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High Globo-H expression associated with poor survival of gastric cancer patients and enriched PD-L1 expression

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BACKGROUND

- Globo series antigens have been implicated in playing important roles in enhancing tumor growth through protecting tumor cells from apoptosis, suppressing T cell activity in the tumor microenvironment and promoting endothelial cell angiogenesis[1-4].
- Globo-H (GH), a globo-series glycosphingolipid (GSL) antigen that is synthesized by key enzymes β1,3-galactosyltransferase V (β3GalT5), fucosyltransferase (FUT) 1 and 2, is highly expressed on a variety of epithelial cancers rendering it a promising target for cancer immunotherapy[1-2, 5].
- GH is reported to associate with the EGFR mutant and PD-L1 expression in non-small cell lung cancer (NSCLC) patients[6].
- GH–targeting antibody–drug conjugate (ADC) OBI-999 has been demonstrated to have an excellent tumor growth inhibition potency in animal models across multiple cancer types including gastric cancer (GC)[7].

OBJECTIVE

- This study aims to investigate the association between GH roles and GC progression.

RESULTS

1. Significant correlations between high mRNA expression of GH-synthesized key enzymes and worse survival for GC patients.

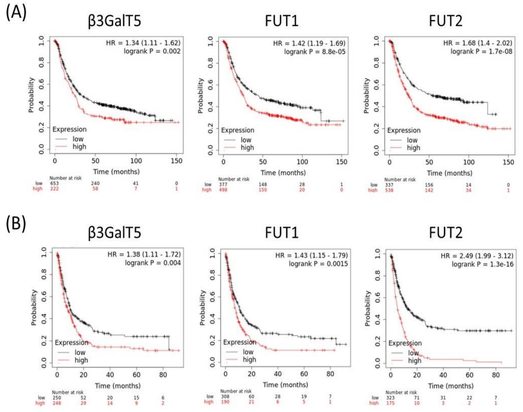


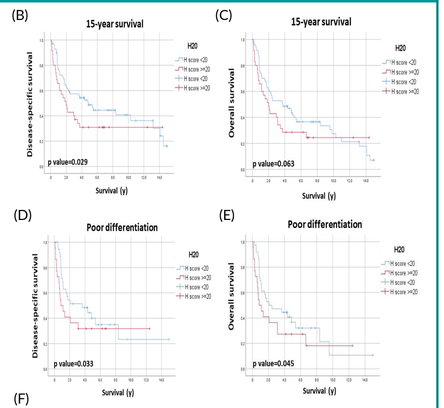
Figure 1. Evaluation of prognostic value of *β3GalT5*, *FUT1* and *FUT2* mRNA expression in www.kmplot.com. The effects of *β3GalT5*, *FUT1* and *FUT2* on (**A**) overall survival and (**B**) post progression survival using gastric cancer samples based on the Kaplan-Meier plotter.

2. Positive GH expression associates with poor survival in GC overall population/poorly differentiated tumors and invasiveness.

(A)

Characteristics of GC patients

		N=105	%
Gender	Male	70	66.7
	Female	35	33.3
Age (y/o) range	33.8--93 (67.1±12.2)		
Stage	1	16	15.2
	2	29	27.6
	3	56	53.3
	4	4	3.8
Differentiation grade	Well	7	6.7
	Moderately	40	38.1
	Poorly	58	55.2
T classification	T1	11	10.5
	T2	17	16.2
	T3	58	55.2
	T4	19	18.1
N status	No	37	35.2
	N1	21	20.0
	N2	14	13.3
	N3	34	31.4
Metastasis	No	102	97.1
	Yes	3	2.9
Chemotherapy	No	80	76.2
	Yes	25	23.8
Survival	Alive	27	25.7
	Death	78	74.3
Survival (y)	0.1-15 (4.1±4.0)		



(F)

	GlobH, H score		p value
	H score<20	H score ≥20	
	Total	70 (66.7%)	35 (33.3%)	
T classification				
T1-T2	28 (26.7%)	24 (34.3%)	4 (11.4%)	0.013
T3-T4	77 (73.3%)	46 (65.7%)	31 (88.6%)	

Figure 2. Kaplan-Meier survival analysis and chi-square test results for GH expression in GC. The level of GH expression was evaluated in clinical adenocarcinoma samples from 105 patients with GC by immunohistochemistry using H-score. (**A**) The patients' clinical features. Kaplan-Meier curves of (**B**) disease specific survival (DSS) and (**C**) overall survival (OS) in the overall population (n=105), and (**D**) DSS and (**E**) OS in the poorly differentiated tumors (n=58). Positive GH expression (H score≥20) was significantly associated with a poor DSS in all samples (at 15 year; P = 0.029) and poor DSS/OS in poorly differentiated tumors (DSS P = 0.033, OS P = 0.045). Prognostic significance of positive GH expression in 15-year OS in entire GC population was not found. (**F**) Relationships between GH expression and T classification in GC. The chi-square test showed that the positive GH expression was correlated with T stage (P = 0.013).

3. Enhanced cell proliferation in sorted GC cells with high GH expression.

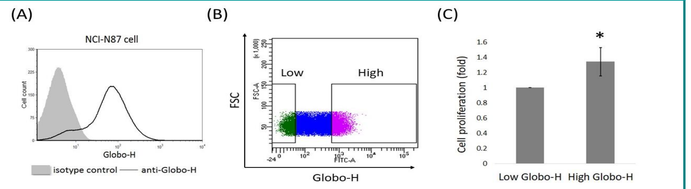
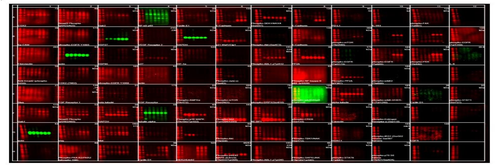


Figure 3. Upregulated cell proliferative activity in high GH–expressing GC cells. (**A**) GH expression on the surface of GC NCI-N87 cells. FACS analysis of GH was performed in cells using mAbVK9. (**B**) NCI-N87 cells were sorted into high-GH and low-GH cells with mAbVK9 by FACSAria. (**C**) Enhanced cell proliferation in high-GH NCI-N87 cells. MTS assay was performed in indicated cells at 48h after cell seeding. Results depict mean ± SD of four independent experiments. The results expressed as fold change of respective low-GH cells (*P < 0.05 versus low-GH cells).

4. Activated AKT/p38/JNK signaling pathways with higher cyclin D1/cyclin E1 levels in high GH-expressing GC cells.



(B)

	Antibodies	Fold change of relative protein abundance (High GH/ Low GH)
Increased	Phospho-AKT (Ser473)	1.342 ± 0.003
	Phospho-SAPK/JNK (Thr183/Tyr185)	1.503 ± 0.008
	Phospho-p38 MAPK (Thr180/Tyr182)	1.41 ± 0.12
	Phospho-NF-kappa-B p65	1.413 ± 0.043
	Cyclin D1	1.714 ± 0.715
	Cyclin E1	1.642 ± 0.095
	Phospho-DRP1(Ser616)	1.213 ± 0.031
	Slug	1.478 ± 0.503
	Phospho--AMPKα (Thr172)	1.186 ± 0.105
	Phospho-p44/42 MAPK (Erk1/2)	1.013 ± 0.156
Decreased	Phospho-Jun(-c) (Ser63)	0.913 ± 0.099

Note: Relative protein abundance was normalized to the average of GAPDH and actin. A fold change of 1.2 or 0.8 in expression between high GH and low GH groups was used as a cutoff.

Figure 4. Analysis of GH-related signaling in gastric cancer cells by micro-western array (MWA) assay. High-GH-expressing and low-GH-expressing NCI-N87 cells were prepared by cell sorting using mAbVK9. Changes in abundance of indicated proteins or their phosphorylated forms were determined by MWA. (**A**) Six samples showed in each well (from left to right) were condition controls (1-4), low-GH cells and high-GH cells, respectively. Artificial coloring differentiates the used secondary antibodies in species (red and green for anti-rabbit and anti-mouse, respectively). (**B**) Selected data of relative protein abundance are listed in Table. Relative protein abundance was normalized to the average of GAPDH and actin. Data are shown as mean ± SD of two independent experiments in which similar results were obtained. A fold change of 1.2 or 0.8 in expression was used as a cutoff for upregulation or down regulation, respectively.

5. High GH expression correlates with higher levels of PD-L1 protein in cancer cells.

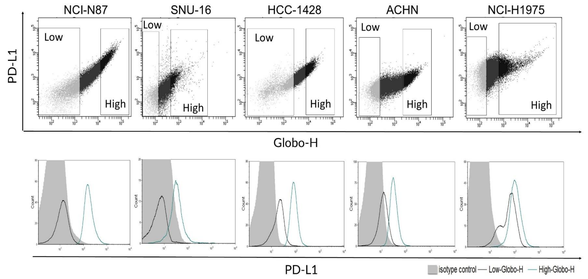


Figure 5. Relatively higher PD-L1 expression in high GH–expressing population of cancer cells. Flow cytometry analysis of cell surface PD-L1 and GH expression in GC NCI-N87, GC SNU-16, breast cancer HCC-1428, renal cell carcinoma ACHN and TKI-resistant NSCLC NCI-H1975 cells (upper panel). PD-L1 expression of gated area for low-GH- and high-GH-expressing populations was measured by flow cytometry analysis (lower panel). Relative higher PD-L1 expression in high GH–expressing population in these five cancer cell lines. The experiment was conducted twice independently with similar obtained results.

6. High GH expression correlates with higher levels of phosphor-EGFR protein in tyrosine kinase inhibitor (TKI)-resistant NSCLC cancer cells.

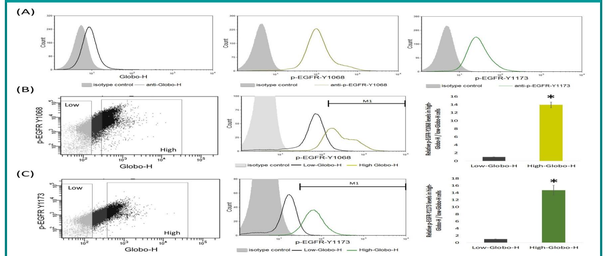


Figure 6. Relatively higher levels of phosphor-EGFR protein in high GH–expressing TKI-resistant NSCLC cells. (**A**) GH, phospho-Y1068 EGFR, and phospho-Y1173 EGFR expression detected by flow cytometry in NSCLC NCI-H1975 cells. High levels of phospho-EGFR were measured. Correlation of GH with the (**B**) phospho-Y1068 EGFR and the (**C**) phospho-Y1173 EGFR, respectively, was investigated by flow cytometry in NCI-H1975 cells. Phospho-EGFR of gated area for low-GH- and high-GH-expressing populations was measured by flow cytometry analysis (middle panel). The overlay histogram (right panel) shows representative histograms of low-GH and high GH. Results depict mean ± SD of four independent experiments. The results expressed as fold change of respective low-GH cells (*P < 0.05 versus low-GH cells).

SUMMARY

- Significant correlations between high mRNA expression of GH-synthesized key enzymes and worse survival for GC patients were measured.
- Positive GH expression associates with poor survival and tumor invasiveness in patients with GC.
- Enhanced cell proliferation in sorted GC cells with high GH expression.
- Upregulated AKT/p38/JNK signaling pathways with higher cyclin D1/cyclin E1 levels in high GH-expressing GC cells were determined.
- Relatively higher PD-L1 expression in high GH–expressing population of cancer cells.
- Relatively higher levels of phosphor-EGFR protein in high GH–expressing TKI-resistant NSCLC cells.

CONSCLUSIONS

GH level was associated with the survival of GC patients and positively correlated with cell surface PD-L1 expression *in vitro*. Therefore, GH-targeting therapy has a potential application for the treatment of GC patients.

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