Dissemination of Human Solid Tumor Cells in the Bloodstream of Immunodeficient Mice

A. D. Mikhailov, A. A. Malakhov, E. S. Revazova, S. A. Moroz, T. V. Piskunova, T. I. Valyakina, and T. V. Yudicheva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 12, pp. 615-618, December, 1995
Original article submitted March 10, 1995

Seven human solid tumor cell lines transplanted into hereditarily immunodeficient mice (nude and beige/nude) were typed for tumor-associated surface antigens and glycoconjugates using fluorescent conjugates of 7 monoclonal antibodies, 5 lectins, and 2 ligands. With this set of 14 selected tumor markers, peripheral blood samples from mice bearing the respective tumors were examined by flow cytofluorimetry for the presence of tumor cells disseminated in their circulation. Tumor cells were detected in the blood of mice carrying a uterine tumor, indicating that the metastatic process can be followed intravitally in immunodeficient animals bearing human solid tumors.

Key Words: *nude mice; beige/nude mice; xenotransplants; metastases; monoclonal antibodies*

The phenomenon of spontaneous metastases in man differs from that in immunodeficient animals. Thus, it is only in rare cases that a well-developed human tumor is seen to metastasize in an immunodeficient animal [5]. The presence of melanoma cells in the bloodstream of animals has been shown to be a reflection of the metastatic process [6]. The aim of the present study was to find promising models for kinetic studies of the metastatic process in experimental animals bearing human tumors and for demonstrating antimetastatic properties of chemotherapeutic agents.

MATERIALS AND METHODS

Cells of eight human solid tumors - one melanoma (Bro [Mel-7]), one neuroblastoma (NeuBl), one breast carcinoma (C_{mam}-4 [MaTu]), three hepatomas (C_{hep}-1 [Alex], C_{hep}-2, and C_{hep}-3), one pulmonary squamous cell carcinoma (C_{pul}) and one uterine carcinoma (C_{ut}) - maintained in cell cultures in the collection of our laboratory at the Cancer Research Center (Moscow) were cultured in DMEM medium supplemented with 10% fetal calf serum and then transplanted, after being separated by centrifugation, to mice subcutaneously into the back. Seven monoclonal antibodies (MAb) were isolated by thiophilic chromatography from ascitic fluids of mice contaminated with the corresponding hybridomas [4]. The oncoprecipitins OP1 and OP2 were isolated from tropical ascidians (*Didemnum ternatum*) collected in the Indian and Pacific Oceans, respectively [3]. The ligands wheat germ agglutinin (WGA), α-lactalbumin (α-LA) (Sigma), and galactosyltransferase (GalT) were isolated and purified as previously described [1].

The MAb, lectins, and ligands were labeled with fluorescein isothiocyanate (FITC) in a 0.1 M carbonate buffer (pH 9.4 or, for GalT, pH 8.5, for 20 h at 4°C) containing protein in concentrations of 2-6 mg/ml, the FITC to protein ratio being 70 μg to 1 mg. The resulting conjugates were then separated from free FITC in an isocratic mode in a 16×400 mm column filled with Superose 12 gel and equili-

Cancer Research Center, Russian Academy of Medical Sciences, Moscow; Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow; Pacific Institute of Bioorganic Chemistry, Far-Eastern Division of the Russian Academy of Sciences, Vladivostok. Address for correspondence: Cancer Research Center, 24 Kashirskoe Shosse, 115478 Moscow, Russia. E-mail: walther@edito.msk.su (Presented by Yu. N. Solov'ev, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0012-1230$12.50

brated with a phosphate-buffered saline containing 0.005% Merthiolate. The cells were stained and their fluorescence was measured as described earlier [2], taking 4000 and 100,000 stained cultured and stained blood cells per measurement, respectively.

RESULTS

As we did not have antibodies produced to tumor-associated surface antigens and specifically recognizing particular tumor cells (perhaps with the exception of the MAb to the tumor-associated melanoma antigen), our first task was to find out which of the tumor-associated antigens, lectins, and ligands could be of help, individually or in combination, in isolating individual cells present in the circulation of immunodeficient mice with spontaneously developing metastases. The MAb to pulmonary carcinoma antigen was expected to provide a marker for pulmonary carcinoma cells; that to carcinoembryonic antigen, a marker for breast cancer, pulmonary carcinoma, and hepatoma cells; that to prolactin, a marker for breast cancer cells; that to α-fetoprotein (α-FP), a marker for hepatoma cells; and that to ovarian carbohydrate antigen (CA-125), a marker for breast and uterine cancer cells. We did not anticipate a narrow specificity from the lectins and ligands but did hope that they could help, when used in combination with other markers, in identifying tumor cells and defining more precisely the carbohydrate profiles of their surfaces.

The results of our measurements are presented in Table 1, where the numerator gives the percentage of antigen-positive cells and the denominator, the ratio of the mean log fluorescence of a stained sample to the mean log autofluorescence of the same sample when unstained. These two results supplement each other, for the ratio of log fluorescences reflects the relative density of binding sites on the surface of antigen-positive cells. In other words, where the numerator has a high value and the denominator a low value (as, for instance, in the case of pulmonary carcinoma (C_{pul} cells stained with WGA), this means that the staining is virtually total and the binding site density is low. The 1.45 ratio of log fluorescences ($Mean_{autofluorescence}+3\,\sigma$) was judged to be the threshold (cutoff value) beyond which the use of a given marker for identifying cells in the circulation is inadvisable.

Our expectations regarding MAb specificity were fulfilled only partially. As expected, the MAb to carcinoembryonic antigen was actively bound by breast cancer cells; the MAb to α-FP, by hepatoma cells of the different lines; and the MAb to tumor-associated melanoma antigen, by melanoma cells. However, the MAb to prolactin and the MAb to pulmonary carcinoma antigen consistently stained

TABLE 1. Mapping of Tumor Cell Surface Antigens and Glycoconjugates against Donor Blood Cells Using Flow Cytofluorimetry

Human tumor cell line	Monoclonal antibodies to							Lectins				Ligands	
	MEI	Loc	CEA	pLac	α-FP D10	α-FP H12	CA-125	OP1l	OP1h	OP2l	OP2h	WGA	α-LA	GalT
BRO (Mel-7)	36.95/2.11	0/1.32	0/0.93	0/1.21	22.7/1.25	0/0.91	0/0.89	0/1.07	91.0/5.50	0/1.15	91.8/6.66	0/1.19	10.9/1.64	0/1.41
NeuBl	25.8/1.68	0/1.03	7.32/1.40	0/1.00	37.2/2.04	30.7/1.77	27.9/1.57	0/0.90	91.6/5.07	0/0.90	71.7/4.90	0/1.01	8.8/1.38	0/1.02
MaTu (C_{mam}-4)	35.3/1.20	0/0.79	33.2/1.56	0/1.18	36.9/1.63	9.1/1.39	4.9/1.30	11.5/1.35	72.8/6.30	13.5/1.40	87.1/5.97	21.6/1.25	10/2.09	56/1.14
Alex (C_{hep}-1)	0/1.01	15.4/1.95	7.4/1.51	12.8/1.81	67.0/1.97	36/1.58	30.5/1.53	0/1.00	-	0/1.06	87.5/9.66	89.9/1.65	23.5/2.05	75.4/1.50
C_{hep}-2	0/0.92	0/0.98	10.2/1.44	0/1.00	48.8/1.60	19.8/1.30	8.8/1.22	0/0.84	0/0.90	0/0.93	85.6/3.90	0/1.03	4.00/1.22	0/1.05
C_{hep}-3	3.65/1.16	0/1.03	2.47/1.20	0/1.05	72.7/2.15	61.2/1.87	43.3/1.83	0/1.18	99.2/4.91	0/1.03	98.6/5.01	0/0.99	5.04/1.40	0/0.95
C_{pul}-4	0/0.98	5.4/1.21	-	3.4/1.30	71.3/1.48	32.3/1.06	0/0.95	0/0.89	5.03/1.25	0/0.91	46.3/3.25	87.0/1.54	3.3/1.35	0/1.01
C_{ut}	0/1.14	0/0.79	1.3/1.09	0/1.26	4.0/1.19	0/1.03	42/2.11	0/1.32	61.2/5.13	0/1.20	79.1/7.55	2.1/1.11	2.4/1.39	0/1.00
Blood from control mouse	0/0.97	3.1/1.13	0/1.00	0/0.98	52.0/10.5	0/0.96	23.2/2.40	0/0.93	4.0/1.15	0/0.88	28.8/2.62	39.5/3.15	11.8/2.03	0/0.98
Blood from healthy human	0/0.93	0/1.01	0/1.00	0/0.97	0/0.95	6.3/1.33	0/1.05	0/0.99	0/1.00	0/1.02	0/1.00	0/1.03	0.6/1.06	0/0.96

Note. Monoclonal antibodies (MAb): MEI, to tumor-associated antigen of human melanoma; Loc, to human pulmonary carcinoma; CEA, to carcinoembryonic antigen; pLac, to prolactin; α-FP D10 and α-FP H12, to different epitopes of human α-fetoprotein; CA-125, to ovarian carbohydrate antigen. Lectins: OP1 and OP2 = two oncoprecipitins from marine invertebrates, low (l) and high (h) molecular-weight fractions; WGA = wheat germ agglutinin. Ligands: α-LA = α-lactalbumin; GalT = galactosyltransferase. A dash indicates that the data are unreliable.

TABLE 2. Marker–Positive Cells per 100,000 Blood Cells of Tumor–bearing Immunodeficient Mice

Marker cells	Mice	
	nude	beige/nude
NeuBl	0	0
	0	0
		0
C_{mam}-4	0	319
	0	0
C_{ut}	12	4970
	350	975

Note. The marker used was α–FP H12.

only hepatoma-1 (C_{hep}-1) cells (13-15% of the cells were antigen-positive). One clone of antibodies to α-FP (D10) stained nearly all the cell lines tested well, and it also stained murine blood cells. The MAb to Ca-125, in addition to staining uterine cancer cells, stained neuroblastoma cells and hepatoma cells of two lines. C_{hep}-1 cells were appreciably stained by all antibodies, which suggests that immunoglobulin-binding sites may be present on the surface of these cells.

The lectins, as anticipated, failed to show high specificity. WGA, which binds to sialic acid terminal residues and to N-acetylglucosamine, had to be used in combination with galactosyltransferase, an enzyme specific for N-acetylglucosamine, to differentiate the terminal residues of sialic acid from N-acetylglucosamine: pulmonary carcinoma-4 (C_{pul}-4) cells, which were stained only by WGA, but not by the other marker, appear to carry only sialic acid residues, whereas hepatoma-1 (C_{hep}-1) and breast cancer (C_{mam}-4) cells, stainable with both markers, carry the terminal carbohydrate residues of N-acetylglucosamine (or both these and sialic acid residues). An unexpected finding was the extraordinarily high activity of the oncoprecipitins from the marine animals *Didemnum ternatum*: they yielded staining rates of 70-99% with most tumor cells (OP1h failed to stain C_{hep}-2 and C_{pul}-4 cells), which had extremely high binding site densities on their surfaces. Unfortunately, the oncoprecipitins also stained to a certain extent (considerable in the case of OP2h) murine (but not human) blood cells, and hence can be used for screening cells in murine blood only in combination with another marker The low-molecular-weight fractions of the oncoprecipitins stained only breast cancer (C_{mam}-4) cells; this is a reflection of their previously demonstrated specificity for trophoblastic globulin [8], which is commonly detected in patients with breast cancer.

α-LA, a ligand specific for galactosyltransferase, stained, as expected, most tumor and nontumor cells, the highest staining rates being observed with breast cancer cells (which is in accord with the reported activity of this enzyme [7]) and C_{hep}-1 cells.

On the basis of the results obtained, the following markers were selected for the detection of human tumor cells of the eight lines in the circulation of immunodeficient mice:

- MEI and its combinations with OP1h and OP2h for Mel-7;
- α-FP D10 and H12 and their combinations with OP1h and OP2h for NeuBl;
- CEA, α-FP D10, OP1l, OP2l and their combinations with OP1h and OP2h for C_{mam}-4;
- GalT in combination with OP2h for C_{hep}-1;
- α-FP H12, CA-125, and their combinations with OP1h and OP2h for C_{hep}-3;
- WGA in combination with OP2h for C_{pul}-4;
- CA-125 and its combination with OP1h for C_{ut}.
- No suitable combination(s) could be found for C_{hep}-2.

Combined staining with more than one marker was not used in this study for technical reasons, although its usefulness is obvious.

Our experiments to detect Mel-7 cells in the blood were published earlier. The results we obtained for human neuroblastoma (NeuBl), breast cancer (C_{mam}-4), and uterine cancer (C_{ut}) cells are presented in Table 2. It can be seen that tumor cells frequently cannot be detected even in the blood of immunologically very weakened animals transplanted with human solid tumor cells. In fact, meaningful numbers of tumor cells in the blood of immunodeficient mice were detected only in the case of uterine cancer. As in our previous experiments with melanoma, the largest numbers of tumor cells were detected in animals with combined immunodeficiency (beige/nude mice), although a propensity for tumor cell dissemination from the primary tumor was also shown by the other mouse strain (nude).

The above-described approach to the monitoring of tumor cell dissemination in the bloodstream of immunodeficient animals may serve as a basis for the development of test models to study the phenomenon of metastasis.

REFERENCES

1. A. D. Mikhailov and K. I. Zhordaniya, *Biokhimiya*, **55**, № 11, 2103-2107 (1990).
2. A. D. Mikhailov, A. A. Malakhov, E. S. Revazova, and T. I. Valyakina, *Byull. Eksp. Biol. Med.*, **118**, № 8, 188-191 (1994).
3. S. A. Moroz, *Dokl. Akad. Nauk SSSR*, **305**, № 5, 1256-1258 (1989).
4. M. Belew, N. Juntti, A. Larson, and J. Poreth, *J. Immunol. Methods*, **102**, 173-182 (1987).
5. T. Furukawa, X. Fu, T. Kubota, *et al.*, *Cancer Res.*, **53**, № 5, 1204-1208 (1993).
6. A. Lozupone, G. Micelli, M. Quaranta, *et al.*, *Anticancer Res.*, **12**, № 6A, 1799 (1992).
7. A. S. Masibay, P. V. Balaji, E. E. Boeggeman, and P. K. Qasba, *J. Biol. Chem.*, **268**, № 13, 9908-9916 (1993).
8. K. A. Myers, V. Rahi-Saund, M. D. Davison, *et al.*, *J. Biol. Chem.*, **269**, № 12, 9319-9324 (1994).