Antigenic Structure of Ovarian Cancer Metastases

P. G. Prokopenko, S. A. Borisenko, and A. A. Terent'ev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 131, No. 6, pp. 665-668, June, 2001
Original article submitted January 31, 2001

Twenty highly specific (organ-specific and tumor-associated) antigens named ovarian metastatic antigens were revealed using polyclonal antibodies isolated from rabbits after immunization with soluble antigens from ovarian cancer metastases. These protein antigens were not detected by precipitating test system (sensitivity 1 mg/liter) in tissues of adult humans (except for organ-specific antigens) and blood plasma from healthy donors. Ovarian metastatic antigens included organ-specific, placenta-specific, tumor embryonic, tumor-associated, and reactive proteins. The precipitating test system identified 15 antigens in the amniotic fluid from pregnant women and 7 antigens in the plasma from patients with ovarian cancer (incidence 16-75%).

Key Words: *human ovarian cancer; antigenic structure of metastases; organ-specific metastatic antigens; tumor-associated antigens*

Studies of the antigenic structure of tumor tissues allow us to understand the molecular mechanisms of carcinogenesis and to identify specific tumor markers in the plasma. Various markers of human ovarian adenocarcinoma, including antigen CA-125, were identified [2,11-15]. However, none of them can be used in clinical and experimental immunodiagnostics of human ovarian carcinoma. Primary human ovarian carcinomas are extensively studied now; however, the antigenic structure of ovarian cancer metastases remains unclear.

Here we studied the antigenic structure of metastases of primary human ovarian carcinoma.

MATERIALS AND METHODS

Specimens of primary epithelial ovarian tumors (n=237) and metastases from ovarian cancer to the omentum (n=89) were analyzed. For evaluation of the distribution of antigens and their specificity, we tested 1721 plasma samples from 617 patients, 162 pregnant women, 94 neonates, and 841 healthy donors (523 women aging 17-45 years and 318 men aging 18-45 years), 1626 extracts from internal organs of adult humans and fetuses, and extracts from benign epithelial ovarian tumors (n=127), uterine cancer (n=85), and uterine myoma (n=23).

Soluble tissue antigens were extracted with Tris-glycine buffer (6 g Tris, 28.8 g glycine, and 1000 ml distilled water, pH 8.3) and Triton X-100 (1:1000). Tissue homogenate (1:2 tissue:buffer, w/v) was frozen, defrosted, and centrifuged. Protein concentration in extracts was ~40 mg/ml.

Rabbits (n=53) were repeatedly immunized (3-10 times at 1.5-2.0-month intervals) with extracts from metastatic tissues. Antimetastatic sera (AMS) were thoroughly absorbed with the plasma and serum from donors, mixture of extracts from internal organs of adult humans, and erythrocyte and leukocyte lysates. γ-Globulin fraction was precipitated from exhausted AMC (35% saturation with ammonium sulfate). The degree of exhaustion was estimated by precipitation in agar gel with individual samples of internal organs from adult humans and blood serum from donors. AMS absorption and γ-globulin superprecipitation were performed to the total exhaustion of AMS antibodies against normal tissue and serum antigens. However, some antisera reacted with organ-specific antigens of the kidneys, brain, and spleen from healthy adult humans (antigens 1-7, Table 1). Antigens detected in

Department of Biochemistry, Russian State Medical University, Moscow

0007-4888/01/1316-0561$25.00

more than 1 organ (interorgan or non-organ-specific antigens) were excluded from the experiment. Protein concentration in AMS γ-globulin fractions was 40-80 mg/ml. Studies of antigens in extracts from metastases and primary ovarian tumor tissues were performed with exhausted AMS. The precipitating test system included the antigen found. The specificity of this antigen was estimated with individual extracts from tissues of internal organs, fetuses, biological fluids, and blood sera from donors. We performed a direct comparison of precipitating test systems for each antigen with known embryonic, tumor-associated, placental, and reactive proteins, including α-fetoprotein, trophoblastic β-glycoprotein (TBG designated as SP-1 or PSG), α_2-globulin from pregnant women, ferritin, lactoferrin, embryonic cancer antigen, brain cancer antigen (BCA), embryonic prealbumin, human chorionic gonadotropin, placental proteins 12 and 14 (PP-12 and PP-14, respectively), β_2-microglobulin, and C-reactive protein [8].

The following methods of immunochemistry and structural protein chemistry were used: precipitation in agar with standard test systems for individual antigens, immunoelectrophoresis, isoelectrofocusing, electrophoresis in PAAG, gel filtration on Sephadex G-100 and G-200, and ion-exchange and affinity chromatography [3,5,9,10].

RESULTS

Rabbit antisera obtained after immunization with soluble antigens from tissues of ovarian cancer metastases detected 20 highly specific (organ-specific and tumor-associated) antigens, ferritin, and TBG. The antigens detected with AMS were named ovarian metastatic antigens (OMA). These protein antigens were

TABLE 1. Ovarian Metastatic Antigens

OMA	Electrophoretic mobility	Molecular weight, kDa		Isoelectric point	Precipitation with ammonium sulfate, % saturation	Localization	
		gel filtration	electrophoresis in PAAG			adult	fetuses
1	α_2-Globulin	600	—	—	25-45	Kidney	Kidney
2	β_1-Globulin	110	—	—	50-70	Kidney	Kidney
3	β_1-Globulin	60	—	—	40-60	Kidney	Kidney
4	α_1-Globulin	115	—	—	50-75	Spleen	Spleen
5	β_1-Globulin	22	—	—	35-55	Spleen	Spleen
6	α_2-Globulin	130	16	—	30-50	Brain	Brain, gastrointestinal tract
7	Prealbumin	24	—	—	60-80	Brain	Brain
8	β_2-Globulin	38	19	6.87	30-50	Placenta	
9	α_1-Globulin	60	60	4.44	30-50	—	Gastrointestinal tract
10	α_2-Globulin	55	52	7.38	30-50	—	Gastrointestinal tract
11	α_2-Globulin	14	—	7.55	30-50	—	—
12	α_1-Globulin	36	18	6.17	30-50	—	—
13	β_2-Globulin	68	—	—	30-50	—	Stomach
14	β_2-Globulin	55	—	—	30-50	—	—
15	β_1-Globulin	32	—	—	50-70	—	Stomach
16	β_2-Globulin	50	—	—	40-60	—	—
17	Prealbumin	21	—	—	50-70	—	—
18	α_1-Globulin	32	—	—	60-80	—	—
19	α_2-Globulin	12	—	—	40-60	—	—
20	β_2-Globulin	100	—	—	30-50	—	—
21	α_2-Globulin (ferritin)	454	18.9	5.4	30-50	All tissues	
22	β_1-Globulin (TBG)*	105	42; 60	4.0	30-50	Placenta	

Note. *Dissociation into major and minor polypeptide chains with molecular weights of 42 and 60 kDa, respectively [6].

not found in tissues from adult humans (except for organ-specific antigens) and in the plasma from healthy donors. Two antigens (OMA 1 and 6) were previously identified as kidney-specific α_2-macroglobulin [6] and BCA [1]. Other antigens had no analogues.

Studies of OMA distribution in various tissues revealed organ-specific (OMA 1 and 7), placenta-specific (OMA 8 and 22), tumor embryonic (OMA 9, 10, 13, and 15), tumor-associated (OMA 11, 12-14, and 16-29), and reactive proteins (OMA 21).

It should be emphasized that OMA 4-20 (except for OMA 6 and 10) were found in the amniotic fluid from pregnant women (22-27 weeks of pregnancy) by immunodiffusion assay (sensitivity 1 mg/liter). However, some antigens (OMA 8 and 12) were detected by this method only in 10-20-fold concentrated samples of the amniotic fluid.

Immunodiffusion assay revealed metastatic antigens (16-75% samples) in the blood serum from patients with ovarian tumors. Therefore, the concentration of tumor tissue protein in the blood serum from patients is high (above 1 mg/liter). These antigens (n=7, OMA 9, 11, 12-14, 16, and 21) were designated as serum OMA.

Kidney-specific proteins found in tumor and metastatic tissues from ovarian cancer are of particular interest.

Taking into account the development of human ovaries during embryogenesis [4], it can be assumed that organ-specific proteins of the kidneys and brain (OMA 1-3, 6, and 7) are the proteins of embryonic ovarian structures (rudiments of primary kidneys, epoophoron, paroophoron, and medullar cords), which can be also expressed in tumor cells. Identification of kidney-specific antigens (OMA 1-3) in embryonic ovaries provide good basis for the development of specific methods for immunodiagnostics of ovarian tumors.

The diversity of highly specific antigens in metastases is probably related to the following facts. First, malignant transformation occurs in primary tumor node carrying a large repertoire of antigens (from normal organ-specific antigens to necrotic antigens of dead cells). Second, primary tumor node is enriched with erythrocytic and leukocytic antigens. And third, primary tumor node also contains true (formed) tumor cells, but their relative content is low and variable. Therefore, immune reactions of normal, erythrocytic, leukocytic, and necrotic antigens interfere (mask) with the antigenic specificity of tumor cells [8]. By contrast, metastatic cells (autogenously transplanted culture of tumor cells [4]) can be considered as pure tumor cells deficient in normal, necrotic, and other antigens hindering antibody isolation. Thus, immunogenicity of tumor cell antigens is manifested to a greater extent.

Our results indicate that metastases of primary tumors is the most perspective object for studies of the antigenic structure of tumor cells and the search for specific tumor markers.

REFERENCES

1. S. A. Borisenko, P. P. Kulagin, and M. A. Chishieva, *Akush. Gin*., No. 7, 63-65 (1976).
2. S. A. Borisenko, *Immunochemical Assay of Antigenic Structure of Human Ovarian Adenocarcinoma*, Abstract of Cand. Med. Sci. Dissertation, Astrakhan (1977).
3. E. Gaal', G. Med'eshi, and L. Veretskei, *Electrophoresis in Separation of Biological Macromolecules* [in Russian], Moscow (1982).
4. M. F. Glazunov, *Ovarian Tumors* [in Russian], Leningrad (1961).
5. *Practical Protein Chemistry* [in Russian], Ed. A. Darbre, Moscow (1989).
6. P. G. Prokopenko, *Byull. Eksp. Biol. Med*., **84**, No. 8, 210-212 (1977).
7. P. G. Prokopenko, Yu. S. Tatarinov, S. A. Borisenko, *et al*., *Ibid*., **109**, No. 6, 573-574 (1990).
8. P. G. Prokopenko, *Identification and Immunochemical Assay of Protein Markers of Ovarian Cancer*, Abstract of Doct. Med. Sci. Dissertation, Moscow (1994).
9. G. V. Troitskii, I. F. Kiryukhin, and V. L. Zav'yalov, *Byull. Eksp. Biol. Med*., **75**, No. 2, 118-120 (1973).
10. N. I. Khramkova and G. I. Abelev, *Ibid*., **52**, No. 12, 107-110 (1961).
11. R. C. Bast, M. Feency, H. Lasarus, *et al*., *J. Clin. Invest*., **68**, No. 5, 1331-1337 (1981).
12. J. Bergmann, J. M. Biclart, M. George, *et al*., *Cancer*, **59**, No. 2, 213-217 (1987).
13. M. Bhattacharia and J. J. Barlow, *Ibid*., **3**, No. 3, 1616-1620 (1978).
14. S. Knauf and Y. Urbach, *Am. J. Obstet. Gynecol*., **127**, No. 7, 705-710 (1977).
15. K. Sonoda, M. Nakashima, T. Kaku, *et al*., *Cancer*, **77**, No. 8, 1501-1509 (1996).