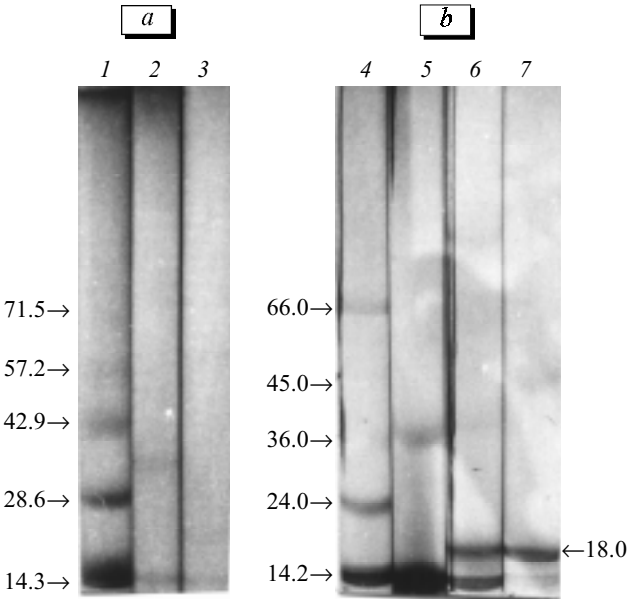

Fig. 2. Electrophoresis of serum onco-ovarian α-1-globulin (SOVA-1) in 10% PAAG with 2% sodium dodecyl sulfate (SDS). *a*) SOVA-1 from ovarian tumor (adenocarcinoma) contents; *b*) SOVA-1 from amniotic fluid. Molecular weight markers: *1*) oligomers with molecular weight 14.3-71.5 kDa (LKB); *4*) 14.2-66 kDa proteins (Sigma). *2*, *3*) 5 μg SOVA-1; *5-7*) 10 μg SOVA-1; *2*, *5*) 2% SDS; *1*, *3*, *4*, *6*, *7*) 2% SDS+2-mercaptoethanol; *7*) polypeptide chains (18 kDa) isolated after electrophoresis from gel stained and repeatedly subjected to PAAG—SDS electrophoresis.

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