GANGLIOSIDES OF PLASMA MEMBRANES FROM NORMAL RAT LIVER AND MORRIS HEPATOMA[1]

Ann M. Dnistrian, Vladimir P. Skipski, Marion Barclay, Edward S. Essner and C. Chester Stock[2]

Memorial Sloan-Kettering Cancer Center
New York, New York 10021

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SUMMARY:

Plasma membranes were isolated from normal rat liver and Morris hepatoma 5123tc by discontinuous sucrose gradient centrifugation. There was an average two and one-halffold enrichment of gangliosides in plasma membranes from normal liver and hepatoma as compared with their respective whole cells. The amount of total gangliosides in plasma membranes from hepatoma was eight times greater than that found in normal liver. This increase resulted from a five-fold increase in hematosides, an eightfold increase in monosialogangliosides and a twenty-twofold increase in disialogangliosides. Trisialogangliosides were present in normal liver but not in hepatoma.

INTRODUCTION

Alterations in ganglioside patterns have been reported in transformed cells (1-3) and in solid tumors (4-8). Although a general similarity in the pattern has been suggested (7), normal liver and hepatoma (6-8) exhibit a more complex ganglioside pattern than that found in rat hepatocyte and hepatoma cell lines (3).

The association of gangliosides with the cell surface plasma membrane is well documented (9-13). However, gangliosides from plasma membranes of solid tumors have not been investigated except by Emmelot (13) who reported a relative estimate of gangliosides in plasma membranes isolated from hepatomas. Therefore, we have made quantitative analyses of the gangliosides present in plasma membranes isolated from the minimally deviated and well differentiated Morris hepatoma 5123tc.

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METHODS:

Isolation of plasma membranes. Plasma membranes were isolated from normal liver (14) and Morris hepatoma (15) as described previously. Plasma membranes to be analyzed for lipids were washed 3x with 1mM $NaHCO_3$ buffer, pH 7.5, 4°C to remove sucrose. They were then dialyzed against the same buffer for 18 hr and centrifuged at 30,000 rpm for 15 min.

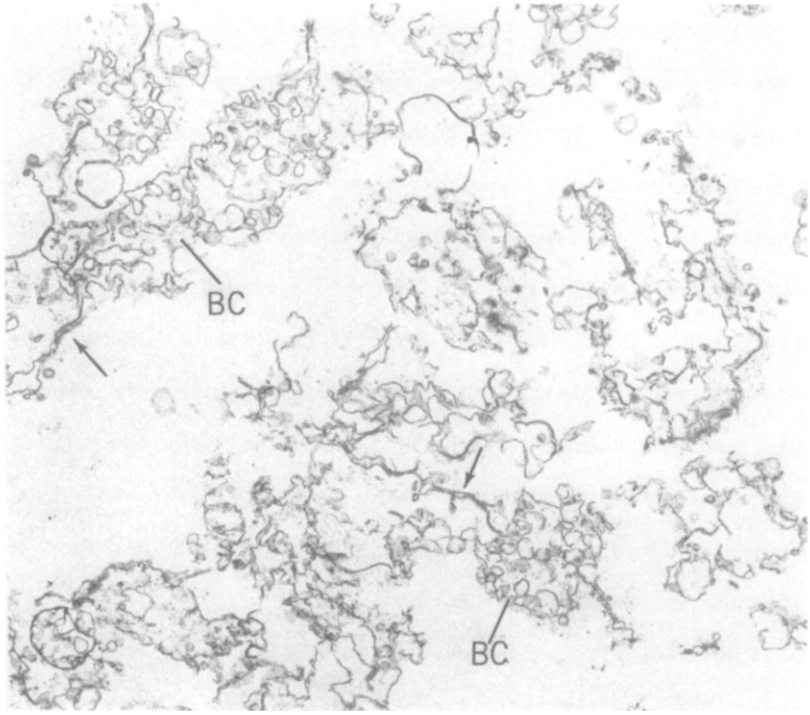
Enzyme assays and protein determinations. Plasma membranes were prepared for enzyme assays as described previously (14). Whole liver homogenate, Morris hepatoma homogenate and plasma membranes from normal liver and Morris hepatoma were tested for activities of 5'-nucleotidase (EC3.1.3.5) (16), glucose-6-phosphatase (EC 3.1.3.9) (17), and cytochrome c oxidase (EC 1.9.3.1) (18). Washed whole membranes were used to determine the amount of total protein (19) and the amount of protein soluble in 0.15 M NaCl (20).

Lipid extraction. Total lipids were extracted from normal rat liver and Morris hepatoma and from their plasma membranes essentially as described previously (21) and partitioned according to Folch et al (22). The upper phase contained water soluble impurities and gangliosides. The lower phase, which contained simple lipids, phospholipids and neutral glycosphingolipids, also contained some hematosides and monosialogangliosides.

Determination of gangliosides. The upper phase from lipid extracts of normal liver and Morris hepatoma was purified by dialysis against water (23). Gangliosides were separated individually by thin layer chromatography and identified according to their mobilities in relation to reference compounds in at least two systems: $CHCl_3$-MeOH-0.25% aqueous $CaCl_2$, 60:35:8 (v/v/v) (24); $CHCl_3$-MeOH-H_2O, 60:45:10 (v/v/v) (25) which effectively separated monosialogangliosides from disialogangliosides; and $CHCl_3$-MeOH-2.5 N NH_4OH 60:45:9 (v/v/v) (26) which effectively separated disialogangliosides from trisialogangliosides. The spots on chromatograms were visualized with primuline spray (27-29), quantitatively eluted, and the eluates analyzed for sialic acid (30,31). Hematosides and monosialogangliosides present in the lower phase were purified and quantitated separately (29).

RESULTS AND DISCUSSION:

Plasma membranes isolated from normal liver and Morris hepatoma 5123tc are relatively pure on the basis of electron microscopic examination (Fig. 1) and assay for marker enzymes (Table I). Activity of 5'-nucleotidase in plasma membranes from normal liver and hepatoma is approximately forty to fifty times greater than in their respective whole cell homogenates. The ratio of lipid to protein (Table II) in plasma membranes from normal liver (.59) does not differ greatly from that calculated for Morris hepatoma (.64) and presumably does not account for the differences in the buoyant densities between these plasma membranes. Emmelot and Benedetti (32) suggested that the lower buoyant density of plasma membranes from hepatomas may result from an increase in structurally bound water. The amount of total protein is only slightly de-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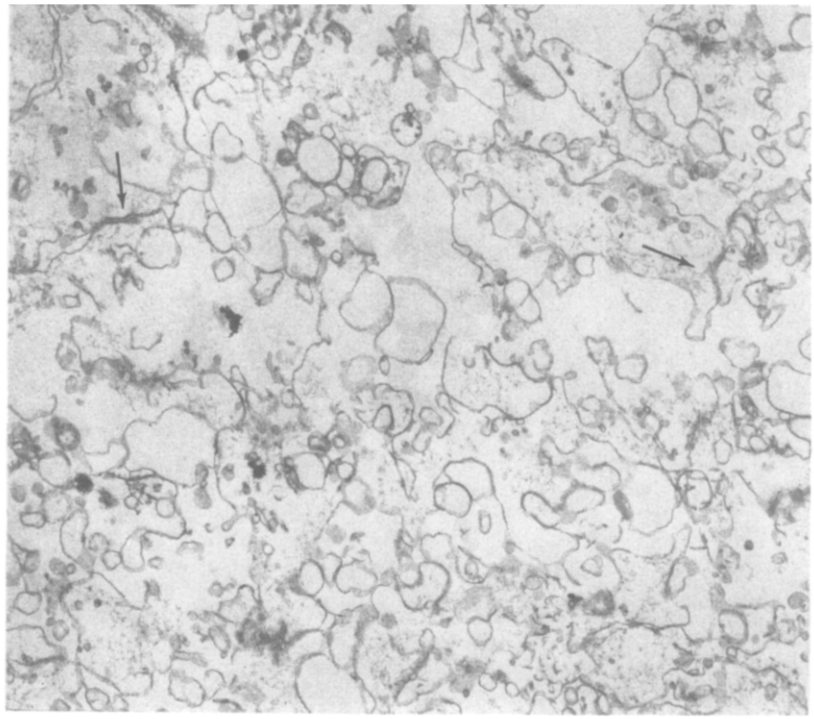

creased in plasma membranes from hepatoma but the amount of soluble protein is greatly decreased (Table II), confirming our previous observations (15). This suggests a possible alteration in the molecular associations of these proteins with other components within the plasma membrane.

Data for gangliosides in normal liver and Morris hepatoma (Table III) show qualitative and quantitative differences. Normal liver contains hematosides, monosialogangliosides, and smaller amounts of disialogangliosides and trisialogangliosides. Morris hepatoma contains predominantly monosialogangliosides and disialogangliosides, whereas trisialogangliosides are absent. There is an average ninefold increase in the total amount of gangliosides in Morris hepatoma as compared with normal liver, resulting from a threefold increase in hematosides, a fifteenfold increase in monosialogangliosides and a thirty-fourfold increase in disialogangliosides.

There is an average two and one-halffold enrichment on a lipid basis (approximately fourfold on a protein basis) of gangliosides in plasma membranes from normal liver and Morris hepatoma as compared with whole cells (Table III). Ganglioside patterns in the plasma membranes reflect those found in whole cells. There is an average eightfold increase in total gangliosides in plasma membranes from hepatoma as compared with those from normal liver. This increase results from a fivefold increase in hematosides, an eightfold increase in monosialogangliosides, and a twenty-twofold increase in disialogangliosides. Trisialogangliosides are not present in plasma membranes from hepatoma.

Fig. 1. Electron micrograph of isolated plasma membranes.
Top, normal rat liver. Fragments of bile canaliculi (BC) and junctional complexes (arrows) between plasma membranes of adjacent hepatocytes are indicated. Stained with lead citrate. X 12,000, Bottom, Morris hepatoma 5123tc. The fraction contains numerous membranous elements. Arrows indicate junctional complexes between tumor cell plasma membranes. Stained with lead citrate. X 14,000, Electron microscopy was carried out essentially as described previously (21).

Table I

Assays of marker enzymes

Fractions	5'-nucleotidase (EC 3.1.3.5)[a]	Glucose -6-phosphatase (EC 3.1.3.9)[b]	Cytochrome c oxidase (EC 1.9.3.1)[c]
	average μmoles P/mg protein/hr		average ΔOD units/ mg protein/min
Normal liver homogenate	3.3	6.87	1.68
Normal liver plasma membrane	174.5	0.20	0.03
Hepatoma 5123tc homogenate	2.9	1.34	2.38
Hepatoma 5123tc plasma membrane	123.3	0.09	0.05

[a] Marker for plasma membranes

[b] Marker for microsomes

[c] Marker for mitochondria

Table II

Quantitative aspects of plasma membranes isolated from normal liver and from Morris hepatoma 5123tc

	Yield[a]	Percent protein	Percent lipid	Lipid/ protein (l/p ratio)	Percent[b] soluble protein	Density[c] (g/ml)
Normal liver	0.75-1.0	62.7	37.3	0.59	24.8	1.16-1.18
Morris hepatoma	0.35	61.3	39.0	0.64	12.6	1.12-1.14

[a] Expressed as dry weight plasma membrane per 1.0 g wet weight starting tissue.

[b] Expressed as percent of total protein soluble in 0.15 M NaCl.

[c] As estimated by sucrose gradient centrifugation.

Table III

Gangliosides isolated from plasma membranes of normal rat liver and Morris Hepatoma 5123tc

	nanomoles/mg lipid			
	Whole tissue		Plasma membranes	
	Normal liver	Morris hepatoma	Normal liver	Morris hepatoma
Hematosides	0.66 (3)[a]	1.88 (4)	1.23 (5)	5.67 (4)
Monosialogangliosides	0.27 (3)	4.06 (6)	1.59 (5)	12.01 (4)
Disialogangliosiades	0.16 (3)	5.41 (6)	0.37 (4)	7.96 (4)
Trisialogangliosides	0.15 (3)	not detected (8)	0.17 (3)	not detected (4)
Total	1.24	11.35	3.36	25.64

[a] Numbers in parenthesis indicate the number of experiments performed.

Comparative data for the alteration of gangliosides in plasma membranes are unavailable since this report constitutes the first quantitative determination of gangliosides in plasma membranes isolated from solid tumors. Concerning whole cells, our results are in agreement with those reported for alterations in gangliosides in hepatomas (6-8). These alterations do not simply reflect differences in growth rate since Siddiqui and Hakomori (7) demonstrated that rapidly growing neonatal liver contains a much higher quantity of trisialogangliosides and a lower quantity of monosialogangliosides than does normal liver. These authors also have shown that the level of disialogangliosides in hepatomas is related to the degree of malignancy.

Hakomori (5) reviewed glycolipids of tumor cell membranes and discussed the possible significance of membrane glycolipid changes. The exact relationship between ganglioside changes and the abnormal behavior of tumor cells remains to be elucidated.

REFERENCES:

1. Hakomori, S., and Murakami, W. T.(1968) Proc. Nat. Acad. Sci. U.S., 59, 254-261.
2. Mora, P. T., Brady, R. O., Bradley, R. M., and McFarland, V. W. (1969) Proc. Nat. Acad. Sci. U.S., 63, 1290-1296.
3. Brady, R. O., Borek, C., and Bradley, R. M. (1969) J. Biol. Chem., 244, 6552-6554.
4. Nigam, V. N. and Cantero, A. (1973) Adv. in Cancer Res. Vol. 17, 1-80 (Klein, G. and Weinhaus, S., eds.) Academic Press, New York.
5. Hakomori, S. (1974) Adv. in Cancer Res. Vol. 18, 265-315 (Klein, G. and Weinhaus, S., eds.) Academic Press, New York.
6. Cheema, P., Yogeeswaren, G., Morris, H. P., and Murray, R. K. (1970) FEBS Letters, 11, 181-184.
7. Siddiqui, B., and Hakomori, S. (1970) Cancer Res., 30, 2930-2936.
8. Dyatlovitskaya, E. V., Novikov, A. M. and, Bergelson, L. D. (1974) Biokhimiya, 39, 552-556.
9. Dod, B. J., and Gray, G. M. (1968) Biochim. Biophys. Acta, 150, 397-404.
10. Renkonen, O , Gahmberg, C. G., Simons, K., and Kääriäinen, L. (1970) Acta Chem. Scand., 24, 733-735.
11. Klenk, H. D., and Choppin, P. W. (1970) Proc. Nat. Acad. Sci. U. S., 66, 57-64.
12. Weinstein, D. B., Marsh, J. B., Glick, M. C., and Warren, L. (1970) J. Biol. Chem., 245, 3928-3937.
13. Emmelot, P. (1973) Europ. J. Cancer, 9, 319-333.
14. Barclay, M., Barclay, R. K., Skipski, V. P., Essner, E. S., and Terebus-Kekish, O. (1972) Biochim. Biophys. Acta, 255, 931-947.
15. Barclay, M., and Terebus-Kekish, O. (1973) J. Nat. Cancer Inst., 51, 1709-1710.
16. Emmelot, P., and Bos, C. J. (1966) Biochim. Biophys. Acta, 120, 369-382.
17. Swanson, M. A. (1955) in Methods in Enzymology, Vol. II, 541-543 (Colowick, S. P. and Kaplan, N. O., eds) Academic Press, New York.

18. Cooperstein, S. J., and Lazarow, A. (1951) J Biol. Chem., 189, 665-670.
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem., 193, 265-275.
20. Barclay, M., Skipski, V. P., and Terebus-Kekish, O. (1972/73) Mech. Age. Dev., 1, 357-365.
21. Ray, T. K., Skipski, V. P., Barclay, M., Essner, E., and Archibald, F. M. (1969) J. Biol. Chem., 244, 5528-5536.
22. Folch, J., Lees, M., and Sloan Stanley, G. H. (1957) J. Biol. Chem., 226, 497-509.
23. Kanfer, J. N., and Spielvogel, C. (1973) J. Neurochem., 20, 1483-1485.
24. van den Eijnden, D. H. (1971) Hoppe-Seyler's Z. Physiol. Chem., 352, 1601-1602.
25. Wagner, H., Hörhammer, L., and Wolff, P. (1961) Biochem. Z., 334, 175-183.
26. Wherret, J. R., and Cumings, J. N. (1963) Biochem. J., 86, 378-382.
27. Wright, R. S. (1971) J. Chromatog., 59, 220-221.
28. Skipski, V. P. (1975) in Methods in Enzymology, Vol. XXXV, 396-425 (Lowenstein, J. M., ed.) Academic Press, New York.
29. Skipski, V. P., and Dnistrian, A. M., manuscript in preparation.
30. Svennerholm, L. (1957) Biochim. Biophys. Acta, 24, 604-611.
31. Miettinen, T., and Takki-Luukkainen, I.-T. (1959) Acta Chem. Scand., 13, 856-857.
32. Emmelot, P., and Benedetti, E. L. (1967) "Carcinogenesis a Broad Critique". Fundam. Cancer Res. 20, 471-533.