IMMUNOCHEMICAL IDENTIFICATION OF β_2-GLOBULIN IN METASTASES OF OVARIAN TUMORS

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Investigations of the antigenic structure of the tissue of human ovarian tumors by traditional immunochemical methods and also with the aid of monoclonal antibodies have led to the identification of several antigens which are promising for the diagnosis of ovarian carcinoma [7, 8, 10, 11]. These include principally CA-125 [7], antigen 19-6 [10], and antigen AOA-1 [8].

Despite extensive immunochemical studies of the tissue of primary ovarian carcinomas, the particular features of the antigenic structure of metastases of ovarian tumors remain unstudied. Metastasization of tumor cells from the ovary occurs most frequently into the peritoneum and greater omentum by cell implantation, and metastatic nodules in the omentum are regarded as an autogenously transplanted culture of ovarian carcinoma cells [2].

This paper describes some of the features of the antigenic structure of metastases of ovarian carcinoma in the omentum and identification of ovarian metastatic β_2-globulin.

EXPERIMENTAL METHOD

Extracts from tissues of internal organs, tumor, and metastases were prepared in Tris-glycine buffer (pH 8.3) in the ratio of 1:2 (w/v). Antisera were obtained [2] by immunizing rabbits with whole and fractionated extracts of metastases of a primary ovarian carcinoma into the omentum. The antisera were adsorbed with plasma from normal blood donors, with a mixture of extracts of tissues from normal organs (liver, kidney, spleen, lungs, heart, brain, thyroid gland, pancreas, prostate, skin, stomach, large and small intestine, adrenal gland, ovary, testis, peripheral nerve, omentum, muscle, cartilage), and with a hemolysate of erythrocytes and leukocytes. From the antisera thus exhausted the γ-globulin fraction was salted out with ammonium sulfate in 35% saturation, and the degree of exhaustion was verified by the agar precipitation test with the above-mentioned organs and with blood serum of individual donors. Specific test systems were modeled and used to study cross reactions with extracts from organs of human adults and fetuses and also with biological fluids (amniotic and ascites fluids, lymph).

Altogether 165 samples of definitive tissues, 63 from embryonic tissues, 61 from primary ovarian carcinomas, 42 from metastases of an ovarian carcinoma into the omentum, 32 from tumors in other situations (uterus, kidney, prostate, liver), 104 from blood serum of individual donors, 8 from neonatal blood serum, 24 from blood serum of pregnant women, 10 from amniotic fluid (8th-41st weeks of pregnancy), 32 from blood serum of patients with ovarian carcinoma, and 36 from blood serum of patients with benign tumors of the ovaries and uterus, were studied.

The general methods of immunochemical analysis were used: immunodiffusion analysis in agar with a standard test system [6], with a sensitivity of 3 mg/liter, immunoelectrophoresis and isoelectric focusing [1], disk electrophoresis [4], gel filtration [3], affinity chromatography [5], and disk-immunoelectrophoresis [4].

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TABLE 1. Physicochemical Characteristics of OMA-8

Property	Characteristics
Electrophoretic mobility (in agar and agarose)	β_2-globulin
Relative electrophoretic mobility	0.26 ± 0.02
Molecular weight, kD	
Gel-filtration method on Sephadex G-100	35 ± 3
Electrophoresis in 10% polyacrylamide gel with sodium dodecylsulfate	α — 18 ± 1 β — 19.5 ± 1
Isoelectric point	6.87
Solubility: in ammonium sulfate at 50% saturation	Insoluble
In 0.6M sulfosalicylic acid	Insoluble
In 0.6M perchloric acid	Insoluble
In 5% TCA	Insoluble
Thermostability	Unstable (65°C, 30 min)
Resistance to enzyme action: protease	Inactivated
Nuclease	No effect on activity
Interaction with: phenyl-sepharose	Binds
Blue sepharose	Is not bound
DEAE-Sephadex (pH 8.0)	The same
Estradiol-sepharose	The same
Testosterone-sepharose	The same
Reaction for: proteins (Coomassie P-250, Amido Black)	Positive
For glycoproteins (PAS reaction)	Negative
Lipoproteins (Sudan B)	Negative
Ferroproteins: $K_4Fe(CN)_6$	Negative

EXPERIMENTAL RESULTS

Analysis of the adsorbed antisera enabled eight antigens to be identified in extracts from metastases of ovarian carcinoma. All these antigens were found with the aid of rabbit antisera, obtained by immunization with extracts from tissue of metastases of ovarian carcinoma into the omentum, and they were therefore called "ovarian metastatic antigens" (OMA). Seven of them gave a cross reaction with antigens of one normal adult human organ (antigens with wider specificity were not taken into account); three gave a cross reaction with kidney antigens, two with splenic antigens, and two with brain antigens. OMA-8, which was not found in tissues from the internal organs of human adults and fetuses, may be regarded as the most interesting.

OMA-8 was identified by immunodiffusion analysis in extracts of metastases of ovarian carcinoma into the omentum in a concentration of 3-180 mg/liter in 55% of cases. It was found in malignant ovarian tumors in 50% of the cases in a concentration of 3-50 mg/liter. It could not be found in tissue extracts from benign tumors of the ovary and uterus or of malignant tumors of other organs (uterus, kidney, prostate, liver). It was also impossible to find OMA-8 by this method in blood serum from normal individuals, from women at different stages of pregnancy, newborn infants, and patients with tumors of the genitalia, or in ascites fluid and lymph from patients with ovarian carcinoma. OMA-8 was found in a low concentration (1-2 μg/g wet weight of tissue) in the chorion (8-20 weeks). Its concentration in the mature placenta (38-40 weeks) was 6-12 μg/g tissue. OMA-8 could not be found in native samples of amniotic fluid at any time of pregnancy, but it could be detected in concentrated samples.

Immunochemical identification using test systems demonstrated the nonidentity of OMA-8 with trophoblastic β-glycoprotein, α-fetoprotein, carcinoembryonic antigen, chorionic gonadotrophin, β_2-microglobulin, transferrin, lactoferrin, and C-reactive protein.

OMA-8 is a protein (Table 1) with molecular weight of 35 ± 3 kD and with electrophoretic mobility of β_2-globulins in agar and agarose (Fig. 1). On electrophoresis in 7.5% polyacrylamide gel OMA-8 forms up to three immunochemically identical fractions in the β-globulin zone. Each of these fractions separately, and also all three fractions pooled, on electrophoresis in 10%

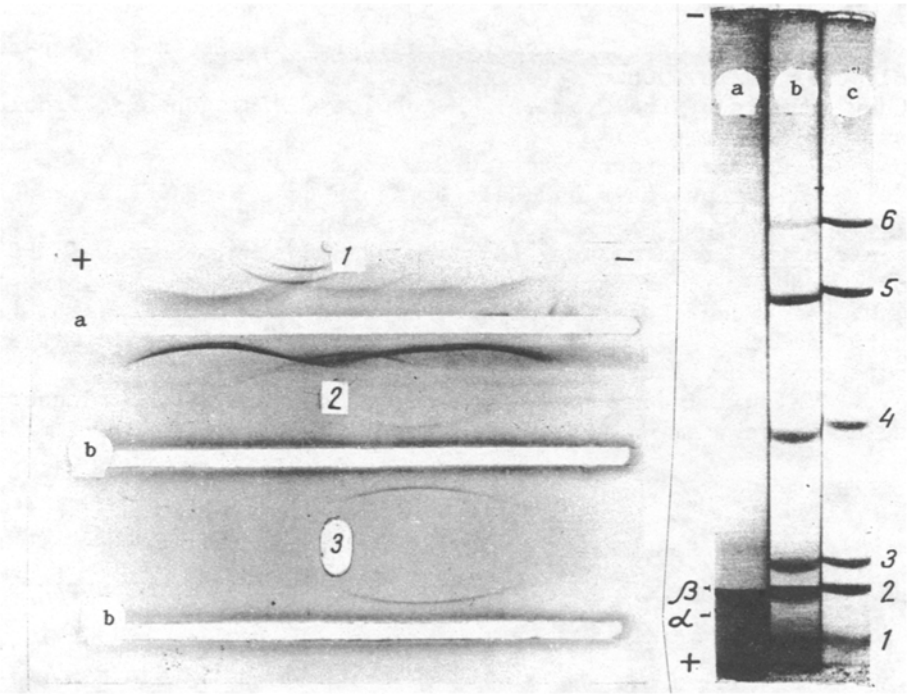

Fig. 1 Fig. 2

Fig. 1. Immunoelectrophoresis of OMA-8 in agar. 1) Blood serum from healthy donor, 2) extract of ovarian carcinoma tissue, 3) extract from tissue of metastasis of ovarian carcinoma into omentum. a) Antiserum to donors' plasma protein, b) antiserum to OMA-8 obtained by immunizing rabbits with tissue extract of metastases of ovarian carcinoma into omentum and exhaustion with plasma from normal blood donors and mixture of tissue extracts of internal organs.

Fig. 2. Disk electrophoresis of OMA-8 in 10% polyacrylamide gel with sodium dodecylsulfate. a) 40 μg OMA-8, b) OMA-8 in mixture of markers for electrophoresis (Pharmacia, Sweden), c) markers for electrophoresis (Pharmacia): 1) α-lactalbumin (molecular weight 14.4 kD), 2) soy trypsin inhibitor (20 kD), 3) carbonic anhydrase (30 kD), 4) ovalbumin (43 kD), 5) bovine serum albumin (67 kD), 6) phosphorylase b (94 kD). α) α-subunit of OMA-8, β) β-subunit of OMA-8.

polyacrylamide gel with sodium dodecylsulfate were found to consist of α- and β-subunits with molecular weight of 18 and 19.5 kD respectively. Moreover, the α-subunit gives a yellowish orange color against the unstained electrophoretic gel, and differs from the β-subunit in staining very weakly for protein (Fig. 2), possible evidence that it contains a prosthetic group. Neither iron, nor lipids, nor carbohydrates, were found in the composition of the α-subunit. The physicochemical properties of OMA-8 (Table 1) are evidence of the marked hydrophobicity and instability of the protein molecule, suggesting that it is enzymic or hormonal in nature.

The distinguishing features of the antigenic structure of ovarian carcinoma metastases into the omentum thus include a marked degree of antigenic divergence (OMA 1-7 are organ-specific) and embryonic reversion (OMA-8 is a specific placental protein). The phenomenon of antigenic divergence evidently reflects the characteristics of formation of the primitive ovaries in the early embryonic period [2]. Accumulation of OMA-8 in tissues of ovarian carcinoma and mature placenta suggests that these biological processes are similar. The possibility cannot be ruled out that OMA-8 takes part in certain common mechanisms, such as those aimed at rejection of the placenta and tumor. The physicochemical and antigenic properties of OMA-8 indicate that it is a new antigen, different from carcinoembryonic and placental proteins already known [9].

The development of sensitive methods of determination of OMA-8 in the blood serum will be interesting in order to assess the diagnostic value of this antigen as a protein marker of ovarian tumors.

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IMMUNOHISTOCHEMICAL STUDY OF CARCINOEMBRYONIC ANTIGEN (CEA) IN NORMAL AND EMBRYONIC HUMAN TISSUES USING THE CEA-SPECIFIC ONCOPRECIPITIN "CRUSTACIN"

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Carcinoembryonic antigen (CEA) [3] is a complex glycoprotein and, as was originally considered, it is produced only by the entoderm of carcinomas of the digestive system and of the embryonic gut. Determination of CEA levels by immunochemical methods has been used successfully to monitor tumor growth after radical treatment, and also as an adjunct to methods of determining the pathology and clinical state of cancer patients [2, 5]. However, this test is ineffective for the early diagnosis of malignant tumors, due to the considerable immunochemical heterogeneity of CEA [8]. Definite progress in increasing the effectiveness of the test for CEA was made by the use of monoclonal antibodies against this antigen [7]. Recently a new class of substances — oncoprecipitins, which interact highly specifically with CEA in the manner of antibodies, has been discovered in marine invertebrates [1]. Oncoprecipitins, as has been shown by immunodiffusion and immunoenzyme methods, do not interact with antigens in extracts of normal human tissues or with other antigens of glycoprotein nature, including with normally cross-reacting antigen (NCA-1) [1].

The aim of the present investigation was to study the possibility of detection of CEA with the aid of an oncoprecipitin, namely crustacin (CR), in sections of human tissues and to compare the results with those of testing with CEA-specific antibodies.

EXPERIMENTAL METHOD

Normal tissues taken at autopsy on persons dying accidentally were studied. Embryonic tissues were obtained from Moscow gynecologic hospitals. Histological material was obtained by acetic–alcohol fixation of tissue fragments followed by embedding in paraffin wax [9]. Some histological material was obtained from the collection of the Department of Pathomorphology of the P. A. Gertsen Moscow Research Institute of Oncology, and was prepared by fixation of tissue fragments in buffered formalin, followed by embedding in paraffix wax. The thickness

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