ONCOLOGY

Cells of Rhabdomyosarcoma PA-23 Tumor Clones with High and Low Metastatic Potential Differ by Activity of Lysosomal Sialidase

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We studied activity of lysosomal sialidase in cells of rhabdomyosarcoma PA-23 tumor clones with high and low metastatic potential. Low activity of lysosomal sialidase was found in clones characterized by high metastatic potential.

Key Words: *sialidase; tumor cells; rhabdomyosarcoma PA-23*

Mammalian sialidases catalyzing removal of sialic acid in glycoproteins and sphingolipids play an important role in the regulation of cell processes. Several types of sialidases were identified, which differ by their intracellular localization and substrate specificity. Activity of different types of sialidases differs in various organs and tissues [4]. For instance, differentiation of striated muscle cells is associated with increased sialidase activity in the cytosol, while activation of plasma membrane-associated sialidase is a marker of neuronal differentiation [7,8]. Abnormal activity of these enzymes leads to pronounced pathophysiological processes, which is clearly seen in sialidoses accompanied by accumulation of oligosaccharides in lysosomes. In tumors, invasive and metastatic properties of malignant cells depend on lysosomal type of sialidase. High activity of these enzymes in rat fibroblasts transformed with Rous sarcoma virus was associated with reduced invasive activity of tumor cells [6]. *In vivo* experiments showed that cells of rhabdomyosarcoma PA-23 tumor clones with high karyotypic instability are characterized by reduced metastatic potential [2].

In the present study, we evaluated activity of lysosomal sialidase in cells of rhabdomyosarcoma PA-23 tumor clones with different metastatic potential.

MATERIALS AND METHODS

Selection of cells of tumor clones of transplanted organotropic rhabdomyosarcoma PA-23 by the incidence of karyotypic abnormalities and evaluation of the metastatic potential were performed as described previously [1,3]. For measuring activity of lysosomal sialidase in cells, the tumor clones (n=10) were homogenized in potassium-phosphate buffer (10 mM) with 0.25 M sucrose and 1 mM EDTA at 4°C. Phenylmethylsulfonylfluoride (0.2 mM) was added immediately before homogenization. The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was separated (fraction I) and 1 ml fraction I was pipetted and

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TABLE 1. Activity of Lysosomal Sialidase in Cells of Rhabdomyosarcoma PA-23 Tumor Clones with High and Low Metastatic Potential

Clone		Activity of lysosomal sialidase, U/mg protein	
		fraction I	fraction II
With high metastatic potential	A 1	0.802	1.42
	A 2	1.000	1.50
	A 3	0.350	1.40
	A 4	0.001	1.16
	A 5	0.620	1.80
Mean value		0.555±0.183	1.46±0.10
With low metastatic potential	B 1	1.330	2.95
	B 2	0.850	2.60
	B 3	0.700	1.84
	B 4	0.790	2.47
	B 5	1.800	3.71
Mean value		1.094±0.304	2.71±0.31*

Note. *$p<0.01$ compared to clones with high metastatic potential.

centrifuged for 1 h at 40,000 rpm at 4°C for 1 h. The supernatant was removed and the pellet (fraction II) was pipetted in a small volume of buffer for homogenization (220 μl). Activity of lysosomal sialidase was measured against 4-methylumbelliferyl-N-acetylneuraminic acid in a final concentration of 2 mM. The reaction mixture (final volume 200 μl) also contained sodium acetate (pH 4.6 or 5.5). After 1-2-h incubation at 37°C the reaction was stopped by adding 2.5 ml glycine buffer (0.25 M glycine-NaOH, pH 10.4) and the reaction product 4-umbelliferone was measured fluorimetrically at λ=450 nm.

The data were processed statistically using Student *t* test.

RESULTS

After isolation of cells with high and low genome instability by the incidence of cells with interphase bridges, five clones with high and five clones with low incidence of interphase bridges (mean number of interphase bridges 18.5 and 0.5%, respectively) were tested for metastatic potential (Fig. 1). For all doses of transplanted cells, the populations of tumor cells with high genome instability had significantly lower metastatic potential (2.80±0.81) than cell populations with low incidence of interphase bridges (29.1±5.3; $p<0.05$). Measuring of sialidase activity in crude lysosomal fraction (fraction I) showed that clones with low metastatic potential are characterized by higher activity of the enzyme (Table 1). In crude lysosomal fraction obtained from clones

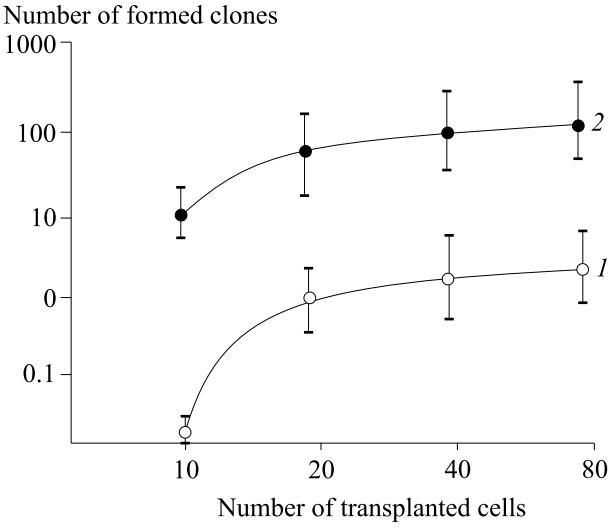

Fig. 1. Metastatic potential of cells of rhabdomyosarcoma PA-23 clones with high (*1*) and low (*2*) incidence of cells with interphase bridges.

with high intensity of metastasizing, activity of sialidase was lower, but the differences between the studied clones of rhabdomyosarcoma PA-23 were insignificant. More than 60% sialidase activity was found in fraction II (irrespective of metastatic potential) obtained after ultracentrifugation, *i.e.* in fraction enriched with lysosomes. In clones characterized by high metastatic potential, activity of lysosomal sialidase was considerably ($p<0.01$) lower than in clones with low metastatic potential (Table 1). These findings agree with the results of previous experiments demonstrating increased activity of lysosomal sialidase in cells of melanoma B16

clones characterized by low metastatic potential [5]. It can be hypothesized that increased content of sialic acid molecules on cell membrane glycoproteins promote metastasizing of tumor cells, whereas high activity of lysosomal sialidase enhances metabolism of sialoconjugates in cell lysosomes, thus reducing the metastatic potential of tumors.

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