Biochemistry (Moscow), Vol. 67, No. 11, 2002, pp. 1258-1261. Translated from Biokhimiya, Vol. 67, No. 11, 2002, pp. 1524-1528.
Original Russian Text Copyright © 2002 by Kobliakov, Somova, Kandyba, Kondalenko, Klim, Dyatlovitskaya.

Comparative Study of Lipid Composition and Proliferative Activity of Rat Cholangiocarcinoma RS1 and Sarcoma M1 Depending on the Transplantation Organ

V. A. Kobliakov[1], O. G. Somova[2], A. G. Kandyba[2], V. F. Kondalenko[1], N. M. Klim[1], and E. V. Dyatlovitskaya[2]*

[1]*Institute of Carcinogenesis, Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Kashirskoe Shosse 24, Moscow, 115478 Russia*
[2]*Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow, 117997 Russia; E-mail: dyatl@ibch.ru*

Received October 22, 2001
Revision received October 31, 2001

Abstract—The proliferative activity and lipid composition (phospholipids, gangliosides) were studied in rat cholangiocarcinoma RS1 and sarcoma M1 transplanted subcutaneously or intrahepatically. The mitotic index was higher in the tumors transplanted into the heterologous organ. The total phospholipid and sphingomyelin contents were higher in the tumors transplanted intrahepatically. GM3 and GD3 were the main gangliosides in both variants of each tumor. A significant amount of GM3 ganglioside lactone was found in the intrahepatic variants whereas it was absent in the subcutaneous tumors. Both the mitotic index and lipid composition of the tumors studied depended on their microenvironment.

Key words: gangliosides, ganglioside lactone, microenvironment, tumor, proliferation, phospholipids

The cell environment is known to play an important role in the regulation of cell functions. In *in vivo* experiments the transplantation organ influenced the morphotype of the tumor transplanted [2], activities of some enzymes [3-5], VEGF gene expression [6], and also levels of sulfhydryl groups and of glutathione [7]. The cell microenvironment was shown earlier to regulate the proliferative status of the tumor (rat hepatoma-27) [8] and the phospholipid and ganglioside composition [8]. In hepatoma-27 transplanted subcutaneously the amounts of phospholipids and gangliosides were significantly higher than in the tumor transplanted intrahepatically, i.e., the heterologous cell environment increased the content of these lipids. In hepatoma-27 transplanted subcutaneously the mitotic activity was also higher than in the tumor transplanted intrahepatically. It was interesting to study the effect of cell microenvironment on the proliferative status and lipid composition of tumors of other genesis under conditions of their subcutaneous and intrahepatic transplantation. Detecting the difference in the content and composition of sphingolipids (sphingomyelin, gangliosides) was especially important because these lipid components are involved in the regulation of cell proliferation and apoptosis.

Therefore, in the present work the proliferative status and lipid composition of rat tumor RS1 (solid cholangiocarcinoma initially obtained by feeding rats with 2-acetylaminofluorene [9]) and sarcoma M1 (obtained by a subcutaneous injection into rats of benz(a)pyrene [10]) were compared under conditions of intrahepatic and subcutaneous transplantation of these tumors. Cholangiocellular cancer originated from cells of bile ducts, and liver is the "maternal" organ for this tumor, whereas for sarcoma M1 a subcutaneous location is related.

MATERIALS AND METHODS

Randomly bred rats with body weight of 80-120 g were used. Earlier it was found that the mitotic index was the same in the case of tumor transplantation into vari-

Abbreviations: CL) cardiolipin; PCh) phosphatidylcholine; PE) phosphatidylethanolamine; PI) phosphatidylinositol; PSe) phosphatidylserine; SM) sphingomyelin; Sia) sialic acid (gangliosides are named according to the nomenclature of Svennerholm [1]).

* To whom correspondence should be addressed.

0006-2979/02/6711-1258$27.00 ©2002 MAIK "Nauka/Interperiodica"

ous organs of either the same or different animals, thus, the effect of one tumor on another was concluded to be minimal. Therefore, to prevent individual variations, both tumor RS1 and sarcoma M1 were transplanted subcutaneously and intrahepatically into the same rat. The transplantation was performed by an injection of the tumor piece with a trocar into the liver tissue and subcutaneously. The animals were decapitated two weeks after the transplantation. The tissues isolated were kept at −70°C.

The proliferation was evaluated by counting the mitotic index on sections stained with hematoxylin-eosin. No difference was found by microscopic examination in the structure of each tumor transplanted subcutaneously or intrahepatically.

Lipids were extracted from the tumor tissues by a repeated treatment with a mixture of $CHCl_3$–CH_3OH (2 : 1, and then 1 : 2 v/v) until they were extracted completely. Small aliquots of the extracts were taken for qualitative and quantitative analyses of phospholipids, and the main part was repeatedly washed with water by the method of Folch et al. [11]. The amount of total phospholipids and the relative content of individual phospholipids were determined as described earlier [12] after TLC on HPTLC-glass plates with silica gel (10 × 10 cm, Merck, Germany) in the system of $CHCl_3$–CH_3OH– HCOOH (65 : 25 : 4) (A).

The ganglioside-containing aqueous phase resulting after the washing of lipid extracts was evaporated in vacuum and in the residue (after purification on a Sep-Pak C_{18} cartridge) the total content of lipid-bound sialic acids was determined [13]. Gangliosides were separated and identified in the system of $CHCl_3$–CH_3OH–H_2O (60 : 40 : 9 v/v) supplemented with 0.02% $CaCl_2$ (B) in the presence of brain gangliosides and GM3 ganglioside lactone as control substances. Gangliosides were destroyed with neuraminidase from *Vibrio cholerae* (Serva, Germany) at 37°C for 24 h [14]. The enzymolysis products were subjected to chromatography on HPTLC-plates (5 × 5 cm) impregnated with NaH_2PO_4 [15] in the system of C_3H_7OH–H_2O–28% NH_4OH (6 : 2 : 1) in the presence of N-acetyl- and N-glycolylneuraminic acids as control substances. GM3 ganglioside lactone in the ganglioside mixtures, in addition to TLC in the presence of the standard, was identified by its conversion to GM3 ganglioside amide by a subsequent TLC by the method described in [16, 17]: gangliosides as a spot were placed onto a HPTLC-plate (5 × 5 cm) and separated in system B (first direction). Then the plate was dried, GM3 ganglioside lactone (standard) was placed onto the starting point, the plate kept for 5 h at room temperature in a desiccator saturated with ammonia vapors, and after a careful removal of ammonia traces the chromatography in the second direction was performed in the same system. Sialo-containing compounds were detected with resorcinol reagent [18]. The relative contents of gangliosides were determined after TLC and detection with the resorcinol reagent using a CS-920 densitometer (Japan) at 580 nm.

Protein was determined by the method of Lowry et al. [19].

RESULTS

Table 1 shows that the mitotic activities were different in the RS1 tumor cells (originated from liver bile ducts) transplanted subcutaneously and intrahepatically: in the intrahepatic tumor the number of cells in mitosis was threefold lower than in the subcutaneous tumor. The ratio was inverse for sarcoma M1 for which the subcutaneous transplantation was transplantation into the originating organ: the cell number in mitosis was 1.5-fold higher in the intrahepatic tumor than in the tumor transplanted subcutaneously. Thus, the mitotic activity of tumors growing in a "heterologous" environment was significantly higher than in the related organs.

Table 2 shows differences between the total phospholipid contents in the tumor variants transplanted subcutaneously or intrahepatically. But both tumors displayed the same tendency in these differences: the absolute amount of phospholipids was somewhat higher in the subcutaneous tumors than in the intrahepatic ones. The same was found for the absolute content of sphingomyelin: in the subcutaneous tumor RS1 the content of SM was 25.9 nmol per mg protein and in the subcutaneous sarcoma M1 it was 30.9 nmol per mg protein, whereas in the intrahepatic tumors the SM contents were 18.7 and 23.3 nmol per mg protein, respectively. Note, that at the different locations the relative contents of SM in each tumor varied insignificantly (Table 2). The contents of PSe + PI in each tumor were only insignificantly different at the subcutaneous or intrahepatic transplantation. However, in both tumors at the intrahepatic transplantation the content of PCh increased and the amount of cardiolipin decreased.

Table 3 shows that the ganglioside content was somewhat lower in the subcutaneous tumors than in the intra-

Table 1. Mitotic activities in cells of rat tumor RS1 and sarcoma M1 depending on the transplantation organ (in parentheses the number of animals; mean values ± mean deviation are presented)

Tumor	Number of mitoses per 1000 cells	
	intrahepatic	subcutaneous
RS1 (15)	1.25 ± 0.22*	3.54 ± 0.25
M1 (12)	4.20 ± 0.14*	2.80 ± 0.15

* $p < 0.05$ (here and further the statistic significance is evaluated in pairs for each tumor type).

Table 2. Content and composition of phospholipids (PL) in rat tumor RS1 and sarcoma M1 (mean data for three determinations ± the standard deviation are presented; in parentheses, number of animals)

Phospholipids	Subcutaneously transplanted tumor		Intrahepatically transplanted tumor	
	RS1 (1)	M1 (2)	RS1 (3)	M1 (4)
PL content, μmol P/mg protein	0.20 ± 0.01	0.26 ± 0.04	0.16 ± 0.03*	0.23 ± 0.05**
Composition of phospholipids, %				
	(1)	(2)	(3)	(4)
CL	10.4 ± 0.6	5.9 ± 0.3	6.5 ± 0.6***	4.4 ± 0.3****
PE	23.5 ± 0.7	23.7 ± 0.7	28.4 ± 0.5***	23.8 ± 0.6****
PSe + PI	5.0 ± 0.2	8.7 ± 0.1	6.3 ± 0.4*	9.0 ± 0.3***
PCh	38.5 ± 2.5	44.6 ± 0.9	45.5 ± 1.2*	51.4 ± 0.9***
SM	13.0 ± 0.5	11.9 ± 0.2	11.6 ± 0.6**	10.6 ± 0.2****
Lyso-PCh	9.6 ± 0.1	5.2 ± 0.3	1.7 ± 0.2**	0.8 ± 0.1**

* $p < 0.05$; ** $p < 0.001$; *** $p < 0.01$; **** $p > 0.1$.

hepatic ones, but this difference was not high. As to the ganglioside composition, GM3 was the main ganglioside in both subcutaneous and intrahepatic variants of both tumors. GD3 ganglioside was also found in rather a great amount. Note that a significant content of an extremely rare GM3 ganglioside lactone was recorded in the intrahepatic variants of tumor RS1 and sarcoma M1. Up to now this lactone was found in insignificant amounts in the mouse melanoma B16 cells [20] and in spontaneous tumors of human stomach and mammary gland [17].

Sialic acids were analyzed in gangliosides of the studied tumors, and only N-acetylneuraminic acid was found in both subcutaneous and intrahepatic variants of tumor RS1, whereas gangliosides of sarcoma M1 in both cases also contained a small amount of N-glycolylneuraminic acid, in addition to N-acetylneuraminic acid.

Table 3. Content and composition of gangliosides in rat tumor RS1 and sarcoma M1 (mean data for three determinations ± the standard deviation are presented; in parentheses, number of animals)

Gangliosides	Subcutaneously transplanted tumor		Intrahepatically transplanted tumor	
	RS1 (1)	M1 (2)	RS1 (3)	M1 (4)
Content, nmol Sia/mg protein	1.6 ± 0.01	1.4 ± 0.03	2.0 ± 0.02****	1.6 ± 0.03*
Composition of gangliosides, %				
	(1)	(2)	(3)	(4)
GM3 ganglioside lactone	—	—	13.0 ± 0.3	13.4 ± 0.8
GM3	73.1 ± 0.6	77.4 ± 0.6	62.3 ± 0.3**	68.1 ± 1.4**
GM2	1.2 ± 0.1	—	2.9 ± 0.4***	—
GM1	3.9 ± 0.1	6.1 ± 0.3	3.1 ± 0.2*	7.5 ± 0.9****
GD3	13.4 ± 0.6	11.3 ± 0.4	11.7 ± 0.1*	6.0 ± 0.8**
GD1a	3.5 ± 0.1	5.2 ± 0.2	4.7 ± 0.3***	5.0 ± 0.4****
GD1b	4.9 ± 0.8	—	2.3 ± 0.1*	—

* $p < 0.05$; ** $p < 0.001$; *** $p < 0.01$; **** $p > 0.1$.

DISCUSSION

The effect of microenvironment on cell functions was studied in rather few works using a model of the tumor transplantation into different organs. In total, these studies suggested that the tumor cells should "feel" changes in the microenvironment not only on the transplantation into the initial or unrelated organs but also on the transplantation into different unrelated organs. Using hepatoma-27 as a transplanted tumor, we have shown earlier that the mitotic index of the tumor was lower during the intrahepatic than during subcutaneous growth [8]. However, in the hepatoma transplanted intrahepatically some differentiation parameters were more pronounced [4, 5] than in the same tumor transplanted into an unrelated organ. The phospholipid levels in the hepatoma-27 transplanted into the liver was more similar to their levels in liver than in the hepatoma transplanted subcutaneously [8]. Thus, our previous findings suggested that in the "maternal" organ a kind of tumor normalization should occur that was manifested by a decrease in the mitotic index and by changes in the lipid composition. However, this effect could also be caused by a specific effect of liver cells on tumor of any genesis. To elucidate what possibility was realized we studied the mitotic index and lipid composition of two tumors of different histogenesis transplanted subcutaneously or intrahepatically. Because tumor RS1 is a cholangiocarcinoma originated from the cells of bile ducts, the intrahepatic transplantation placed it into the organ of its origin although into the environment of other type cells compared to cells of the tumor RS1 origin. Sarcoma M1 was obtained as a result of subcutaneous injection of a carcinogen; therefore, its subcutaneous transplantation was a model of its growth in the "maternal" organ, whereas the intrahepatic transplantation was a transplantation into an unrelated organ. In the present work the mitotic index was higher in the tumor RS1 transplanted subcutaneously than intrahepatically, and this ratio was inverse for sarcoma M1; thus, this has confirmed the earlier findings on the decreased tumor proliferation in the case of its transplantation into the initial organ compared to the transplantation into an unrelated organ [8].

The lipid compositions of the tumor transplanted into different organs were also different, suggesting an influence of the microenvironment. Unlike the mitotic index, these changes in the tumors of different histogenesis were directed similarly: the total lipid and sphingomyelin content was higher in the subcutaneous tumors than in the intrahepatic ones. The ganglioside contents in the two variants of each tumor were not very different, and in both cases the ganglioside content was somewhat higher in the intrahepatic tumors than in the subcutaneous ones. The identification of GM3 ganglioside lactone in the intrahepatic variants of rat tumor RS1 and sarcoma M1 seemed to be the most interesting because it is detected extremely seldom. It seemed that the hepatic microenvironment of each tumor, independently of its histogenesis, promoted the generation of this lactone.

Thus, the mitotic index of tumors of different histogenesis transplanted subcutaneously and intrahepatically was higher in the case of proliferation in the heterologous organ, whereas the effects of microenvironment on the phospholipid and ganglioside compositions were similar independently of histogenesis of the tumor cells.

This work was supported by the Russian Foundation for Basic Research (grant No. 01-04-48088).

REFERENCES

1. Svennerholm, L. (1964) *J. Lipid Res.*, **5**, 145-155.
2. Dingemans, K., Zeeman-Boeschoten, I., Keep, R., and Das, P. (1993*) Int. J. Cancer*, **54**, 1010-1016.
3. Scheid, B. (1990) *Oncology*, **47**, 278-281.
4. Sultatos, L., and Vessel, E. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 600-604.
5. Kobliakov, V., Kulikova, L., Kolyada, A., Chemeris, G., and Turusov, V. (1993*) Xenobiotica*, **23**, 703-708.
6. Fukumura, D., Yuan, F., Monsky, W., Chen, Y., and Jain, R. (1997) *Am. J. Pathol.*, **151**, 679-688.
7. Ahmad, S., Mulberg, A., Alian, J., and Vistica, D. (1986*) Biochem. Pharmacol.*, **35**, 1697-1701.
8. Kobliakov, V. A., Somova, O. G., Kondalenko, V. F., Ostashkina, N. M., Kandyba, A. G., Dubovaya, T. K., and Dyatlovitskaya, E. V. (2001) *Biochemistry (Moscow)*, **66**, 603-607.
9. Malyugina, L. L. (1958) *Vopr. Onkol.*, **4**, 600-604.
10. Shabad, L. M., and Blokh, M. I. (1947) *Byull. Eksp. Biol. Med.*, **24**, 246-249.
11. Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957) *J. Biol. Chem.*, **226**, 497-508.
12. Lemenovskaya, A. F., Koen, Ya. M., Perevoshchikova, K. F., Zbarskii, I. B., Dyatlovitskaya, E. V., and Bergelson, L. D. (1976) *Biokhimiya*, **41**, 1000-1003.
13. Dyatlovitskaya, E. V., Zablotskaya, A. E., Volgin, Yu. V., Azizov, Yu. M., and Bergelson, L. D. (1979) *Biokhimiya*, **44**, 1623-1629.
14. Dyatlovitskaya, E. V., Zablotskaya, A. E., Azizov, Yu. M., and Bergelson, L. D. (1980) *Eur. J. Biochem.*, **110**, 475-483.
15. Malykh, Ya. N., Dyatlovitskaya, E. V., Lemenovsakaya, A. F., Sorokin, M. M., Indzhiya, L. V., Yakovleva, L. A., and Bergelson, L. D. (1991) *Biokhimiya*, **56**, 1049-1056.
16. Sonnino, S., Ghidoni, R., Chigorno, V., Masserini, M., and Tettamanti, G. (1983) *Analyt. Biochem.*, **128**, 104-114.
17. Tekieva, E. A., and Dyatlovitskaya, E. V. (1993) *Biochemistry (Moscow)*, **58**, 1207-1209.
18. Svennerholm, L. (1963*) Meth. Enzymol.*, **6**, 459-462.
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randell, R. J. (1951) *J. Biol. Chem.*, **193**, 265-275.
20. Nores, G., Dohi, T., Taniguchi, M., and Hakomori, S. (1987) *J. Immunol.*, **139**, 3171-3176.