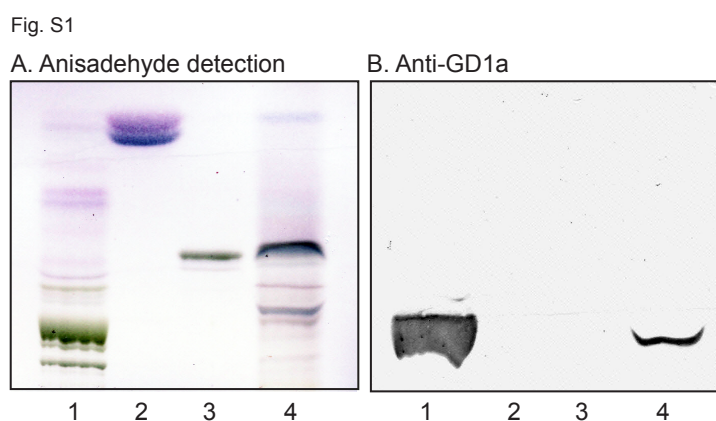


SUPPLEMENTARY MATERIAL

Characterization of Human Medullary Thyroid Carcinoma Glycosphingolipids Identifies Potential Cancer Markers

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Supplementary figures

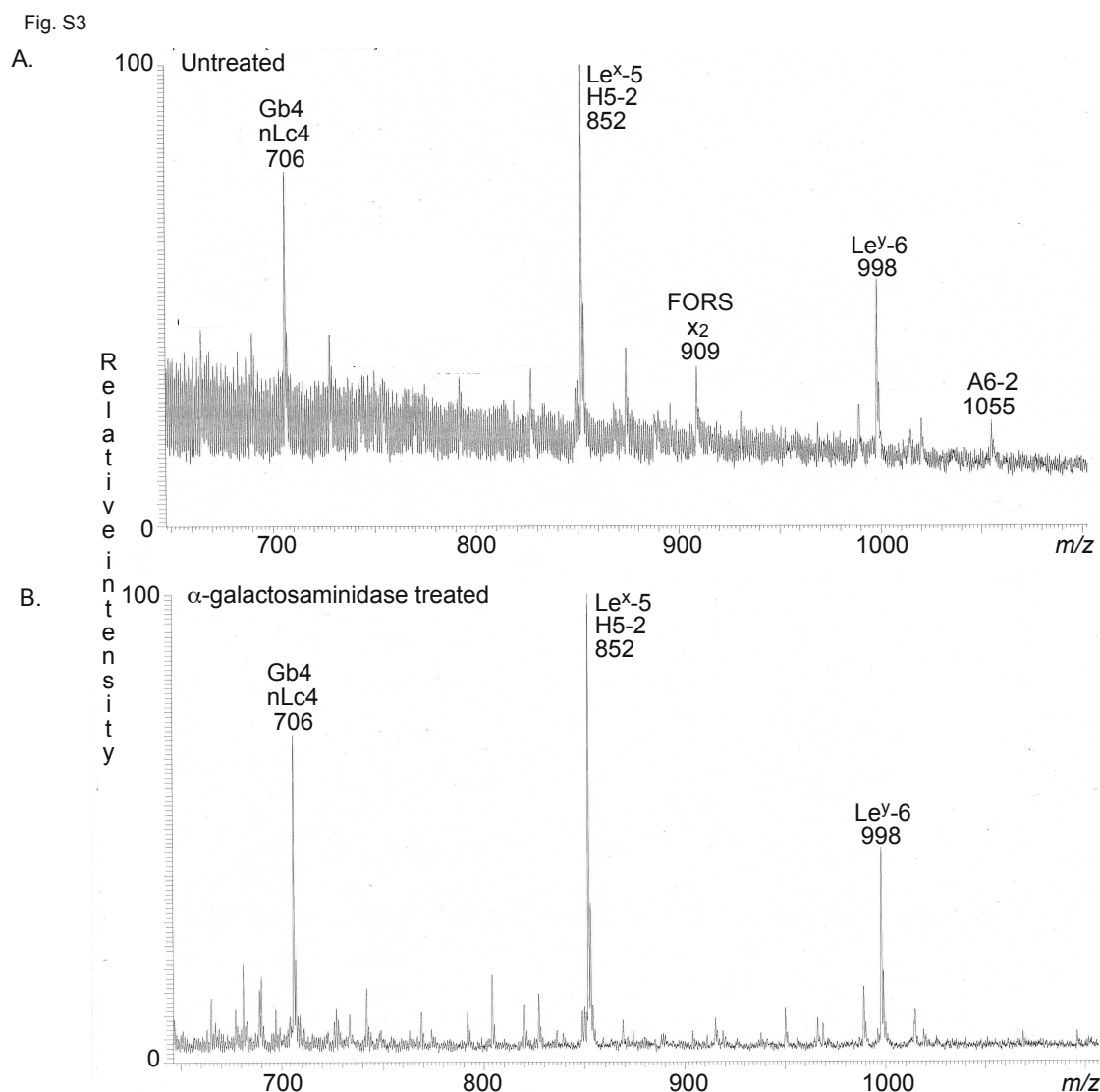


Supplementary Figure S1. Binding of monoclonal anti-GD1a antibodies to the acid glycosphingolipids from human medullary thyroid cancer. Thin-layer chromatogram after detection with anisaldehyde (A), and autoradiogram obtained by binding of monoclonal anti-GD1a antibodies (B). The lanes were: lane 1, calf brain gangliosides , 40 μ g; lane 2, sulfatide ($\text{SO}_3\text{-3Gal}\beta\text{1Cer}$), 4 μ g; lane 3, GM3 ganglioside ($\text{Neu5Ac}\alpha\text{3Gal}\beta\text{4Glc}\beta\text{1Cer}$), 4 μ g; acid glycosphingolipids from human medullary thyroid cancer, 40 μ g.

Fig. S2

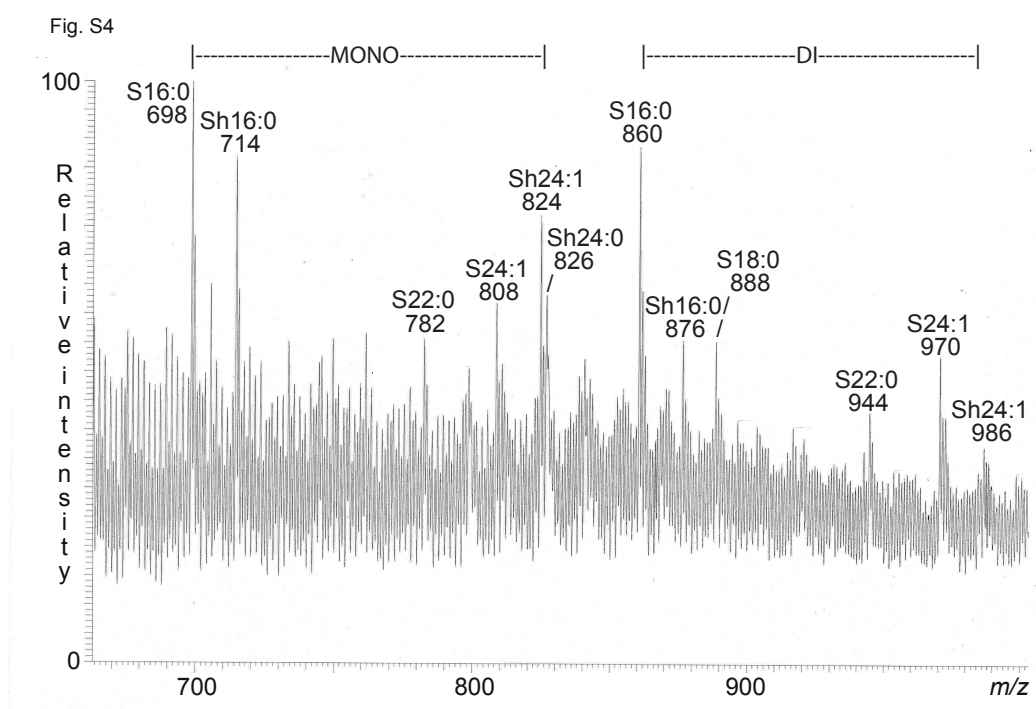
A. <i>m/z</i> 706	HexNAc-O- 220 C ₁	Hex-O- 382 C ₂	4Hex-O- 544 C ₃	4Hex	Globotetra
B. <i>m/z</i> 706	Hex-O-4HexNAc-O- 382 C ₂	Hex-O- 544 C ₃	4Hex		Neolactotetra
C. <i>m/z</i> 852	Hex-O-4HexNAc-O- O Fuc	Z _{3β} 528 C ₂	690 C ₃	4Hex	Le ^x penta
D. <i>m/z</i> 852	Fuc-O-Hex-O- 325 C ₂	4HexNAc-O- 528 C ₃	Hex-O- 690 C ₄	4Hex	H type 2 penta
E. <i>m/z</i> 909	HexNAc-O-HexNAc-O- 423 C ₂	4Hex-O- 585 C ₃	4Hex-O- 747 C ₄	4Hex	Forssman
F. <i>m/z</i> 909	HexNAc-O-Hex-O- 382 C ₂	4HexNAc-O- 585 C ₃	Hex-O- 747 C ₄	4Hex	x2 penta
G. <i>m/z</i> 998	Fuc-O-Hex- 325 C _{2α}	O-4HexNAc-O- O Fuc	Z _{3β} 836 C ₄	4Hex	Le ^y hexa
H. <i>m/z</i> 1055	HexNAc-O-Hex-O- O Fuc	4HexNAc-O- 528 C ₂	Hex-O- 731 C ₃	4Hex 893 C ₄	A type 2 hexa
I. <i>m/z</i> 1201	HexNAc-O-Hex-O- O Fuc	4HexNAc-O- 528 C _{2α}	3 O Fuc	Z _{3β} 877 C ₃ 1039 C ₄	A type 2 hepta

Supplementary Figure S2. Interpretation formulas for Figure 3 and Figure 5.



Supplementary Figure S3. α -N-acetylgalactosaminidase hydrolysis of the oligosaccharides derived from the total non-acid glycosphingolipid fraction from human medullary thyroid cancer by hydrolysis with endoglycoceramidase II from *Rhodococcus* spp. (A) Molecular ion profile from LC-ESI/MS of the untreated oligosaccharides. (B) Molecular ion profile from LC-ESI/MS of the oligosaccharides after α -galactosaminidase hydrolysis.

The oligosaccharides identified in the chromatogram were: Gb4, GalNAc β 3Gal α 4Gal β 4Glc; nLc4, Gal β 4GlcNAc β 3Gal β 4Glc; Le^x-5, Gal β 4(Fuc α 3)GlcNAc β 3Gal β 4Glc; H5-2, Fuc α 2Gal β 4GlcNAc β 3Gal β 4Glc; FORS, GalNAc α 3GalNAc β 3Gal α 4Gal β 4Glc; x₂, GalNAc β 3Gal β 4GlcNAc β 3Gal β 4Glc; Le^y-6, Fuc α 2Gal β 4(Fuc α 3)GlcNAc β 3Gal β 4Glc.



Supplementary Figure S4. Molecular ion profile from LC-ESI/MS of fraction T1. In the shorthand nomenclature for fatty acids and bases, the number before the colon refers to the carbon chain length and the number after the colon gives the total number of double bonds in the molecule. Fatty acids with a 2-hydroxy group are denoted by the prefix h before the abbreviation, as *e.g.* h16:0. S designates sphingosine (d18:1) long chain base.

Supplementary tables

Supplementary Table S1. Glycosphingolipid preparations

	Dry weight	Total acid glycosphingolipids	Total non-acid glycosphingolipids	mg acid glyco-sphingolipids/g dry weight	mg non-acid glyco-sphingolipids/g dry weight
Medullary thyroid cancer	5.4 g	84.4 mg	24.4 mg	15.6	4.5

Supplementary Table S2. Carbohydrate binding ligands used in chromatogram binding assays.

Ligand	Clone	Manufacturer	Dilution	Binding specificity
Anti-sialyl-Le ^a	116-NS-19-9	Thermo Fischer	1:30	Neu5Ac α 3Gal β 3(Fuc α 4)GlcNAc
Anti-GD1a	GD1a-1	Merck Millipore;	1:100	Neu5Ac α 3Gal β 3GalNAc β 4(Neu5Ac α 3)Gal β 4Glc
<i>H. pomatia</i> lectin	-	Sigma-Aldrich	-	GalNAc α
Anti-blood group A	HE-195	Sigma-Aldrich	1:500	GalNAc α 3(Fuc α 2)Gal
Anti-blood group H type 2	A583	DakoCytomation Norden A/S	1:100	Fuc α 2Gal β 4GlcNAc
Anti-Lewis ^x	P12	Merck Millipore	1:100	Gal β 4(Fuc α 3)GlcNAc
Anti-Lewis ^y	F3	Merck Millipore	1:100	Fuc α 2Gal β 4(Fuc α 3)GlcNAc
Anti-Forssman	M1/87	Santa Cruz	1:100	GalNAc α 3GalNAc β 3Gal α 4Gal β 4Glc
Anti-GloboH	VK9	eBioscience/Invitrogen	1:100	Fuc α 2Gal β 3GalNAc β 3Gal α 4Gal β 4Glc

Supplementary Table S3. Characteristics of patients studied by immunohistochemistry

Patient No.	Number of sections	Blood group	Tumor type	Hereditary
1	2	A(Rh+)	Primary tumor	MEN2A ^a
2	4	O(Rh+)	Lymf node metastasis	-
3	1	B(Rh+)	Lymf node metastasis	MEN2B ^b
4	2	O(Rh+)	Primary tumor + Lymf node metastasis	-
5	1	A(Rh+)	Primary tumor	-

^aMultiple neuroendocrine neoplasia type 2A

^bMultiple neuroendocrine neoplasia type 2B