ONCOLOGY

Phenotypic Characteristics of Lewis Lung Carcinoma Cells

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We studied adhesive properties and physiological activity *in vivo* of cells from Lewis lung carcinoma and its metastases. These cells differed in tumorogenic activity and metastatic potential in the syngeneic system. *In vivo* non-metastasizing cells are characterized by a lower content of surface lectins to tetrasaccharides SiaLe^x [Neu5Acα2-3Galβ1-4(Fucα1-3) GlcNAcβ] and SiaLe^a [Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ] and trisaccharide HSO_3Le^x [$HSO_3$2-3Galβ1-4(Fucα1-3)GlcNAcβ] compared to cells forming metastases in the syngeneic system. Metastatic cells with low tumorogenic activity weakly expressed lectins to disaccharide ligands 6-SiaLac [Neu5Acα2-6Galβ1-4Glc], 6-HSO_3LacNAc, and A-di [GalNAcα 1-3Galβ] and trisaccharides H-type 1 [Fucα1-2Galβ1-3GlcNAc and Le^x [Fucα1-3(Galβ 1-4)GlcNAc] compared to cells that initiated tumor formation in the syngeneic system (similarly to transplanted tumors). We hypothesized that cell receptors to these carbohydrate determinates are involved in the development and growth of primary tumors, while lectins to SiaLe^x, SiaLe^a, and HSO_3Le^x play a role in the progress of tumor process and metastasizing.

Key Words: *pectins; carbohydrate ligands; phenotype; metastatic potential; tumorogenicity*

The development of malignant tumors is a complex multistage process that includes cell invasion into host tissues, penetration into lymphatic and blood vessels, adhesion to cells in target organs, and growth of secondary tumors. Cell receptors play an important role at various stages of the "host-tumor" interaction [8,9]. These receptors interact with microenvironmental ligands and regulate expression of genes controlling the synthesis of proteolytic enzymes (plasminogen activator, collagenase, and cathepsin B) involved in cell invasion and metastasizing [3].

Tumor cells differ in their morphology, genotype, protein expression, and other characteristics. Only few circulating tumor cells (about 0.01%) form metastases [5]. These cells differ from cells not forming metastases by surface proteins [7]. Some authors hypothesized that metastatic cells with highly specialized phenotype initially present in the heterogeneous cell population of primary tumors [6].

Taking into account the important role played by lectins in the development of malignant neoplasms, we compared receptors, tumorogenic activity, and metastatic potential of Lewis lung carcinoma and its metastases.

MATERIALS AND METHODS

Cells obtained from primary node (PLMG-T) and metastases (PLMG-M1 and PLMG-M2) of Lewis lung carcinoma were cultured in RPMI-1640 medium with 7% fetal bovine serum.

Tumorogenic activity and metastatic potential of cells were studied in a syngeneic system (BDF1 mice aging 8-10 weeks). Experiments were repeated twice. The cells were implanted subcutaneously (10^6 cells) in

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0.2 ml RPMI-1640 medium. The mixture of PLMG-T and PLMG-M2 cells contained 5×10^5 cells of both types. Control mice received subcutaneously 0.3 ml suspension of minced tumor tissue (1 g sample was minced and suspended in 10 ml RPMI-1640 medium). Tumor volume and mortality rate were determined daily. The number of metastases in the lungs was estimated after autopsy.

For evaluation of the adhesive properties of cell lectins, the cells were harvested using 0.2% EDTA and washed with phosphate buffered saline. Washed cells were transferred on slides, dried on air, fixed in 10% formaldehyde vapors for 3 min, mixed with fluorescently-labeled neoglycoconjugates (0.3 mg per ml phosphate buffered saline), and incubated in a moist atmosphere at 37°C for 1 h. The dye was washed out, and the preparations were dried on air. Binding of cells to conjugates was determined visually by the intensity of fluorescence under a Leitz Orthoplan microscope at the excitation and emission wavelengths of 495 and 530 nm, respectively. We used 24 probes, whose carbohydrate components included mono-, di-, tri-, and tetrasaccharides.

Fluorescently labeled glycoconjugates on a polyacrylamide carrier were obtained from the Syntesome GmbH Company [1].

Binding of cells with some carbohydrate probes ($SiaLe^x$ and Le^x) was quantitatively assayed on an Epics Elite flow cytofluorometer (Coulter Electronics). The cells were harvested using 0.2% EDTA, washed, resuspended in 2 ml phosphate buffer, and mixed with 0.2 ml glycoconjugate solution (0.3 mg per 1 ml phosphate buffered saline). After 5-min incubation at 37°C the cells were washed with phosphate buffer and analyzed on a cytofluorometer (no less than 500 cells). The average intensity of fluorescence was measured and expressed in arbitrary units (MultiGraph, IMMUNO, Coulter Electronics).

RESULTS

Tumorogenicity of PLMG-T cells did not differ from the control (by the period of formation and dynamics of tumor growth, Fig. 1). We revealed no differences in the lifespan of control animals and mice receiving PLMG-T cells. However, PLMG-T cells did not induce the formation of lung metastases (Table 1). PLMG-M1 cells had a complete malignant phenotype. Tumorogenic activity and metastatic potential of these cells and lifespan of mice did not differ from the control (Table 1, Fig. 1).

PLMG-M2 cells were characterized by low tumorogenicity. Induration developed 12 days postinjection and gave rise to a tumor node only on day 25. However, metastases were revealed in 100% animals (Table 1, Fig. 1). The effect produced by a mixture of cells with incomplete malignant phenotype (PLMG-T and PLMG-M2) did not differ from the control (Table 1, Fig. 1). Our results are consistent with published data that tumors contain cells with incomplete malignant phenotype. These cells complement each other, maintain malignant growth, and act cooperatively [8].

TABLE 1. Metastatic Potential and Lifespan of Mice ($M\pm m$, n=6)

Experimental conditions	Metastatic potential*	Lifespan, days
Control	5/5	40.4±2.9
PLMG-T	1/6	36.7±5.2
PLMG-M1	6/6	43.4±4.8
PLMG-M2	6/6	67.8±11.4
PLMG-T and PLMG-M2	6/6	43.5±6.1

Note. Numerator: number of metastases. Denominator: number of mice in group.

As differentiated from PLMG-M2 cells possessing no tumorogenicity, PNMG-T and PLMG-M1 cells with tumorogenic activity bound to carbohydrate determinates 6-Sia-Lac, 6-HSO_3LacNAc, A-di, H-type 1, and Le^x (Table 2). PLMG-M1 and PLMG-M2 cells with metastatic potential expressed lectins to carbohydrate ligands $SiaLe^x$, $SiaLe^a$, and HSO_3Le^x (unlike tumor cells). Probably, cell receptors for haptens $SiaLe^x$, $SiaLe^a$, and HSO_3Le^x are responsible for metastatic activity, while receptors for 6-SiaLac, 6-HSO_3LacNAc, A-di, H-type 1, and Le^x are involved in primary tumor formation.

PLMG-M1 and PLMG-M2 cells with metastatic potential more efficiently bound to $SiaLe^x$ that PLMG-

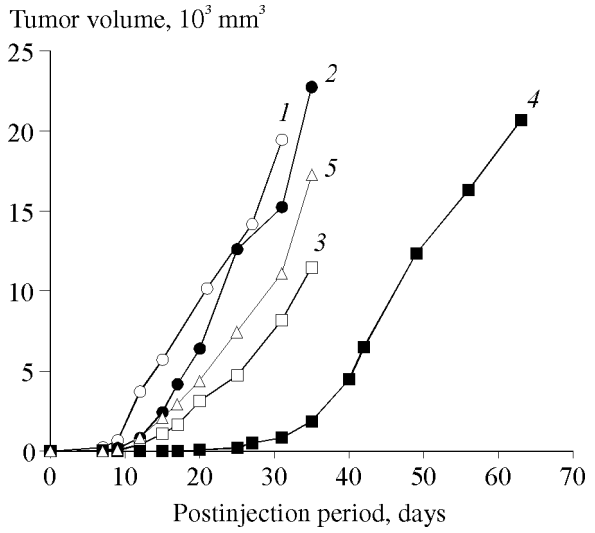

Fig. 1. Tumor growth in the control (*1*) and after administration of PLMG-T (*2*), PLMG-M1 (*3*), and PLMG-M2 cells (*4*) and mixture of PLMG-T and PLMG-M2 cells (*5*).

TABLE 2. Binding of Carbohydrate Probes (Sug-PAA-Flu) to Cells

Sug	PLMG-T	PLMG-M1	PLMG-M2
6-SiaLac [Neu5Acα2-6Galβ1-4Glc]	+++	+	++
$SiaLe^x$ [Neu5Acα2-3Galβ1-4(Fucα1-3) GlcNAcβ]	+	+++	+++
GlcNAcβ1-4GlcNAc	0	++	+++
HSO_3Galβ	+	++	+++
6-HSO_3GlcNAc	+++	++	+++
6-HSO_3LacNAc	+++	++	++
$(Neu5NAc\alpha2\text{-}8)_3$	+	++	+
Galα1-3GalNAcα	0	+	++
Neu5Acα2-3Galβ1-4Glc	+	+	++
$SiaLe^a$ [Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ]	+	+++	+++
Galβ1-3Galβ1	+	+	++
B-trisaccharide [Galcα1-3(Fucα1-2)Galβ]	0	+	++
H type 1 [Fucα1-2Galβ1-3GlcNAc]	++	0	0
A-di [GalNAcα1-3Galβ]	+++	+	+
Le^x [Fucα1-3(Galβ1-4)GlcNAc]	+++	++	+
B_{di} [Galα1-3(Fucα1-2)Galβ]	0	+	+
Neu5Acα	++	+	+++
L-Rhaα	+	+	+
HSO_3Le^x [$HSO_3$2-3Galβ1-4(Fucα1-3) GlcNAcβ]	+	+++	+++
HSO_3Le^a [$HSO_3$2-3Galβ1-3(Fucα1-4) GlcNAcβ]	+	+	+++
A-tri [GalNAcα1-3(Fucα1-2)Galβ]	+++	+	+++
Le^a [Galβ1-3Fucα1-4)GlcNAcβ]	+	+	++
D-Galβ	+	+	+
L-Fucα	++	+	++

Note. 0: no fluorescence. "+", "++", and "+++": weak, moderate, and intensive fluorescence, respectively.

T cells (by 8.8 and 12.9 times, respectively). The intensity of binding of PLMG-T cells to Le^x surpassed that of PLMG-M1 and PLMG-2 cells by 4 and 6 times, respectively.

Our results suggest that primary tumor cells (PLMG-T) cannot adhere and invade the basal membrane or bind lung tissue and form a new colony die to the absence of surface proteins to $SiaLe^x$, $SiaLe^a$, and HSO_3Le^x ligands. By contrast, metastatic PLMG-M2 cells in concentrations much lower than those of primary tumor cells express lectins to 6-SiaLac, 6-HSO_3LacNAc, A-di, H-type 1, and Le^x, which probably explains slow tumor growth after injection of PLMG-M2 cells. PLMG-M1 cells express receptors that do not differ from those present on PLMG-T and PLMG-M2 cells. Therefore, PLMG-M1 cells possess a complete malignant phenotype (similarly to the mixture of PLMG-T and PLMG-M2 cells). $SiaLe^x$, $SiaLe^a$, and 3-O-HSO_3Le^x act as receptors for endothelial E lectins and efficiently bind metastasizing cells [1,4].

Our results show that cells of primary Lewis lung carcinoma and its metastases differ by the receptor repertoire, which probably explains their different physiological activities. Further studies of the phenotype of tumor and metastatic cells and evaluation of their surface properties are important for the development of antitumor preparations [2,10].

REFERENCES

1. N. V. Bovin, *Bioorg. Khim*., **22**, No. 9, 643-663 (1996).
2. E. L. Vodovozova, S. V. Khaidukov, G. P. Gaenko, *et al*., *Ibid*., **24**, No. 10, 760-767 (1998).
3. A. Barendsz-Janson, A. Muller, H. Lichtenbeld, *et al*., *Tumor Biol*., **19**, 104-112 (1998).
4. A. De Frees, L. Phillips, L. Guo, *et al*., *J. Am. Chem. Soc*., **118**, 6101-6104 (1996).
5. I. J. Fidler and I. R. Hart, *Science*, **217**, 998-1001 (1982).
6. L. A. Liotta, K. Tryggvason, S. Garbisa, *et al*., *Nature*, **284**, 67-68 (1980).
7. L. A. Liotta, P. S. Steeg, and W. G. Stetler-Stevenson, *Cell*, **64**, 327-338 (1991).
8. Sh. McBride, P. Meleady, A. Baird, *et al*., *Tumor Biol*., **19**, 88-103 (1998).
9. M. Nakajama, D. Lotan, M. Baig, *et al*., *Cancer Res*., **49**, 1698-1708 (1989).
10. E. L. Vodovozova, G. P. Gaenko, V. I. Razinkov, *et al*., *Biochem. Mol. Biol. Int*., **44**, 543-553 (1998).