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GANGLIOSIDES OF NORMAL AND NEOPLASTIC HUMAN MELANOCYTES

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The major ganglioside component isolated from diploid human melanocytes is sialosyllactosylceramide (G_{M3} 86-91% of total sialic acid). The corresponding disialo derivative (G_{D3}) is found as a minor component (2-6% of total sialic acid) in the membranes of these cells. In human melanoma cells, grown in tissue culture, G_{D3} is the predominant ganglioside component (48-63% of total sialic acid). Withdrawal of TPA from the culture medium of normal melanocytes or addition of TPA to the medium of melanoma cells had no significant effect on G_{M3}/G_{D3} ratios. We conclude that the difference between the composition of gangliosides is related to the normal vs transformed phenotypes of melanocytes.

G_{M3} and G_{D3} are the major ganglioside components of human melanoma cells (2,3). Since melanoma cells studied so far show a relatively simple pattern of ganglioside composition (4), the question arises whether this pattern is characteristic of malignant melanomas or whether it is a marker for the pigment cell phenotype. With the recent development of a method for the growth of human diploid melanocytes in culture (5), this question can now be answered. Comparative studies on the ganglioside composition of human foreskin melanocytes (HFC) and melanoma cells are described in this report.

METHODS

Diploid human melanocytes were obtained from foreskins of newborn Caucasians and grown in culture medium that contained TPA (65nM) and cholera toxin (10nM), as described by Eisinger and Marko (5). The three lines used in these studies were grown in culture for five - six months. The cells were karyotyped after several months of cultivation and found to contain fewer than 5% of cells with higher than diploid chromosome numbers (P.Krass and R. Halaban, unpubl.). The three human melanoma cells lines were obtained from surgical specimens of regional lymph node (Y-Mel 130/80 and Y-Mel 550/82) or small bowel mural

*Abbreviations: Svennerholm's nomenclature (1) is used for gangliosides (see Figure 2 for details). TPA, 4-0-methyl-12-0-tetradecanoyl-phorbol-13-acetate; HFC, human foreskin melanocytes; Cer, ceramide, Glc, glucosyl; Gal, galactosyl; SA, sialic acid; $GalNA_C$, N-acetylgalactosaminosyl.

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metastasis (Y-Mel 621/82). The patients from whom the metastatic lesions were obtained had received surgical therapy, surgery and immunotherapy, and interferon alpha + dacarbazine therapy, respectively. The tumor specimens, acquired at surgery, were identified as melanomas by means of growth in nude mice and pigmentation in monolayer culture, as well as cell morphology. Sterile specimens were initially disrupted mechanically and dissociated with Clostridium histolyticum collagenase (0.1 U/ml, Sigma Chemicals, St. Louis) and DNAse in EDTA (0.5 mM) with trypsin (0.25%, Sigma Chemicals) for 4-12 hours at 37° in a humidified atmosphere of 5% CO_2. The cell lines used in these experiments were propagated in monolayers in F10 medium (Gibco, Grand Island, NY) plus 10% fetal calf serum (Gibco). In order to study the effects of TPA on the ganglioside composition, melanoma were grown in the presence of TPA for 7-11 days and HFC were grown without TPA for 3 days as indicated in Figure 1. The isolation and purification of the gangliosides from lyophilized cells was based on a previously published method,[6] which was modified to deal with smaller sample sizes. Briefly, after a dry cell weight was obtained, an equivalent amount of distilled water was added to the cell pellet to replace that which was lost during lyophilization. The total lipids were then extracted by adding approximately 10 volumes of 1:1 chloroform: methanol. The chloroform- methanol extract was adjusted to a composition of 30:60:8 chloroform: methanol: water (solvent A) and applied to a DEAE - Sephadex A-25 column (bed volume, 1.0 ml - 2.5 ml, depending upon sample size). The DEAE column was rinsed with 10 column volumes of solvent A to ensure complete removal of all neutral lipids. The acidic lipids were then eluted with 10 column volumes of 30:60:8 chloroform: methanol: 0.8M sodium acetate. The eluant was concentrated by rotary evaporation and the residue was base-treated with 0.1N sodium hydroxide in methanol. After the methanol was removed, the resulting residue was dialyzed against distilled water and then lyophilized. Final purification of the gangliosides was achieved by Iatrobead column chromatography. Ganglioside compositions were analyzed by thin-layer chromatography-densitometry according to Ando, Chang and Yu.[7] Gangliosides were identified by comparing the mobilities of the resorcinol-positive bands with those of the standards in at least two different solvent systems.

RESULTS AND DISCUSSION

Figure 1 shows the chromatographic separation, on silica gel thin layer plates, of gangliosides from human melanoma cells and diploid melanocytes. The well characterized gangliosides of human brain were used as standards. The major ganglioside in HFC is G_{M3} which accounts for over 90% of the total ganglioside sialic acid. In comparison, the proportion of G_{M3} is decreased and that of G_{D3} increased in all melanoma cell lines examined (Figure 1). The identity of G_{M3} and G_{D3} was confirmed by TLC in several solvent systems. The doublet bands of G_{M3} and G_{D3} represent gangliosides containing the same carbohydrate residues attached to lipid chains of different lengths. Multiplicity of bands of gangliosides, due to different lipid chains has been found before (8). In sample #2 (melanoma 621/82) the doublet of G_{D3} ran more slowly than in the rest of the samples because of the interference of an unidentified material that did not stain with resorcinol. As shown in the chromatogram, the relative amounts of G_{M3} and G_{D3} are

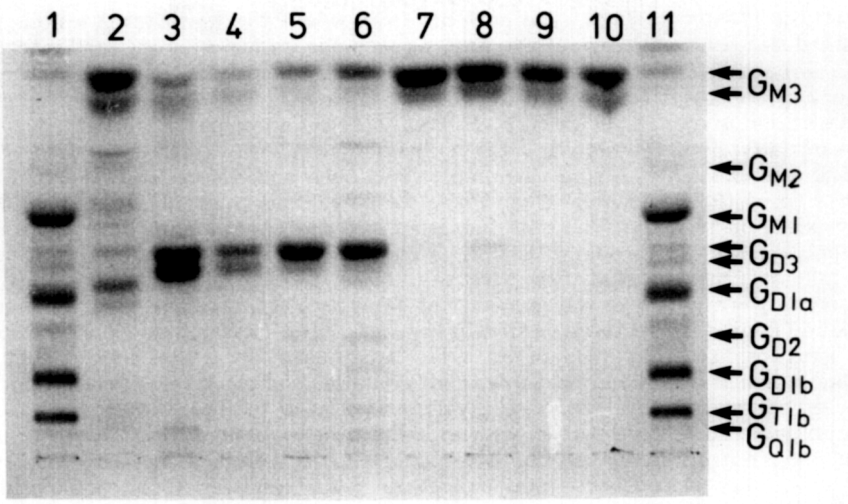

Figure 1 Separation of gangliosides by thin layer chromatography. Samples: #1, #11, were standards from human brain (white matter); samples 2-6 were human (Y-Mel) melanoma cells (#2, 621/82; #3, 550/82; #4, 550/82 + TPA (11 days). #5, melanoma 130/80; #6, the same with TPA(7 days); samples #7-10 were HFC cells (#7, HFC-7, no TPA for 3 days; #8, HFC-7; #9, HFC-29; #10, HFC-9).

strikingly different in melanoma cells (samples #2-6) as compared to HFC (samples #7-10). The amount of ganglioside present in the bands of TLC plates was quantitated by densitometry and these data are summarized in Table I. In agreement with earlier reports (2-4), all three melanoma cell lines contained G_{M3} and G_{D3} as the major ganglioside components. On a molar basis, the G_{M3}/G_{D3} ratio was 0.5-0.9 for melanoma cells. In contrast, the normal diploid melanocytes yielded G_{M3} as the major component (86-90% of total gangliosides), whereas the G_{M3}/G_{D3} ratio was 18-56. Since it is known that phorbol esters such as TPA affect the composition of gangliosides in other cell lines (9-11), we investigated whether the difference between the ganglioside composition of normal and neoplastic pigment cells was due to the presence of TPA in the medium that is used for the growth of non-malignant pigment cells from human foreskin (5). As is shown in Figure 1 and Table 1, withdrawal of TPA from the medium of normal melanocytes (sample #7) or addition of TPA to the medium of melanoma cells (samples #4,6) did not alter the difference in the ganglioside composition of HFC and melanoma cells.

Table 1
Ganglioside composition of melanoma cells and diploid melanocytes

	Melanoma cells				Diploid Melanocytes			
	550/82	550/82 (+TPA)	130/80	130/80 (+TPA)	HFC-9	HFC-29	HFC-7	HFC-7 (-TPA)
Gangliosides	Ganglioside content, % of total							
G_{M3}	25.1	34.5	29.0	33.9	90.4	90.0	85.9	91.4
G_{M2}	3.0	1.7	2.7	5.4	1.5	0.6	1.1	1.0
G_{M1}	*	*	1.0	2.5	*	*	0.8	1.0
G_{D3}	63.0	55.4	56.0	48.6	2.0	2.8	6.1	2.7
G_{D1a}	*	*	*	*	2.3	1.8	1.6	0.9
G_{D2}	*	*	2.1	3.0	*	*	*	*
G_{D1b}	*	*	1.9	2.5	*	*	*	*
G_{T1b}	*	*	2.9	1.8	*	*	*	*
G_{Q1b}	2.3	0.8	1.4	1.4	0.6	0.9	0.7	*
Unidentified**	6.6	7.6	3.0	0.9	3.2	3.9	3.8	3.0
G_{M3}/G_{D3} (mol. ratio)	0.5	0.8	0.6	0.9	40.3	56.2	17.6	42.3
Total sialic acid content***	0.027	0.014	0.041	0.049	0.142	0.031	0.165	0.034

Notes: Bands of gangliosides, separated by TLC (Figure 1) were quantitated by densitometry.
*Ganglioside content less than 0.5%.
**Minor bands that were not identifiable on TLC plates.
***Per cent of sialic acid content/dry weight of cells.

The difference between the levels of G_{M3} and G_{D3} extracted from normal and malignant pigment cells could be due to differences in the levels of the degradative or synthetic enzyme(s) that are responsible for the hydrolysis or synthesis of G_{D3} (Figure 2). Thus, it is possible that in normal human melanocytes a specific neuraminidase removes the terminal sialic acid from G_{D3}, converting it to G_{M3} (arrow #2 of Figure 2). However, since most of the biosynthetic derivatives of G_{D3}, such as G_{D2}, G_{D1b} and G_{T1b} are missing from HFC and, at the same time, G_{D1a}, the biosynthetic derivative of G_{M2} is absent from melanoma cells (Table 1), it is more likely that coincidently or subsequently to the transformation of normal melanocytes a sialyl transferase is induced or activated,

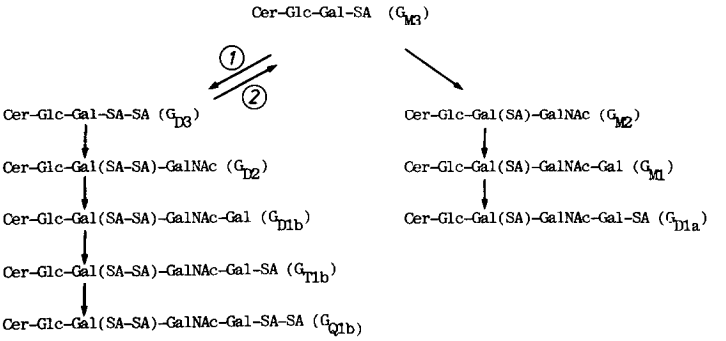

Figure 2 Biosynthesis of gangliosides.

which is responsible for the conversion to G_{M3} to G_{D3} in melanoma cells (arrow #1 of Figure 2). Analysis of the enzymes responsible for the synthesis, hydrolysis and conversion of G_{D3} in HFC and melanoma cells should answer this question. It is well known that oncogenic transformation of cells frequently alters the metabolism of cell surface gangliosides (12,13).

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