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Fucosyl-G_{M1} - A Ganglioside Associated with Small Cell Lung Carcinomas

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Characterization of monosialogangliosides of a small cell lung carcinoma showed a unique composition. The tumour contained G_{M2} and Fucosyl-G_{M1} (Fuc-G_{M1}) with 2-hydroxy fatty acids as major ganglioside components. Three out of four other small cell carcinomas analysed contained also Fuc-G_{M1} as a characteristic ganglioside. Fuc-G_{M1} is suggested to be a small cell lung carcinoma associated ganglioside antigen.

Gangliosides are sialic acid-containing glycosphingolipids found in a high concentration in nervous tissue, but they are normal constituents in all mammalian cell membranes. Their localization in the outer layer of the plasma membrane, with the carbohydrate chain facing the extracellular space and the large structural diversity suggests them to be specific mediators of cellular communication, including regulation of cell growth and differentiation [1]. Malignant transformation has been shown to be accompanied by changes in the ganglioside composition. These changes are the result of either an inhibition of certain glycosyltransferases or an activation of glycosyltransferases normally unexpressed in adult tissues [1].

During the last decade there has been an intense search for tumour-associated ganglioside antigens expressed in malignant tissues. Using the monoclonal antibody technology, human tumour-associated ganglioside antigens have been described in melanomas [2, 3], gastrointestinal and pancreatic carcinomas [4] and carcinomas in general [5]. This study demonstrates a unique ganglioside composition and a possible novel ganglioside antigen associated with human small cell lung carcinomas.

Nomenclature: The gangliosides have been designated according to Svennerholm [25]; G_{M3}, II^3NeuAc-LacCer; G_{D3}, II^3(NeuAc)$_2$-LacCer; G_{M2}, II^3NeuAc-GgOse$_3$Cer; G_{M1},II^3NeuAc-GgOse$_4$Cer; Fuc-G_{M1}, αFucIV^2NeuAcII^3-GgOse$_4$Cer; $3'L_{M1}$, IV^3NeuAc-nLcOse$_4$Cer; $6'L_{M1}$, IV^6NeuAc-nLcOse$_4$Cer.

Materials and Methods

Clinical Material

The tumour tissue was obtained at autopsy from an 82 year old male smoker, who died of lung cancer with multiple metastases. The patient had not received any chemotherapy or radiation treatment against his tumour. Histopathological examination established the diagnosis of small cell lung carcinoma and the subtype as oat cell carcinoma [6]. Only the primary tumour was analysed.

In addition, tumour tissue of 4 other patients who had died from disseminated small cell lung cancer was examined.

Chemicals

Silica Gel 60, (230-400 mesh) and TLC- and HPTLC-plates (Silica Gel 60), were obtained from Merck AG, Darmstadt, W. Germany. Sephadex G-25 was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. The anion exchange resin Spherosil-DEAE-Dextran was a gift from Institute Mérieux, Lyon, France [7]. α-L-Fucosidase (EC 3.2.1.51) was obtained from Boehringer, Mannheim, W. Germany. Light petroleum (boiling range 42-45°C) used for GLC analyses of fatty acids and sphingosine bases, was redistilled and tested for purity as previously described [8]. All organic solvents and chemicals were of analytical quality and used without further purification.

Isolation and Characterization of the Gangliosides

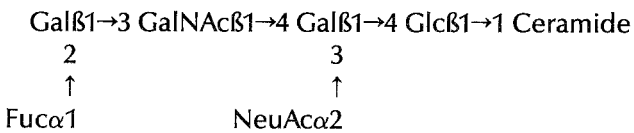
The tumour (250 g) was homogenized in a scissor homogenizer and extracted twice with 10 volumes (w/v) of chloroform/methanol/water (C/M/W) 4/8/3 (v/v/v) [9]. The total lipid extract was evaporated to dryness and dissolved in C/M/W 60/30/4.5 (v/v/v). Low molecular contaminants were removed by chromatography on Sephadex G-25 [10]. Gangliosides were isolated from the purified total lipid extract by anion exchange chromatography on a Spherosil-DEAE-dextran column. Neutral lipids were eluted from the column with C/M/W 60/30/4.5, and mono- and oligosialogangliosides with a discontinuous gradient of potassium acetate in methanol [7]. Individual monosialogangliosides were isolated by silica column chromatography and preparative TLC.

Gangliosides were quantified with the resorcinol assay [11], and the distribution of individual gangliosides was determined by densitometry after TLC separation in the following solvent systems; a). C/M/0.25% KCl 50/40/10 (v/v/v); b). C/M/2.5M Ammonia 50/40/10(v/v/v); c). n-Propanol/2.0 M Ammonia 7/3(v/v); d). n-Propanol/ 0.25% KCl 3/1(v/v). The carbohydrate composition of the individual gangliosides was quantitatively determined by GLC as their corresponding alditol acetates [8]. Long-chain bases were quantitatively determined with the methyl orange method [12]. The ceramide was characterized by GLC analyses of the fatty acid and long chain base composition [13]. Partial acid hydrolysis with trichloroacetic acid and enzymic hydrolyses with specific glycosidases were performed as in [14]. Permethylated sugars were analysed by GC-MS as described in [14].

Results

The ganglioside concentration and the distribution of the major gangliosides are given in Table 1. An HPTLC-plate of the total monosialoganglioside fraction and the isolated major monosialogangliosides is shown in Fig. 1. For comparison, monosialogangliosides isolated from normal lung tissue and from other types of lung carcinomas are shown in Fig. 2. Compared to normal lung tissue this small cell carcinoma had about three times higher concentration of monosialogangliosides, (91 nmol/g and 297 nmol/g respectively). Ganglioside G_{M3} was the dominating monosialoganglioside in the normal lung tissue, representing approximately 85% of the monosialogangliosides.

In the small cell lung carcinoma three major monosialogangliosides were found, which made up more than 80% of the total ganglioside sialic acid. Structural analyses showed two of these gangliosides to be G_{M3} and G_{M2}. The third ganglioside contained sphingosine, glucose, galactose, *N*-acetylgalactosamine, fucose and *N*-acetylneuraminic acid in the molar ratio 1.0:1.0:2.1:1.1:1.0:1.1. The fucose residue was completely removed by α-fucosidase hydrolysis, which gave a ganglioside migrating on HPTLC as a double band slightly slower than reference brain G_{M1}. After hydrolysis with trichloroacetic acid a major product was isolated, which migrated on TLC as a double band slightly slower than gangliotetraosylceramide. This product contained glucose, galactose and *N*-acetylgalactosamine in the molar ratio 1:2:1. The results of the permethylation analyses of the fucose-containing ganglioside and the corresponding defucosylated, desialylated substance are given in Table 2. The structural data suggest that the fucose-containing ganglioside was Fuc-G_{M1} (αFucIV^2 NeuAcII^3-GgOse$_4$Cer):



Characterization of the ceramide composition showed that the three gangliosides contained both normal and 2-hydroxy fatty acids, but there was a marked difference in the degree of hydroxylation of the fatty acids between the three gangliosides. In G_{M3} there were about equal proportions of normal and 2-hydroxy fatty acids, in G_{M2} the proportion of 2-hydroxy fatty acids was approximately 70%, while Fuc-G_{M1} contained >90% 2-hydroxy fatty acids (Table 3). The 2-hydroxy fatty acids were of similar chain length in the three gangliosides, with C-16, C-22 and C-24 2-hydroxy fatty acids as dominating components. The normal fatty acids in G_{M3} and Fuc-G_{M1} had a similar chain length to the 2-hydroxy fatty acids, while in G_{M2} oleic acid and stearic acid were the major fatty acids (Table 3). The sphingosine base composition was similar in the three gangliosides; 4-sphingenine made up 85-88 molar% of the long chain bases.

Preliminary characterization of the gangliosides in tumour tissue of 4 other patients with small cell lung carcinoma revealed the presence of Fuc-G_{M1} in 3 of these tumours.

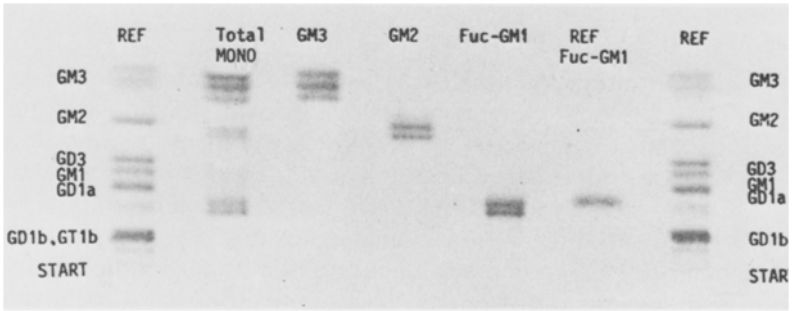


Figure 1. Total monosialoganglioside fraction and major individual monosialogangliosides of a small cell lung carcinoma.

The gangliosides were separated by HPTLC, using C/M/2.5 M Ammonia 50/40/10 as developing solvent. Visualization of the gangliosides was performed with the resorcinol reagent [11]. Ref Fuc-G_{M1} was isolated from minipig brain as described in [18].

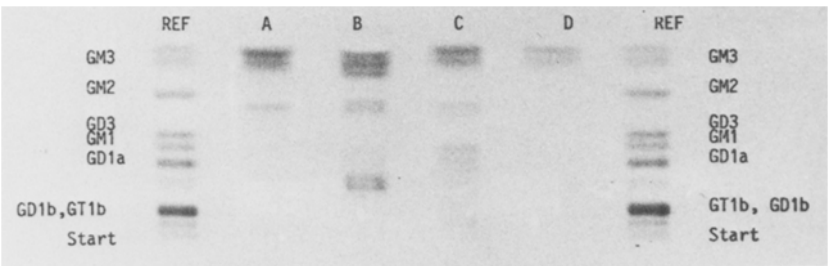


Figure 2. Monosialogangliosides isolated from normal lung and different lung carcinomas.

A. Normal lung; B. Small cell lung carcinoma, this case; C. Squamous cell carcinoma; D. Adenocarcinoma of the lung. The gangliosides were separated by HPTLC developed in C/M/2.5 M Ammonia, 50/40/10, and visualization with resorcinol [11].

Discussion

This small cell lung carcinoma showed a unique ganglioside composition not previously demonstrated in any human tissue. As shown in Fig. 2 the ganglioside composition was also different from that found in other types of lung carcinomas. In normal human adult organs of visceral origin (including lung) the dominating gangliosides are G_{M3} and G_{D3}. The major part of the complex gangliosides in these tissues is related to the *neo*lactotetraose structure, while gangliosides of the gangliotetraose series are minor components. In this tumour G_{M2} and Fuc-G_{M1}, both related to the gangliotetraose structure,

Table 1. Ganglioside concentration and distribution of major gangliosides in a small cell lung carcinoma.

	nmol NeuAc/g	%NeuAc
Monosialogangliosides	297	84
G_{M3}	155	44
G_{M2}	54	15
Fuc-G_{M1}	79	22
Minor components[a]	9	3
Oligosialogangliosides	57	16
G_{D3}	32	9
Total	354	

[a] in this fraction G_{M1}, 3'-L_{M1} and 6'-L_{M1} were identified

Table 2. Results of the permethylation study of the fucose-containing ganglioside isolated from a small cell lung carcinoma.

	2,3,6-Me_3 glc	2,3,4-Me_3 fuc	2,3,6-Me_3 gal	2,3,4,6-Me_4 gal	2,6-Me_2 gal	3,4,6-Me_3 gal	4,6-Me_2 galNAc
Intact ganglioside	+	+	—	—	+	+	+
Substance isolated after acid hydrolysis	+	—	+	+	—	—	+

Table 3. Fatty acid composition of major monosialogangliosides isolated from a small cell lung carcinoma.

			% of each fatty acid							
		%	16:0	18:0	20:0	22:0	22:1	23:0	24:0	24:1
G_{M3}	N	60	25	4	4	18	2	10	15	19
	H	40	34	7	8	17	—	10	15	6
G_{M2}	N	30	36	39	5	11	—	4	4	1
	H	70	40	7	9	16	—	10	13	7
Fuc-G_{M1}	N	<10	45	8	6	20	—	8	10	1
	H	>90	42	5	9	16	—	9	12	3

N = normal fatty acids; H = 2-hydroxy fatty acids

represented about 40% of the ganglioside sialic acid. Thus the malignant transformation of this tissue has lead to a totally changed ganglioside biosynthesis.

Fuc-G_{M1} has been found in small amounts in a variety of mammalian tissues [15-18], and has been shown to be involved in the tumourogenesis of chemically induced rat hepatomas [19]. The ganglioside has also been detected in human brain, but has to our knowledge previously not been reported in other human tissues.

Characterization of the ceramide of Fuc-G_{M1} and G_{M2} revealed a fatty acid composition not described in gangliosides of the ganglio- series of human origin. These gangliosides normally contain only normal fatty acids, with stearic acid as the dominating fatty acid in both brain and extraneural tissues [13, 20]. 2-Hydroxy fatty acids have been found in G_{M3} isolated from adult human kidney and liver [20, 21], and in gangliosides of the lacto- and *neo*lactotetraose series in human fetal intestine (meconium) [22], but this is the first demonstration of 2-hydroxy fatty acids in gangliosides of the ganglio series.

The ceramide composition of glycolipids has been proposed to be developmentally regulated and associated with specific glycosyltransferases, resulting in the synthesis of "specific" glycolipids with a characteristic ceramide composition during cell differentiation [23]. The low concentration of G_{M1} (< 1%), in contrast to the high concentration of Fuc-G_{M1}, indicates a high fucosyltransferase activity in the tumour, leading to the immediate synthesis of Fuc-G_{M1} from G_{M1}. The presence of almost exclusively 2-hydroxy fatty acids in Fuc-G_{M1} suggests a ceramide specificity of the glycosyltranferases synthesizing Fuc-G_{M1}, with preferential glycosylation of precursors with ceramides containing 2-hydroxy fatty acids. The accumulation of Fuc-G_{M1} in this tumour might be the result of a developmentally regulated synthesis of ceramides containing 2-hydroxy fatty acids associated with specific galactosyl-, *N*-acetylgalactosaminyl- and fucosyltransferases, found only during a short period of cell differentiation, but also present in the undifferentiated cells of this tumour. A shift of glycolipid synthesis from glycolipids of the ganglio- series in undifferentiated cells towards glycolipids of the lacto- series and globo- series during differentiation has been described in murine leukemia cells [24]. A similar switch in glycolipid synthesis might also occur during differentiation of human lung, and thus explain the high concentration of gangliosides of the ganglio- series in the undifferentiated cells of this tumour, although they occur in very low amounts in normal adult lung.

Small cell lung carcinomas are a heterogenous group of tumours with different subtypes [6]. Studies are in progress to determine if Fuc-G_{M1} is a general small cell lung carcinoma-associated ganglioside antigen, and as such could be of importance for immuno-diagnosis and therapy of these tumours. So far Fuc-G_{M1} has been found as a characteristic ganglioside in four out of five small cell lung carcinomas analysed. Monoclonal antibodies have also been raised against Fuc-G_{M1} for further studies concerning its tissue distribution and potential use in immunodiagnosis and therapy (Brezicka *et al.*, in preparation).

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