Supplementary Material

Glycomic analysis of gastric carcinoma cells discloses glycans as modulators of RON receptor tyrosine kinase activation in cancer

Stefan Mereiter 1, 2, 3; Ana Magalhães 1, 2; Barbara Adamczyk 4; Chunsheng Jin 4; Andreia Almeida 5, 6; Lylia Drici 7; Maria Ibáñez-Vea 7; Catarina Gomes 1,2; José A. Ferreira 1, 2, 8; Luis P. Afonso 9; Lúcio L. Santos 8, 10; Martin R. Larsen 7; Daniel Kolarich 5; Niclas G. Karlsson 4; Celso A. Reis 1, 2, 3, 11,#

1. I3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Portugal; 2. Institute of Molecular Pathology and Immunology of the University of Porto - IPATIMUP, Porto, Portugal; 3. Institute of Biomedical Sciences of Abel Salazar - ICBAS, University of Porto, Portugal; 4. Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Sweden; 5. Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany; 6. Free University Berlin, Berlin, Germany 7. Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark; 8. Experimental Pathology and Therapeutics Group, Portuguese Institute of Oncology of Porto, Portugal, 10. Department of Surgical Oncology, Portuguese Institute of Oncology of Porto, Portugal 11. Medical Faculty, University of Porto, Portugal

#To whom correspondence should be addressed: Celso A. Reis

Supplementary table 1

		Mock (N = 2)		ST3GAL4 (N = 2)		
Gene	Encodingfor	Avg	SD	Avg	SD	T-test
MGAT3	GNT-III	0.078	0.090	0.041	0.011	0.666
MGAT5	GNT-V	2.936	0.304	2.553	0.229	0.300
ST6GAL1	ST6Gal-I	10.505	3.385	8.686	0.923	0.584
ST6GAL2	ST6Gal-II	0.000	0.000	0.000	0.000	
MST1R	RON	15.374	1.052	15.391	0.507	0.987
ACTB	Beta-actin	737.975	53.375	852.531	64.743	0.198
GAPDH	GAPDH	1154.628	84.734	1283.145	115.757	0.342
TUBB	Tubulin beta chain	126.554	3.703	110.503	4.211	0.057

Supplementary table 1: RNASeq of selected genes

Selected target genes assessed by quantitative RNASeq data analysis of ST3GAL4 overexpressing cells and the mock transfected control. Read counts of each RNASeq experiment were normalized to the total number of reads and are represented as average (Avg) and standard deviation (SD) of the two biological replicates. The selected target genes show no significant alterations (p < 0.05). Statistical significance was calculated by the two-tailed, unpaired t-test.

Supplementary table 2

Case	Туре	RON	SLe ^x	PLA
1		++	++	+
2		++	-	na
3		+	+	na
4		++	-	na
5	Intestinal	++	++	++
6	mestinai	++	+	+
7		++	+	+
8		+	+	+
9		+	+	na
10		+	+	+
11		+	+	++
12		+	++	na
13	Diffuse	+	-	na
14		+	+	na
15		+	+	+
Total	+	60%	60%	75%
	++	40%	20%	25%
	++ and +	100%	80%	100%

Supplementary table 2: Gastric tumor samples

15 paraffin embedded tumor samples were examined for the expression of RON and SLe^x and classified according to the strength of their IHC staining. 6 intestinal type cases and 2 diffuse type cases were selected for further analysis of RON-SLe^x colocalization by *in situ* proximity ligation assay. All cases showed colocalization with two cases being highly positive. Each case contained non-cancerous, adjacent mucosa which acted as an internal negative control for the PLA experiments and which were exclusively negative. Staining strength and distribution was classified in -: negative; +: positive; ++: very positive. na: not analyzed.