Am J Cancer Res 2023;13(10):4811-4821
www.ajcr.us /ISSN:2156-6976/ajcr0152290

Brief Communication

Globo H ceramide is an independent prognostic marker for gallbladder cancer

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Received July 18, 2023; Accepted September 23, 2023; Epub October 15, 2023; Published October 30, 2023

Abstract: In recent studies, there has been growing interest in developing cancer therapeutics targeting Globo H ceramide, which is considered as the most prevalent tumor-associated carbohydrate antigen in epithelial cancers. In this study, we aimed to evaluate the expression of Globo H and investigate its prognostic significance in gallbladder cancer (GBC). The tumor specimens and clinical characteristics of GBC patients were collected from the tumor bank and database of Chang Gung Memorial Hospital. Globo H in tumor specimens was detected by immunohistochemistry (IHC) and mass spectrometry analysis. Through data mining, it was discovered that FUT1 and FUT2, which are key enzymes involved in the biosynthesis of Globo H, were significantly up-regulated in human gallbladder cancer (GBC). Consistent with this finding, Globo H expression was detected in 86% (128 out of 149) of GBC specimens using immunohistochemical (IHC) staining. This was the highest frequency among Globo H expressing cancers. Patients with tumors exhibiting higher Globo H expression (H-score ≥ 80) demonstrated significantly shorter disease-free survival (DFS) and overall survival (OS) (P = 0.0001 and P = 0.0004, respectively). In a multivariable Cox regression analysis, elevated Globo H expression was identified as an independent unfavorable predictor for DFS and OS (hazard ratio: 2.29 and 2.32, respectively, P = 0.008 and 0.001) in primary GBC. Globo H is an independent prognostic marker for GBC.

Keywords: Globo H, gallbladder cancer, immunotherapy, prognosis, immunohistochemistry staining

Introduction

Gallbladder cancer (GBC) is an aggressive form of cancer that poses a significant healthcare issue in specific regions of the world [1]. The prevalence of GBC and its healthcare burden are greater in South Asia, Eastern Asia, Southeastern Asia, Latin America, and Eastern Europe [2]. The development of gallbladder cancer is influenced by a variety of factors. Gallstones and chronic inflammation of the gallbladder are the most common risk factors for gallbladder cancer [3]. A diet high in saturated fats and low in fiber may increase the risk of gallbladder cancer [4]. From a genetic perspective, family history and specific genetic mutations or syndromes, such as Lynch syndrome and BRCA gene variants, have been associated with a higher risk of gallbladder cancer [5]. In addition, environmental factors, such as heavy metals, tobacco, and radon, may also contribute to gallbladder cancer [6]. Unfortunately, most GBC cases are diagnosed at advanced stages, and the response to chemotherapy and radiotherapy is generally limited, resulting in modest improvements in overall survival (OS) [7, 8]. Traditionally, GBC patients have been included in clinical trials for biliary tract cancer [9]. Similar to cholangiocarcinoma, the first-line systemic chemotherapy options for GBC, typically involve gemcitabine and cisplatin, while FOLFOX is considered as a second-line

therapy [10]. Notably, the recent approval of durvalumab, a PD-L1 inhibitor, in combination with chemotherapy by the US FDA for adult patients with locally advanced or metastatic GBC represents a significant development [11, 12]. Molecular profiling studies have revealed important genetic differences between gallbladder cancer and cholangiocarcinoma, influencing the choice of targeted therapy. Frequent genetic alterations in GBC, such as Her2/neu amplification, PIK3CA mutations, EGFR mutations, Hedgehog pathway aberrations, increased expression of VEGFs, and abnormalities in MAPK and DNA repair genes, provide actionable targets for therapy [13-17]. Molecularly targeted strategies have the potential to enhance the effectiveness of conventional chemotherapy and alter the natural history of this fatal disease. However, clinical trials assessing the therapeutic efficacy of these novel agents in chemoresistant and recurrent GBC have yielded disappointing results, likely due to the heterogeneity in tumor etiology and genetic alterations [17].

The success of dinutuximab, an anti-GD2 (disialoganglioside) antibody, for the treatment of neuroblastoma has opened new horizons for cancer therapy in targeting tumor-associated glycans [18, 19]. High correlation with poor outcomes in various cancers has been observed in cases of overexpression or de novo expression of O-GalNAc glycans, Lewis blood group-related antigens, and N-glycans [20-23]. Recently, unique N-glycans were found in primary culture cell lines derived from GBC tumors [24]. We have recently reported that Globo H is a poor prognostic factor for intrahepatic cholangiocarcinoma (iCCA) and anti-Globo H monoclonal antibody displays significant therapeutic efficacy for thioacetamide-induced cholangiocarcinoma in rats [25]. Besides iCCA, Globo H is overexpressed on the surface of various cancers, including cancers of breast, lung, colon, and pancreas [26-28]. However, it is not expressed in normal tissues, except for the apical portion of epithelial cells at the lumen border, which is considered not accessible to immune system [29]. Thus, Globo H is an ideal target for cancer immunotherapy. However, its prognostic significance has yet to be investigated in most cancers. Whether Globo H is expressed in GBC and its potential prognostic value has yet to be explored.

In this study, we showed that Globo H is expressed in GBC, as in most other epithelial cancers, and its expression correlated with inferior survival. The finding points to a new direction for the treatment of GBC by targeting Globo H.

Materials and methods

Clinical samples and array data

Biopsies and formalin-fixed, paraffin-embedded (FFPE) tumor of 149 GBC patients with American Joint Committee on Cancer stages I to III GBC collected between 1990~2022 in Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan. Patient written informed consents, experiment protocols, and regulations were approved by the Institutional Review Board of the Chang Gung Medical Foundation (reference numbers: 201304768B0 and 201901150044). Array data were downloaded from NCBI's Gene Expression Omnibus (accession number GSE76633). GSE76633 contains microarray data of normal and cancer part tissue from 9 GB patients.

Immunohistochemistry (IHC) staining

The primary antibody used in this study was VK9, a monoclonal antibody against Globo H (hybridoma kindly provided by Dr. Govindaswami Ragupathi, Memorial Sloan-Kettering Cancer Center, New York, NY). Globo H IHC staining was performed on FFPE human and PDX tissues by using BOND RXm staining machine (Leica Biosystems, Vista, CA) and the Leica BOND™ Polymer Refine Detection Kit (DS9800, Leica Biosystems, Vista, CA) following manufacturer's instructions. Sections were examined by one pathologist (Y Huang) and digital images were captured by Aperio Scope AT Turbo Slide Scanner (Leica Biosystems, Vista, CA) at 40× magnification. Y Huang performed the interpretation of the IHC results and calculate the H score using the following formula: 3 times the percentage of strong staining, plus 2 times the percentage of moderate staining, plus the percentage of weak staining. This calculation yields a range of values between 0 and 300.

Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) analysis of permethylated GSLs

GSLs were extracted from GBC PDX tissues as previously described [30]. Briefly, 25 mg fresh PDX was homogenized and extracted with methanol and chloroform for four times. The extracted was anion-exchange chromatography

was used to extract neutral GSLs from supernatants. Permethylated GSLs were mixed with matrix 2,5-dihydroxybenzoic acid (Sigma-Aldrich, Co., St. Louis, MO, USA) and acquired the mass spectrum profile by MALDI/TOF (SCIEX 5800 TOF/TOF) with positive-ion mode and accumulated 4000 laser shots with a random sampling mode [32].

Statistical analysis

Data were presented as mean ± SD, count, and percentages. Mann-Whitney U test and Pearson Chi-square test were employed for group difference comparisons. Kaplan-Meier method and the log-rank test were used to analyze the survival curves by using Prism 6.0 (GraphPad Software, La Jolla, CA, USA). To verify the independent prognostic factors in GBC, Cox proportional-hazards regression analysis was performed in the analysis by using SPSS (SPSS Inc., Chicago, IL, USA) software.

Results

Expression of Globo H in GBC tissues

Data mining of Human Transcriptome Array datasets, GSE76633 [31], for GBC indicated significantly higher expression of six genes involved in the biosynthesis of Globo H [32], UGCG, B4GALT5, B4GALT6, B3GALNT1, FUT1 and FUT2 in GBC tumor than non-tumor part by 1.1-fold (P = 0.001), 1.1-fold (P = 0.01), 1.3-fold (P = 0.03), 1.2-fold (P = 0.002), 1.2-fold (P = 0.02) and 6.9-fold (P = 0.004), respectively (Supplementary Figure 1). This finding prompted us to evaluate the expression of Globo H on GBC specimens by IHC staining with a monoclonal antibody against Globo H. Representative positive and negative IHC stainings of GBC specimens were shown in **Figure 1A**. Similar to iCCA [25], Globo H-positive cells were observed in clusters within the tumor, with staining in the membrane, cytoplasm, and occasionally nucleus. The presence of Globo H was confirmed by mass spectrometric analysis of GSLs extracted from the GBC tumor tissue that stained positively for Globo H on IHC. As analyzed by MALDI-TOF mass spectrometry, the signals of FucHex4HexNAc1Cer (m/z = 1,867/1,979) that represented Globo H were found in the neutral fraction of the GSL extraction (**Figure 1B**).

Globo H expression correlates with poor clinical outcome

Using IHC staining, Globo H expression with H-score ≥ 1 was detected in 128/149 (86%) of GBC tumor specimens. The relationship between Globo H expression and clinical pathological parameters in patients with stage 1, 2 and 3 GBC was examined. As shown in **Table 1**, higher Globo H expression levels (H-score ≥ 80) were significantly correlated with higher CA19-9 (P = 0.003), vascular invasion (P < 0.001), relapse (P = 0.01) and death (P < 0.001), indicating that Globo H expression is associated with aggressive tumor behaviors.

We next investigated whether higher expression of Globo H was a significant predictor for recurrence of GBC. Kaplan-Meier analysis revealed that patients with higher Globo H expressing GBC (H-score ≥ 80) had significantly lower rate of disease-free survival (DFS) than those with lower Globo H expression (P = 0.0001): 60.9% vs. 71.9% at 1 year, 26.8% vs. 59.6% at 3 years and 21.9% vs. 53.7% at 5 years (**Figure 2A**). Similarly, OS was also significantly shorter for patients with higher expression of Globo H (P = 0.0004): 45.9% vs. 73.3% at 1 year, 36.3% vs. 65.9% at 3 years, and 32.2% vs. 60.4% at 5 years (**Figure 2B**). Even in patients with early stage (stage 1 and stage 2) GBC, higher expression of Globo H significantly correlated with shorter 5 years DFS (47.1% vs. 82.4%, P = 0.001) (**Figure 3A**) and OS (52.3% vs. 87.3%, P = 0.01) (**Figure 3C**). In addition, DFS in stage 3 patients with higher expression of Globo H had shorter 5 years DFS (4.1% vs. 28.6%, P = 0.009) (**Figure 3B**). OS in stage 3 patients with higher expression of Globo H was also significantly shorter than those with low expression of Globo H (5 years OS: 9.3% vs. 34.1%, P = 0.006) (**Figure 3D**).

Globo H expression is an independent risk factor for GBC

Univariate and multivariate cox proportional hazard regression analyses were performed to assess the potential prognostic value of Globo H expression in GBC. As shown in **Figure 4**, DFS correlated with higher grade (Hazard Ratio (HR): 2.78, 95% CI: 1.56-4.96, P = 0.001), advanced stage (HR: 5.24, 95% CI: 2.96-9.27,

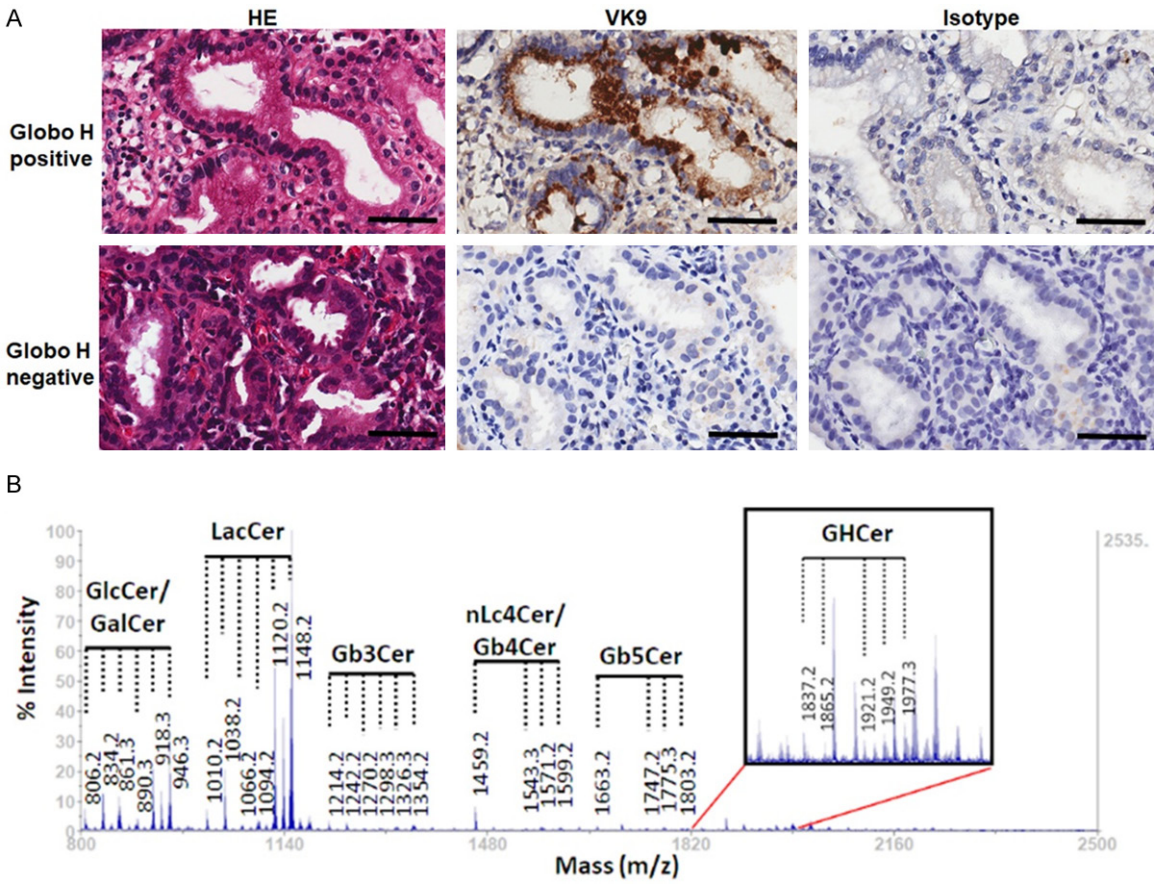

Figure 1. Globo H was detected in FFPE GBC cancer tissues. A. Representative histological images of Globo H-positive (top panels) and -negative (bottom panels) cases were shown. H&E (left panels), Globo H IHC (middle panels) and isotype IHC (right panels) staining were shown. All scale bars, 60 μm. B. GSLs extracted from Globo H positive GBC tissue were separated into neutral and acidic fractions, permethylated and analyzed by MALDI/TOF mass spectrometry. The major GSLs in the neutral fraction were GlcCer, LacCer, Gb3Cer, Gb4Cer, Gb5Cer, and Globo H were found in the neutral fraction.

P < 0.001), presence of vascular invasion (HR: 2.24, 95% CI: 1.29-3.87, P = 0.004), in line with the known adverse impacts of these parameters. Importantly, higher expression of Globo H is associated with increased risk of tumor recurrence in GBC patients (HR: 2.41, 95% CI: 1.47-3.94, P < 0.001). Furthermore, OS correlated with elderly age (HR: 3.36, 95% CI: 1.47-7.68, P = 0.004), higher grade (HR: 2.29, 95% CI: 1.47-3.53, P < 0.001) and advanced stage (HR: 3.58, 95% CI: 2.37-5.41, P < 0.001), tumor size greater than 5 cm (HR: 1.76, 95% CI: 1.19-2.61, P = 0.005), presence of vascular invasion (HR: 2.43, 95% CI: 1.59-3.73, P < 0.001), consistent with the known prognostic impacts of these parameters. Additionally, OS of GBC patients was significantly associated with higher expression of Globo H (HR: 2.48, 95% CI: 1.67-3.69, P < 0.001) (**Figure 4**).

Next, we selected the covariates which showed statistical significance in the univariate analysis for multivariable Cox regression analysis to identify the independent variables associated with poor DFS and OS. Several known clinical pathological factors were found to be independent risk factors. These include higher grade for DFS (HR: 2.21, 95% CI: 1.20-4.05, P = 0.01) and OS (HR: 1.91, 95% CI: 1.21-3.600, P = 0.005), advanced stage for DFS (HR: 4.26, 95% CI: 2.15-8.44, P < 0.001) and OS (HR: 2.45, 95% CI: 1.47-4.01, P = 0.001), and tumor size greater than 5 cm for OS (HR: 1.61, 95% CI: 1.02-2.54, P = 0.04). Notably, higher Globo H expression was an independent risk factors for both DFS (HR: 2.29, 95% CI: 1.24-4.25, P = 0.008) and OS (HR: 2.32, 95% CI: 1.43-3.78, P = 0.001) (**Figure 5**). These findings identified higher Globo H expression as a novel and inde-

Table 1. Correlation of Globo H expression with clinical-pathological parameters in 149 patients with GBC

Characteristics	N (range)	Globo H Expression		
		Low (n = 108)	High (n = 41)	*p* value
Age (Median and range) (years)	68 (27-93)	65 (27-93)	68 (42-86)	0.13
Platelet, 10^9/L	230 (54-422)	239 (54-422)	226 (71-417)	0.8
BUN, mg/dL	13 (3.3-75.5)	13.4 (3.3-75.5)	13.6 (3.3-70)	0.64
Creatinine, mg/dL	1.00 (0.27-7.97)	0.9 (0.27-5.6)	0.9 (0.41-7.97)	0.89
Hemoglobin, g/dL	13 (7.4-17.0)	12.7 (7.4-17.0)	12.4 (8.3-15.6)	0.43
Albumin, g/dL	4.0 (1.9-5.1)	3.8 (1.9-5.1)	3.9 (2.4-4.9)	0.72
AST, U/L	28 (9-1882)	28.5 (9-1064)	26.0 (14-1889)	0.66
ALT, U/L	30 (6-1847)	27.5 (6-772)	36.5 (8-1847)	0.42
Total bilirubin, mg/dL	1.0 (0.2-23.2)	0.9 (0.2-23.2)	0.7 (0.2-12.4)	0.09
ALK-P	92 (23-1097)	92 (32-1097)	86 (23-530)	0.86
CA19-9, U/ml	36 (0.6-10000)	21 (0.3-577.6)	50 (2-5066)	0.003
CEA, ng/ml	2.0 (0.5-593)	2.08 (0.4-159.2)	2.19 (0.6-356.3)	0.06
Sex	N (%)			
Male	60 (40.3%)	63	26	0.71
Female	89 (59.7%)	45	15	
Grade[a]				
I	51 (36.4%)	40	11	0.33
II-III	92 (63.6%)	64	28	
Stage				
I-II	69 (46.3%)	52	17	0.58
III	80 (53.7%)	56	24	
Tumor size				
≤ 5 cm	99 (66.4%)	69	30	0.33
> 5 cm	50 (33.6%)	39	11	
Gall stone				
Without	81 (54.4%)	58	23	0.85
With	68 (45.6%)	50	18	
Vascular invasion[b]				
No	114 (79.1%)	95	19	< 0.001
Yes	30 (20.9%)	10	20	
Relapse (Mean ± SD and range) (months)	66.2±77.1, 0.95-276.6			
No	81 (54.4%)	66	15	0.01
Yes	68 (45.6%)	42	26	
Death (Mean ± SD and range) (months)	70.9±74.8, 1.87-276.6			
No	41 (27.5%)	41	0	< 0.001
Yes	108 (72.5%)	67	41	

[a]Data not available in 6 patients. [b]Data not available in 5 patients. BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine transaminase; ALK-P, Alkaline phosphatase; CEA, carcinoembryonic antigen; CA19-9, Carbohydrate Antigen 19-9.

pendent predictor for DFS and OS in GBC patients, suggesting that Globo H may play a critical role for GBC progression.

Discussion

Overexpression of Globo H in many epithelial cancers and its absence of Globo H expression in vital organs make Globo H an ideal target for cancer immunotherapy. Thus, it will be important to assess the level of Globo H expression as a criterion for the patient's eligibility for Globo H-based immunotherapy. We have developed a simple and reproducible IHC method to determine Globo H expression in FFPE samples, using monoclonal antibody VK9 and

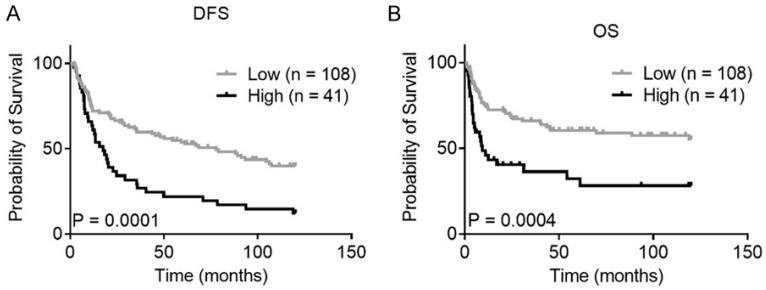

Figure 2. Kaplan Meier Curve of GBC in relation to Globo H expression. (A, B) DFS (A) and OS (B) between GBC patients with low and high Globo H expression. Gray line, patients with low Globo H expression; Black line, patients with high Globo H expression.

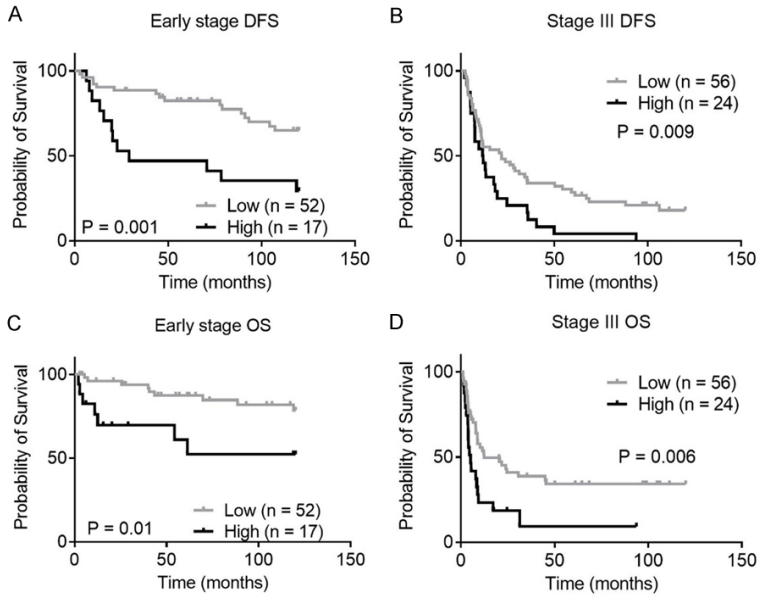

Figure 3. Kaplan-Meier curve of different stage gallbladder cancer in relation to Globo H expression. DFS (A, B) and OS (C, D) between early stage (A, C) and stage 3 (B, D) GBC patients with low and high Globo H expression. Gray line, patients with low Globo H expression; Black line, patients with high Globo H expression.

BondMAX autostainer. In our previous study, we found that Globo H was expressed in 41% of iCCA and that its expression was an independent prognostic factor [25]. Others have reported the detection of Globo H was detected in 38.6% stage 1 non-small cell lung cancer, 76.4% pancreatic cancer, 65.6% Esophagus cancer, 58.9% gastric cancer, 58.8% breast cancer, 50.7% colon cancer, 24.7% liver cancer and 16.4% thyroid cancer [27, 33-35]. While Globo H expression may not be an independent prognostic factor in lung and thyroid cancer, there have been studies indicating associations between Globo H expression and clinical outcomes in specific subtypes of these cancers. For instance, in patients with lung squamous cell carcinoma, positive Globo H expression was found to correlate with a lower survival rate [27]. In papillary thyroid cancer, Globo H expression was positively associated with invasion and relapse [34]. However, these findings may not be conclusive due to the small sample sizes in these studies. In this study, Globo H positive staining was found in 86% (128 of 149) GBC patients. So far, this is the highest frequency of Globo H expression among all cancers. More importantly, higher Globo H expression is an independent poor prognostic indicator for GBC.

In recent years, a number of strategies for Globo H-directed cancer immunotherapy are being pursued. The first approach is active immunotherapy with Globo H vaccines which consist of Globo H linked to either Hemocyanin-Keyhole Limpet (KLH) to form OBI-822 or non-toxic mutant of diphtheria toxin, CRM197 to form OBI-833. Administration of these vaccines with adjuvant OBI-821 has been shown to trigger immune system to produce antibodies against Globo H [36]. An international phase II randomized trial of OBI-822 indicated that breast cancer patients who produced higher titer of anti-Globo H IgG/IgM had better outcome [26, 37]. Recently, a humanized Globo H-targeting monoclonal antibody, OBI-888, was shown to exhibit tumor suppressive effects in murine models of various cancers [38]. Subsequently, an antibody-drug conjugate, OBI-999, was developed by conjugation of OBI-888 with monomethyl auristatin E (MMAE). OBI-999 exhibits superior growth inhibition in a dose-dependent manner in xenografts of breast, gastric, and pancreatic cancer, as well as patient-derived xenograft

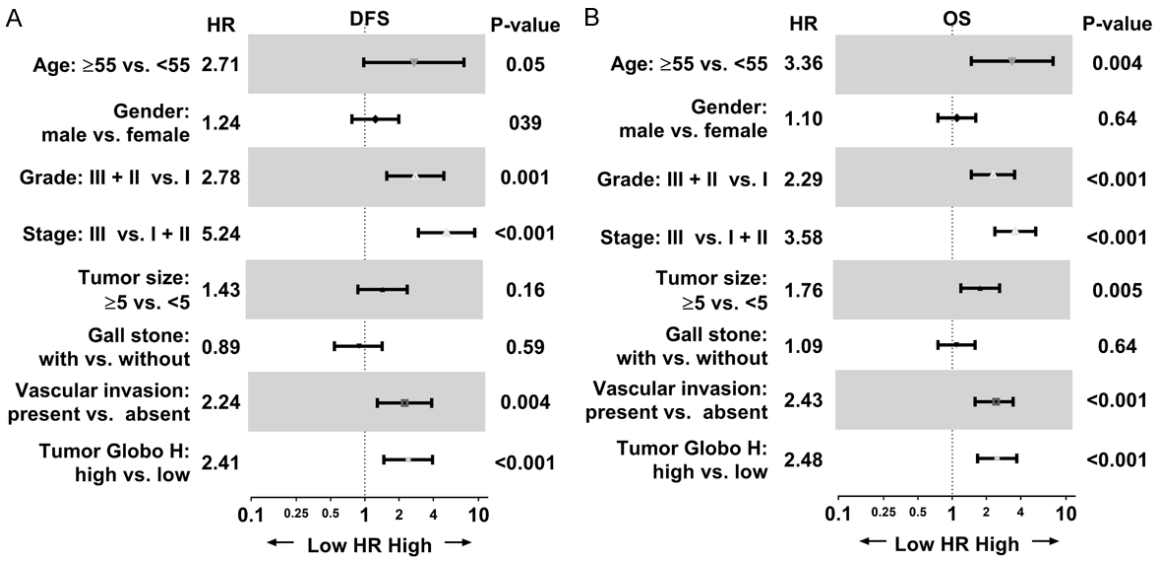

Figure 4. Univariate Cox regression analysis of higher expression of Globo H as a prognostic factor in GBC patients. Forest plot of univariate analysis is shown. Covariates tested in univariate cox models were age, gender, grade, stage, tumor size, gall stone, vascular invasion, and Globo H expression for (A) DFS and (B) OS. *P* values were calculated using the Cox regression model.

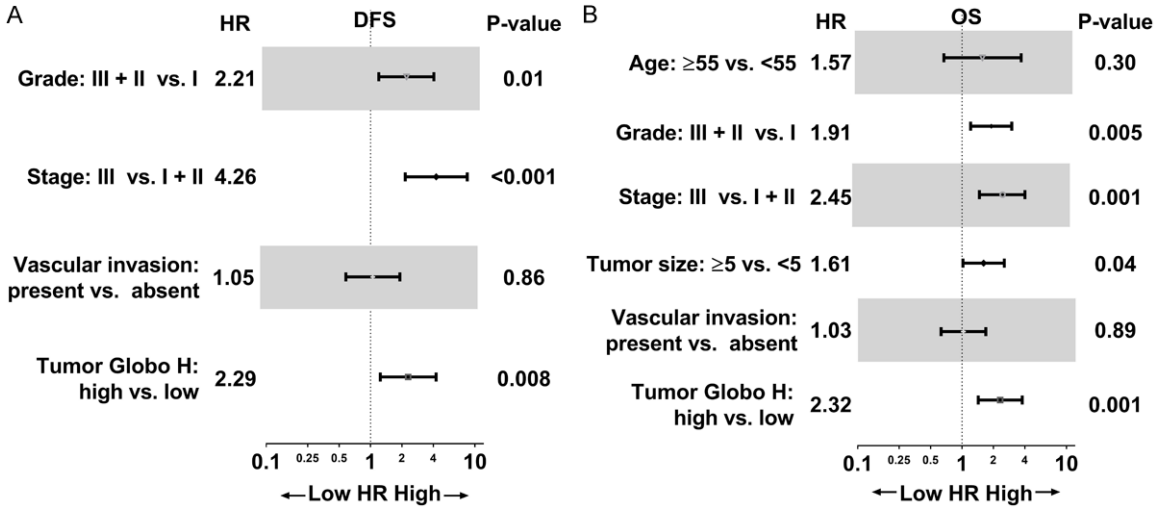

Figure 5. Multivariate Cox regression analysis of higher expression of Globo H as a prognostic factor in GBC patients. Forest plot of multivariate analysis is shown. Covariates tested in multivariate cox models were age, grade, stage, tumor size, vascular invasion, and Globo H expression for (A) DFS and (B) OS. *P* values were calculated using the Cox regression model.

models of lung cancer [39]. Currently, OBI-999 is in phase 1/2 trials for pancreatic, gastric, colorectal, and esophageal cancers [28, 33]. The safety profiles and encouraging early findings suggest that these immunotherapeutic agents have therapeutic potential for GBC in the near future. Recently, Globo H targeted chimeric antigen receptor T cells have been shown to exhibit anti-tumor activities against various types of cancers both *in vitro* and *in vivo* [40].

The biological functions of Globo H ceramide (GHCer) are not entirely clear. We have demonstrated that it is shed into the extracellular vesicles. Upon incorporation into lymphocytes, GHCer can facilitate tumor escape from

immune elimination by suppressing Notch signaling and T cell activation [41]. Uptake of GHCer by the endothelial cells in tumor microenvironment promotes angiogenesis, which is mediated through binding of GHCer with translin-associated factor X (TRAX) to release phospholipase C beta1 sequestered by TRAX [42, 43]. Activation of angiogenesis is common in malignancy and increased vascularization may facilitate local invasion, tumor progression and metastasis, and clinical outcome [44]. In this context, we found a correlation of higher expression of Globo H with increased vascular invasion, which is an adverse prognostic factor in GBC [43, 45]. This is consistent with our previous report that Globo H expression positively correlated with vascular invasion in iCCA and hepatocellular carcinoma FFPE samples [25, 35]. Taken together, these findings provide further scientific rationales for the development of Globo H targeting immunotherapy [26, 37].

In this study, we noted that Globo H expression correlated with the circulating levels of CA19-9, consistent with our previous findings in iCCA [25]. CA19-9 (also known as sialyl-Lewis A, SLe^a), is highly expressed in lung, pancreatic, colorectal, gastric, breast, thyroid and biliary tract cancers [46, 47]. Biosynthesis of SLe^a involves the addition of β1,3Gal residues to GlcNAcβ1-3Galβ1-4GlcNAcβ1-R branched chains of N-glycans by Beta-1,3-galactosyltransferase 5 (B3GALT5) [48, 49], followed by addition of fucose by fucosyl transferases 1 and 2 (FUT1 and FUT2) to generate Le^a and final addition of sialic acid to form sLe^a. This is consistent with the reported positive correlation of B3GALT5 expression with CA19-9 in several cancers [50, 51]. As to the biosynthesis of Globo H, B3GALT5 is responsible for transferring a galactose to the terminal GalNAc of GB4 to generate SSEA-3, which is then fucosylated by FUT1 and FUT 2 to form Globo H [32]. The sharing of similar glycosyltransferases for the biosynthesis of Globo H and CA19-9 underscores the association of higher circulating CA19-9 levels in cancer patients with higher Globo H expressing tumors. Like Globo H, higher expression of CA19-9 is also associated with poor outcome in GBC [52, 53]. In this context, CA19-9 might have the potential to be a screening marker to identify patients with Globo H expressing tumors for Globo H directed therapy.

Conclusions

Herein, we demonstrated the expression of Globo H expression in 86% of GBC clinical samples, which represented the highest frequency of Globo H expression in cancers. Importantly, we provided the first evidence for Globo H expression to be an independent risk factor for GBC recurrence and overall survival, suggesting that inclusion of GBC patients in the ongoing clinical trials of Globo H-directed immunotherapeutics is warranted.

Acknowledgements

We are indebted to Tissue Bank and Biobank of Linkou Chang Gung Memorial Hospital for providing the GBC samples and related clinical data for our study. This work was supported by grants from the Chang Gung Medical Foundation (OMRPG3C0048 for ALY), National Science and Technology Council (MOST111-2314-B-182A-086 and NSTC 112-2314-B-182A-148 for ALY; NSTC111-2321-B-182-002 for JY; NSTC110-2314-B-182A-063-MY3 for CNY; NSTC109-2314-B-182-080-MY3 for CE-W), Chang Gung Memorial Hospital at LinKou (CMRPG3N0181 for JTH; CMRPG3M1231 for THH; CMRPG3M0171~2 and CMRPG3N0221 for CNY; CMRPG3L1621 for YH; CMRPG3L-0911~2 for CEW).

Disclosure of conflict of interest

Alice L. Yu received a research grant from OBI Pharma Inc., which is developing Globo H directed cancer immunotherapy. Other authors have no conflict in the manuscript.

Abbreviations

GBC, gallbladder cancer; OS, overall survival; DFS, disease free survival; iCCA, intrahepatic cholangiocarcinoma; FFPE, formalin-fixed, paraffin-embedded; IHC, Immunohistochemistry; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; HR, hazard ratio; TRAX, translin-associated factor X; B3GALT5, Beta-1,3-galactosyltransferase 5; FUT1, fucosyl transferases 1; FUT2, fucosyl transferases 2.

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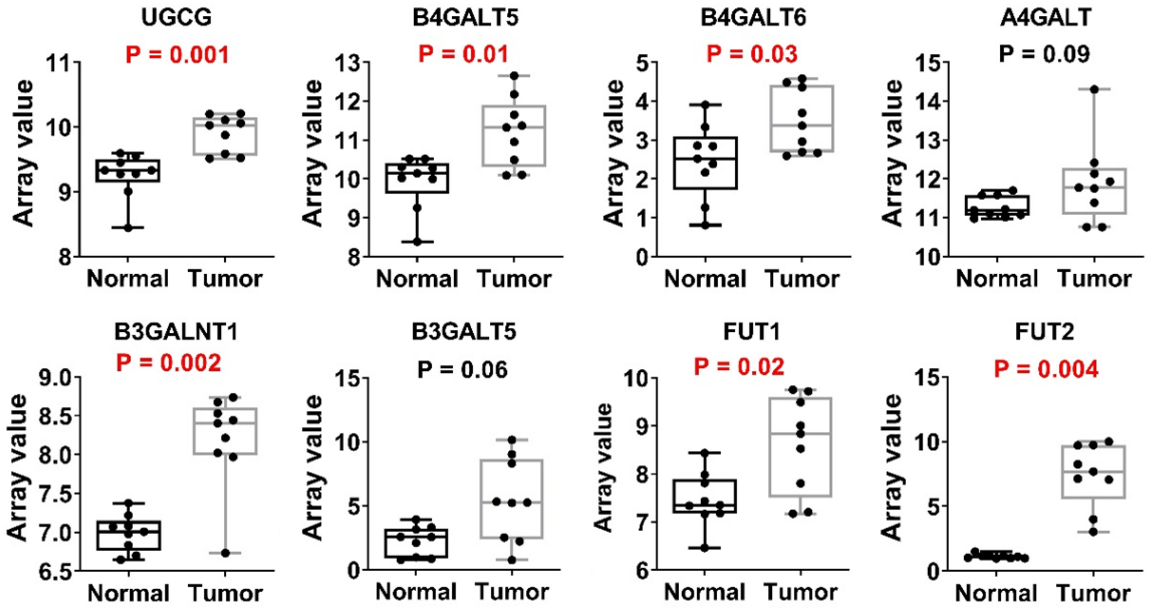

Supplementary Figure 1. The Globo H biosynthetic enzymes were up-regulated in human GBC. RNA expression levels of UGCG, B4GALT5, B4GALT6, A4GALT, B3GALNT1, B3GALT5, FUT1 and FUT2 in GBC tumors were analyzed from the Human Array datasets, GSE76633. The data are expressed as the mean ± SEM.