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Accumulation of globotriaosylceramide in a case of leiomyosarcoma

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Analysis of the glycosphingolipid composition in one case of uterine leiomyosarcoma metastasized to the liver showed an accumulation of globotriaosylceramide as compared with normal liver and uterus from which the tumour originated. The structure and the amount of glycosphingolipids were established by using specific glycosidases, permethylation analysis and h.p.l.c. The reason for the accumulation of globotriaosylceramide in leiomyosarcoma remains to be answered.

INTRODUCTION

It has been well documented that oncogenic transformation is associated with changes in g'ycosphingolipid composition in cells (Hakomori, 1981; Yogeeswaran, 1983). Changes in glycosphingolipid pattern have been observed in tumour cells transformed by DNA and RNA viruses and chemical carcinogens and in spontaneous tumours including human cancer (Hakomori, 1973, 1981; Yogeeswaran, 1983). It has been suggested that two kinds of changes occur in carbohydrate chains during oncogenic transformation (Hakomori, 1975). One involves the block in the synthesis of certain glycosphingolipids, resulting in the accumulation of their precursors, and the second is the enhanced synthesis of a unique glycosphingolipid not present in the parent cells due to the activation of a glycosyltransferase. In this study, we have made a detailed analysis of the glycosphingolipid composition of uterine leiomyosarcoma metastasized to the liver. The results showed a massive accumulation of $GbOse_3Cer$ in this tumour.

MATERIALS AND METHODS

Glycosphingolipid standards

GlcCer, LacCer, $GbOse_3Cer$, $GbOse_4Cer$, $LcOse_3Cer$, and sialosyl-lactoneotetraosylceramide were isolated from human erythrocytes (Kundu *et al.*, 1979). Forssman glycolipid ($GbOse_5Cer$) was prepared from sheep erythrocytes (Naiki *et al.*, 1972). $nLcOse_4Cer$ and asialoG_{M1} were prepared from sialosyl-lactoneotetraosylceramide and G_{M1}, respectively, by formic acid hydrolysis (Svennerholm *et al.*, 1973).

T.l.c. was performed on precoated silica gel 60 plates (E. Merck). Diphenylamine/aniline (Harris & McWilliam, 1954) and resorcinol reagents (Svennerholm, 1957) were used to detect neutral glycosphingolipids and gangliosides, respectively. The quantification of the neutral glycosphingolipids on the plate was accomplished by scanning the plate with a Corning 760 fluorometer/densitometer as described by Ando *et al.* (1978). G.l.c. analyses were carried out on a Hewlett–Packard gas chromatographic unit, model no. 7610 A, equipped with a model 3370 electronic integrator.

Tumour specimens

The human liver with metastatic leiomyosarcoma (7 kg) was collected post mortem from a 45-year-old female with uterine leiomyosarcoma. The tumour mass, free from the neighbouring tissue, was dissected from the metastatic liver and used for this study.

Isolation and analysis of glycosphingolipids

Glycosphingolipids from normal liver, uterus, and the tumour were isolated as described previously (Kundu & Roy, 1978; Kundu *et al.*, 1979). In brief, the total lipids were extracted with chloroform/methanol (2:1, 1:1 and 1:2, v/v), and dried. The total lipid extract was subjected to mild alkaline treatment to remove the alkali-labile lipids. Then the neutral glycosphingolipids were separated from gangliosides by using DEAE-Sephadex A-50 chromatography (Kundu & Roy, 1978; Ledeen *et al.*, 1973). The neutral glycosphingolipids were further purified by the acetylation procedure of Saito & Hakomori (1971). Quantitative analysis of neutral glycosphingolipids was performed by h.p.l.c. (Suzuki *et al.*, 1980). The individual neutral glycosphingolipids from normal liver or the tumour were isolated by preparative t.l.c. with chloroform/methanol/water (12:6:1, by vol.) as developing solvent. The silica gel scrapings of individual glycosphingolipids were eluted with chloroform/methanol/water (50:5:1, by vol.). The amount of glycosphingolipid in each preparation was determined by the phenol/H_2SO_4 reaction (Dubois *et al.*, 1956). The sugar compositions of the purified glycosphingolipids were determined by g.l.c. (Esselman *et al.*, 1972). The procedures for the analysis of the permethylated neutral sugars have been described in detail (Lindberg, 1972).

Abbreviations used: GlcCer, glucosylceramide; LacCer, lactosylceramide; $GbOse_3Cer$, globotriaosylceramide or ceramide trihexoside; $GbOse_4Cer$, globotetraosylceramide or globoside; $GbOse_5Cer$, globopentaosylceramide or Forssman hapten; $LcOse_3Cer$, lactotriaosylceramide; $nLcOse_4Cer$, neolactotetraosylceramide or paragloboside; $GgOse_4Cer$, asialoG_{M1}.

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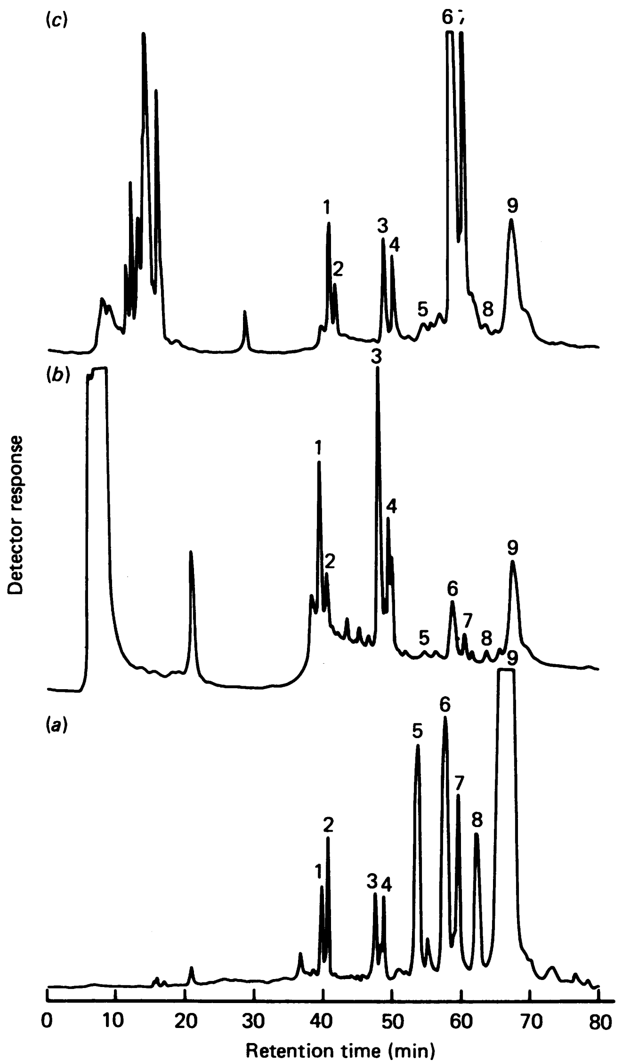

Fig. 1. H.p.l.c. of *O*-acetyl-*N*-*p*-nitrobenzoyl derivatives of neutral glycosphingolipids derived from normal human liver and leiomyosarcoma

Columns, Zorbax SIL; flow rate, 0.5 ml/min. Elution was performed as follows: 5 min with 1% (v/v) propan-2-ol in hexane/dichloroethane (2:1, v/v); 55 min with a linear gradient from 1% to 5% (v/v) of propan-2-ol in hexane/dichloroethane (2:1, v/v) and 20 min with 5% (v/v) propan-2-ol in hexane/dichloroethane (2:1, v/v). (*a*) Standard mixture containing GlcCer (peaks 1 and 2), LacCer (peaks 3 and 4), nLcOse$_3$Cer (peak 5), GbOse$_3$Cer (peaks 6 and 7), nLcOse$_4$Cer (peak 8) and GbOse$_4$Cer (peak 9); (*b*) neutral glycosphingolipid derivatives from normal liver; (*c*) neutral glycosphingolipid derivatives from an equal quantity of tumour mass obtained from the liver with leiomyosarcoma.

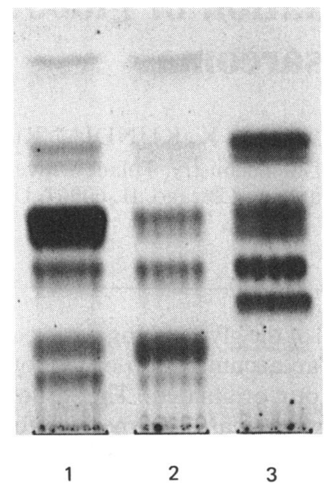

Fig. 2. T.l.c. of neutral glycosphingolipids in leiomyosarcoma and normal uterus

The neutral glycosphingolipid fractions were prepared by DEAE-Sephadex A-50 chromatography and acetylation. Lane 1, neutral glycosphingolipids from leiomyosarcoma; lane 2, neutral glycosphingolipids from normal uterus; lane 3, standards (from top to bottom): LacCer, GbOse$_3$Cer, GbOse$_4$Cer and GbOse$_5$Cer. The plate was developed with chloroform/methanol/water (60:35:8, by vol.). The glycolipids were visualized by spraying the plate with the diphenylamine/aniline spray reagent.

RESULTS AND DISCUSSION

The neutral glycosphingolipid fractions of normal liver and tumour, which were purified by using DEAE-Sephadex A-50 chromatography and acetylation, were analysed by h.p.l.c. as shown in Fig. 1. The quantitative data for each neutral glycosphingolipid are summarized in Table 1. Qualitatively, the number of neutral glycosphingolipids in normal liver (Fig. 1, panel *b*) and in leiomyosarcoma (Fig. 1, panel *c*) were the same. However, the content of GbOse$_3$Cer (Fig. 1, panel *c*, peaks 6 and 7) in the leiomyosarcoma is approximately eight times that of the normal liver. Among neutral glycosphingolipids, LacCer, GbOse$_3$Cer and GbOse$_4$Cer were individually isolated from preparative t.l.c. and their identifications were based on h.p.l.c. retention times, sugar composition by g.l.c., and methylation analysis. In the methylation analysis, the GbOse$_3$Cer isolated from the tumour resulted in a 50–60% overall yield of the following partially methylated alditol acetates: 1,5-di-*O*-acetyl-2,3,4,6,-tetra-*O*-methyl-galactitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylgalactitol and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol. These alditol acetates were identical with those produced from the GbOse$_3$Cer isolated from normal liver or human erythrocytes. Moreover, the GbOse$_3$Cer isolated from the tumour was hydrolysed by fig α-galactosidase (Li & Li, 1972) to produce LacCer which was demonstrated by t.l.c. analysis. The identity of GbOse$_3$Cer was further confirmed by complement fixation assay using purified rabbit IgG antibodies against GbOse$_3$Cer (Naiki & Taketomi, 1969). The identification of the purified nLcOse$_4$Cer was based on chromatographic mobility, sugar composition by g.l.c. and enzymic analysis (Li, 1979) using exoglycosidases and endo-β-galactosidase. nLcOse$_4$Cer was converted into a trisaccharide and GlcCer by endo-β-galactosidase. It was sequentially hydrolysed by β-galactosidase, β-hexosaminidase, and again β-galactosidase to produce nLcOse$_3$Cer, LacCer and GlcCer, respectively. Since the tumour originated from uterus, we compared the glycosphingolipid profile of the leiomyosarcoma with that of normal uterus. Both the neutral glycosphingolipids and the gangliosides

Table 1. Concentration of neutral glycosphingolipids in normal liver and the leiomyosarcoma

The individual glycosphingolipids were separated and quantified by h.p.l.c. (Suzuki *et al.*, 1980).

	Amount (μmol/g dry tissue) of:					
	GlcCer	LacCer	$GbOse_3Cer$	$nLcOse_3Cer$	$GbOse_4Cer$	$nLcOse_4Cer$
Normal liver	0.45	0.48	0.21	0.04	0.34	0.03
Leiomyosarcoma	0.26	0.16	1.70	0.02	0.33	0.03

obtained from DEAE-Sephadex A-50 chromatography were compared. We found that the ganglioside profile of leiomyosarcoma was practically identical with that of the normal uterus. The neutral glycosphingolipid fraction of the tumour showed the same number of glycolipid bands as that of the normal uterus (Fig. 2); however, the amount of $GbOse_3Cer$ in the tumour was approximately six times that found in a normal uterus. In addition, LacCer was about twice as abundant in the tumour than in the normal uterus. Two glycosphingolipids with t.l.c. mobilities slower than $GbOse_4Cer$ were detected both in the uterus and the tumour. The structures of these two glycolipids are yet to be determined. The lack of availability of normal human uterus in large quantity precluded the detailed studies of glycosphingolipids in this tissue.

Based on the same amount of tissue, the leiomyosarcoma showed an extensive accumulation of $GbOse_3Cer$ as compared with the tissue of origin, the uterus, and the metastasized tissue, the liver. This suggests that the elevation in $GbOse_3Cer$ may be indeed tumour-associated. It is of interest to note that the $GbOse_3Cer$ has been identified as a glycolipid antigen associated with Burkitt lymphoma (Nudelman *et al.*, 1983; Wiels *et al.*, 1984). The reason for such a massive accumulation of $GbOse_3Cer$ in leiomyosarcoma is not known at the present time.

As leiomyosarcoma is a low-incidence tumour, we were not able to analyse more cases to determine whether the accumulation of $GbOse_3Cer$ can be considered as a marker for this tumour. Since we have a large amount of tumour tissue available, we will be able to share it with other investigators in order to compare this case with others and to define more precisely the biochemical basis for the changes in glycosphingolipids in leiomyosarcoma and other related tumours.

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