GANGLIOSIDE PATTERNS OF THREE MORRIS MINIMAL DEVIATION HEPATOMAS*

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Received 28 September 1970

1. Introduction

Gangliosides, long recognised as important constituents of brain and a few other tissues [1, 2], are also present in most extraneural tissues including liver [1, 3]. Abnormalities of the pattern of neutral glycolipids in tumour tissues are well-documented [4, 5]. The studies of Hakomori and Murakami [6] first indicated that significant changes of ganglioside pattern occur in virally-transformed cells. Mora et al. [7] have also reported dramatic simplifications of ganglioside patterns in mouse cells transformed by SV-40 virus. Cells from the chemically-induced hepatoma H 5123 when grown in tissue culture have been shown to display a simplification of ganglioside pattern as compared with cultured hepatocytes [8]. Similar types of changes of ganglioside pattern have also been observed in chick embryo cells transformed by Rous sarcoma virus and in unspecified minimal deviation hepatomas [9]. In the present communication we describe the ganglioside patterns of three of the Morris minimal deviation hepatomas, a series of tumours that has been subjected to extensive biochemical investigations [10–13]. The tumours used were grown *in vivo*. The ganglioside patterns of all three hepatomas are shown to differ from that of normal rat liver, but the patterns are generally more complex than those previously described for neoplastic cells [6–9]. The observations appear of interest in relation to the interpretation of alterations of glycosphingolipid patterns in chemically-transformed as opposed to virally-transformed cells and also in relation to biochemical differences between tumour cells grown *in vivo* and *in vitro*.

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2. Materials and methods

Adult male Buffalo control rats and animals carrying hepatomas 7777, 7800 and 5123 D inoculated intramuscularly were shipped by air express from Washington to Toronto. The first hepatoma is a relatively fast-growing tumour and the two latter are slower-growing [14]. Control animals and animals carrying the tumours were sacrificed by decapitation and 3–4 g portions of control livers, host livers and the hepatomas were washed in ice-cold 0.1 M tris-HCl (pH 7.4) and frozen at -70° until analysed. The hepatomas were first carefully dissected free of necrotic tissue if present. The general methodology for extraction and analysis of the gangliosides was as described for analysis of cultured mouse cell gangliosides [15]. The extraction procedure used was that of Suzuki [16] Lipid-bound sialic acid was measured by the procedure of Svennerholm [17] as modified by Miettinen and Takki-Luukkainen [18]. Aliquots of ganglioside fractions from control and host livers and the hepatomas containing approximately 20 μg of lipid-bound sialic acid were subjected to thin layer chromatography (TLC) on glass plates coated with silica gel G and developed in chloroform–methanol–ammonia–water (60 : 35 : 1 : 7, by volume) as described by Wherrett and Cumings [19]; the ganglioside bands were detected by the resorcinol reagent of Svennerholm [17]. Aliquots of human brain gangliosides were used as standards. The nomenclature used for description of the gangliosides is that of Svennerholm [20, 21].

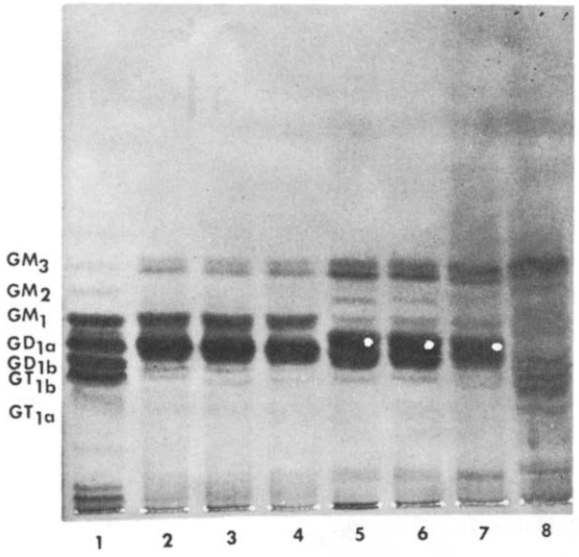

Fig. 1. Thin layer chromatogram of various ganglioside preparations. Slot 1: human brain gangliosides; Slots 2–4: ganglioside fraction from three individual hepatomas 5123 D; Slots 5–7: ganglioside fraction from three individual hepatomas 7777; Slot 8: ganglioside fraction from control rat liver. TCL and detection of the gangliosides was performed as described in the text. The nomenclature for the gangliosides is that of Svennerholm [20, 21]; the slowest-migrating ganglioside band in control rat liver is assigned the symbol GT_{1a}. The band indicated by the white dot in slots 5–7 is tentatively designated as a disialohematoside on the basis of its chromatographic migration [22].

Table 1
Lipid-bound sialic acid content of rat liver and hepatomas

Tissue	No. of samples	Lipid-bound sialic acid (μg per g wet weight)
Control liver	4	21 ± 3
Host liver	3	35 ± 5
Hepatoma 5123 D	3	64 ± 6
Hepatoma 7800	2	27
Hepatoma 7777	4	38 ± 2

Results are expressed as mean ± standard error except in the case of hepatoma 7800 for which the results are the average of two samples. All determinations were performed in duplicate.

3. Results

The quantitative data on lipid-bound sialic acid (expressed per g wet weight of tissue) are given in table 1. In general the hepatomas showed higher values than control liver. Fig. 1 is a photograph of a thin layer chromatogram of the ganglioside pattern of human brain, hepatomas 5123 D and 7777 and control liver. The latter (slot 8) has a complex pattern, exhibiting bands corresponding in migration to GM_3, GM_2, GM_1, GD_{1a}, GD_{1b} and GT_{1b}; an additional band migrating slower than GT_{1b} and tentatively assigned the symbol GT_{1a} [21] is also observed. This pattern was observed in all of six control livers examined. The principal ganglioside bands seen in hepatoma 5123 D extracts (slot 2–4) are those corresponding in migration to GM_3, GM_1 and GD_{1a}; traces of bands corresponding in migration to GD_{1b} and GT_{1b} are also observed. The principal bands exhibited by the ganglioside fraction of hepatomas 7777 (slots 5–7) are those corresponding in migration to GM_3, disialohematoside [22] and GD_{1a}; traces of bands corresponding in migration to GM_2, GM_1 and GT_{1b} are also visible. The ganglioside pattern of hepatoma 7800 is not shown but was similar to that of hepatoma 5123 D. Thus, the major differences from control liver observed in the ganglioside fractions of all three hepatomas were the accumulation of GD_{1a} and diminutions of GD_{1b}, GT_{1b} and GT_{1a}. Four samples of the ganglioside fraction of hepatomas 7777 and 5123 D and three samples of hepatoma 7800 were examined by TLC; the pattern of each of the hepatomas was constant. The ganglioside pattern of four host livers was also examined; two of four samples showed a diminution of GT_{1b} and GT_{1a} but otherwise the pattern was identical to that of control liver.

4. Discussion

There has recently been considerable interest in the ganglioside pattern of neoplastic cells, stimulated by the finding of Hakomori and Murakami [6] that, as compared with control cells, polyoma-transformed baby hamster kidney cells showed a marked decrease of hematoside (GM_3) and an accumulation of its precursor ceramide lactoside. Mora et al. [7] reported that SV-40 transformed cells showed a lack of the more complex gangliosides found in control cells. These workers also observed that a spontaneously-

transformed cell line did not show changes of ganglioside pattern and thus postulated that the simplification of ganglioside pattern might result from the presence of the viral genome. Further investigations have suggested that these changes could be explained by a deficiency of the galactosaminyltransferase converting GM_3 to GM_2 [23]. Brady et al. [8] have described the ganglioside pattern of Morris hepatoma H 5123 grown in tissue culture; the major findings were a marked decrease of GD_{1a} and an accumulation of GM_3 and GM_1 as compared with control hepatocytes. The present observations also show that chemically-transformed hepatocytes growing *in vivo* demonstrate alterations of ganglioside pattern as compared with control liver. However, the changes are different from those described above for neoplastic cells in that the hepatomas studied here preserve a relatively complex ganglioside pattern and appear to accumulate GD_{1a} but show reductions of GD_{1b}, GT_{1b} and GT_{1a}. Alterations of the activities of glycosidases degrading gangliosides or of the various glycosyl transferases involved in the biosynthesis of the more complex gangliosides [24] could explain the differences of ganglioside patterns observed in the hepatomas. It is relevant to note that hepatomas 7777 and 7800 have been shown to display decreases of glycoprotein sialyl transferase activities [25]. It is also possible that the alterations of ganglioside patterns observed in the hepatomas could reflect a change in cell population in the tumours as compared with normal livers (e.g. alterations in the amount of bile duct or reticulo-endothelial cells). Since the observations reported here were made we have been informed by Dr. S. Hakomori that his laboratory has found that the ganglioside patterns of hepatomas 7800, 5123 W and 5123 C are generally similar to those described here [26], Klenk and Choppin [27] and Weinstein et al. [28] have recently demonstrated that some or all of the gangliosides of cultured cells are located on the plasma membrane. We have found (D. Bailey and R.K. Murray, unpublished observations) that the ganglioside pattern of the microsomal fraction of rat liver is identical to that of whole liver; however, the concentration of ganglioside in the smooth endoplasmic reticulum is approximately four times that in rough endoplasmic reticulum (expressed per mg protein). Smooth endoplasmic reticulum is known to be contaminated by plasma membrane [29] so that these observations leave open the possibility that all of the gangliosides of rat liver are represented in the plasma membrane. Dod and Gray [30] have reported the presence of at least one ganglioside in a plasma membrane fraction of rat liver. Various workers have speculated that the alterations of gangliosides in surface membranes [6–8] may be related to the abnormal biological properties of tumour cells. At the present time this remains an attractive but unproven hypothesis. Further studies are in progress in our laboratory on the structure, subcellular location and metabolism of gangliosides in rat liver and minimal deviation hepatomas.

Acknowledgments

This work was supported by grants from the Medical Research Council of Canada and the National Cancer Institute of Canada; a period of support from the Ontario Cancer Institute is also acknowledged. H.P.M. is supported by USPHS Grant No. CA-10729. The authors are grateful to Dr. S. Hakomori for making a copy of his manuscript [26] available to us prior to publication. The assistance of Mr. L. Lerner in some of these studies is also appreciated. We are also indebted to Dr. John Wherrett for various discussions and advice regarding methodology.

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