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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Expression of GOLM1 Correlates with Prognosis in Human Hepatocellular Carcinoma

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ABSTRACT

Background. Serum Golgi membrane protein 1 (GOLM1) is a novel biomarker for hepatocellular carcinoma (HCC). However, few studies have investigated the relationship between GOLM1 protein expression and clinicopathologic features in HCC patients. The aim of this study was to investigate the expression of GOLM1 in human HCC and its correlation with clinicopathologic parameters.

Methods. Clinicopathologic data were obtained through a detailed retrospective review of the medical records of 193 patients with HCC who had undergone surgical resection between 1990 and 2006 at the Taipei Veterans General Hospital. Another 120 HCC tissue samples provided by the Taiwan Liver Cancer Network were used as validation cohort. Immunohistochemical staining was used to determine the expression of GOLM1 in archived formalin-fixed, paraffin-embedded tissue specimens.

Results. GOLM1 expression was significantly higher in resected HCC tumor tissues than in corresponding normal liver tissues ($p < 0.01$). After a median follow-up of 51 months, multivariate analysis showed that portal vein invasion (hazard ratio [HR], 1.515; 95 % confidence interval [95 % CI], 1.008–2.277; $p = 0.046$) and high GOLM1 protein expression (HR, 1.696; 95 % CI, 1.160–2.479; $p = 0.006$) were independent prognostic factors for poor overall survival. High GOLM1 protein expression still significantly correlates with worse overall survival as well as disease-free survival in the validation cohort ($p < 0.001$ and $p = 0.002$).

Conclusions. Overexpression of GOLM1 is associated with poor prognosis in human HCC.

Hepatocellular carcinoma (HCC) is the most common liver malignancy and one of the leading causes of cancer death worldwide. HCC is an aggressive cancer with a 5-year survival rate below 12 %.[1] Surgical resection is the main form of therapy for HCC; however, this is not a viable option for many patients because of the difficulty in diagnosing early-stage HCC.[2,3] Moreover, high recurrence rates of up to 70 % are observed after curative resection of HCC.[4]

Diagnostic imaging is currently the best approach for the early detection of relapsed HCC and future localized treatment plans.[2,5] Alpha fetoprotein (AFP), a serum biomarker, has been widely used as a surveillance tool for HCC recurrence.[6,7] However, serum AFP is not an ideal screening method because of the low sensitivity and specificity.[8] Recently, several studies confirmed that serum Golgi membrane protein 1 (GOLM1) is a novel and

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promising biomarker for early HCC detection and follow-up after resection.[9–12] GOLM1, a type II transmembrane protein of the Golgi cisternae, is typically expressed in the epithelial cells of normal human tissues.[13,14] Serum GOLM1 has been reported to be a more reliable biomarker for the early diagnosis of HCC than current biomarkers such as AFP.[11] Although overexpression of GOLM1 protein should first be observed in the cytoplasm before protein secretion by exocytosis, few studies have investigated the value of GOLM1 protein expression as a prognostic biomarker in HCC.[15] In the present study, we analyzed GOLM1 expression in resected human HCC tumors by immunohistochemical staining and investigated the relationship of GOLM1 expression with clinicopathologic parameters. In our study, a relatively homogenous population from a single institute with standardized treatment and medical care and a long-term follow-up were used to further clarify the relationship between GOLM1 expression and HCC prognosis. In addition, the prognostic role of GOLM1 expression was further validated in another validation cohort.

MATERIALS AND METHODS

Patient Clinicopathologic Data

This study has been approved by the Ethics Committee of the Taipei Veterans General Hospital (201010021IC). Clinicopathologic data were obtained through a detailed retrospective review of the medical records of 193 patients with HCC who had undergone surgical resection between 1990 and 2006 at the Taipei Veterans General Hospital. Patients who suffered from tumor recurrence could receive surgery, transarterial chemoembolization, and percutaneous ethanol injection before 2002. After 2002, patients had other therapeutic choices, such as radiofrequency tumor ablation and liver transplantation. No patients in this study received sorafenib because the drug was unavailable from 1990 to 2006.

The HCC tissue samples of validation cohort were provided by the Taiwan Liver Cancer Network (TLCN). The TLCN is funded by the National Science Council to provide primary liver cancer tissue and associated clinical information. The use of the 120 HCC tissues in this study was approved by our Institutional Review Board and the TLCN User Committee.

HCC Microarray Dataset Analysis

GSE6764 microarray datasets were collected from Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6764).[16] The series contain gene expression profiles of 75 samples that represent a panel of carcinogenesis progression from normal liver, cirrhosis, dysplasia to HCC in background of HCV infection. The raw intensities in *.CEL files were normalized by robust multichip analysis, and fold-change analysis was performed using GeneSpring GX11 (Agilent Technologies). Differentially expressed genes were identified by applying a twofold change cutoff with $p < 0.01$ as threshold for significance.

Tissue Microarray Construction and Immunohistochemical Staining

Hematoxylin and eosin staining of all 193 samples was reviewed by a pathologist. The HCC tissue microarrays were constructed by obtaining 3 1-mm-diameter cores from each tumor. Archived formalin-fixed, paraffin-embedded tumor specimens were used for immunohistochemical staining. Briefly, specimens were cut into 5-μm sections, dewaxed in a 60 °C oven, deparaffinized in xylene, rehydrated through serial dilutions of alcohol, and washed in phosphate-buffered saline (pH 7.2). A polyclonal anti-GOLM1 antibody (dilution 1:200; ABNOVA) was used for immunohistochemical staining. Immunohistochemical staining was performed on the fully automated Bond-Max autostainer (Leica Microsystems) using onboard, heat-induced antigen retrieval in citrate buffer (ER1 protocol) for 20 min and VBS Refine polymer detection system (Leica Microsystems). Immunohistochemical staining was developed using diaminobenzidine (Leica Microsystems) as a chromogen and hematoxylin as a counterstain.

Scoring of GOLM1 Expression

The intensity of staining was scored using a 4-tier scale and defined as: 0, no staining; 1+, weak staining; 2+, moderate staining; 3+, strong staining. The extent of staining was scored by the percentage of positive cells (0–100 %). The final IHC scores (0–300) were obtained by staining intensity score multiplied by the percentage of positive cells. All cases were divided into two groups according to the final IHC scores. High GOLM1 expression level was defined as a score more than and including 150 itself and a score less than 150 was defined as low GOLM1 expression. All of the immunohistochemical staining results were reviewed and scored independently by two pathologists. In the case of discrepant results from the same slide, a final consensus was obtained after a discussion between the two pathologists. In order to further validate our data, we used an automated image analysis system (Aperio Technology, Vista, CA), which uses a color deconvolution algorithm to visualize GOLM1 protein expression in HCC.[17,18] Quantification of immunohistochemical staining was performed with color translation and

an automated thresholding algorithm from Aperio Technology (Fig. 1).

Statistical Analysis

Statistical analysis was performed using SPSS (version 15.0; SPSS Inc., Chicago, IL). Fisher exact and chi-squared tests were used to assess the association between GOLM1 expression and the various clinicopathologic parameters. Overall survival (OS) was defined as the time between date of diagnosis and date of death. Disease-free survival (DFS) was defined as the time between date of diagnosis and date of recurrence or death. Univariate survival analysis was performed using the Kaplan–Meier method. Multivariate analysis was performed using the Cox regression model. A p value of < 0.05 was considered significant.

RESULTS

Patient Characteristics

The median age of the patients was 63 years (range, 21–83 years; mean, 60.9 years). Follow-up was available in all cases and ranged from 0.7 to 172 months (median, 51 months; mean, 58.2 months). During the follow-up period, 151 patients presented with evidence of disease recurrence (78.2 %). The latest survival data were collected on August 31, 2011. The total survival rate was 71.8 % at 5 years and 45.4 % at 10 years. GOLM1 protein expression was not correlated with other clinicopathologic features, and the relationship between GOLM1 protein expression and the clinicopathologic features of HCC are summarized in Table 1.

Identification of GOLM as 1 of the Potential Biomarker from HCC Microarray Analysis

To identify potential early HCC markers, we analyzed HCC microarray datasets from GSE6764 that contained 10 normal liver samples, 13 cirrhosis samples, 17 dysplasia samples, and 35 HCC samples.[16] We filtered the genes that were differentially expressed in early cirrhosis and those were found to be persistently overexpressed in dysplasia and HCC. Using this strategy, 120 probes representing 87 genes were identified as potential early markers for HCC (Fig. 2a). After conducting a review of the literature related to these 87 genes, we decided to investigate *GOLM1*, which secretes a protein considered to be a novel and promising biomarker for early HCC detection. Few studies have investigated the value of GOLM1 protein expression as a prognostic biomarker. *GOLM1* was then found to be upregulated 4.08-fold in cirrhosis, 4.72-fold in dysplasia, and 2.87-fold in HCC compared with the expression rate in normal liver (Fig. 2b). We therefore verified its prognostic value in HCC patients by IHC analysis.

GOLM1 was Significantly Overexpressed in Human HCC Tumor Tissues Compared with Adjacent Nontumor Tissues

Tumor and adjacent nontumor tissues were clearly distinguishable in the hematoxylin and eosin staining (Fig. 3,

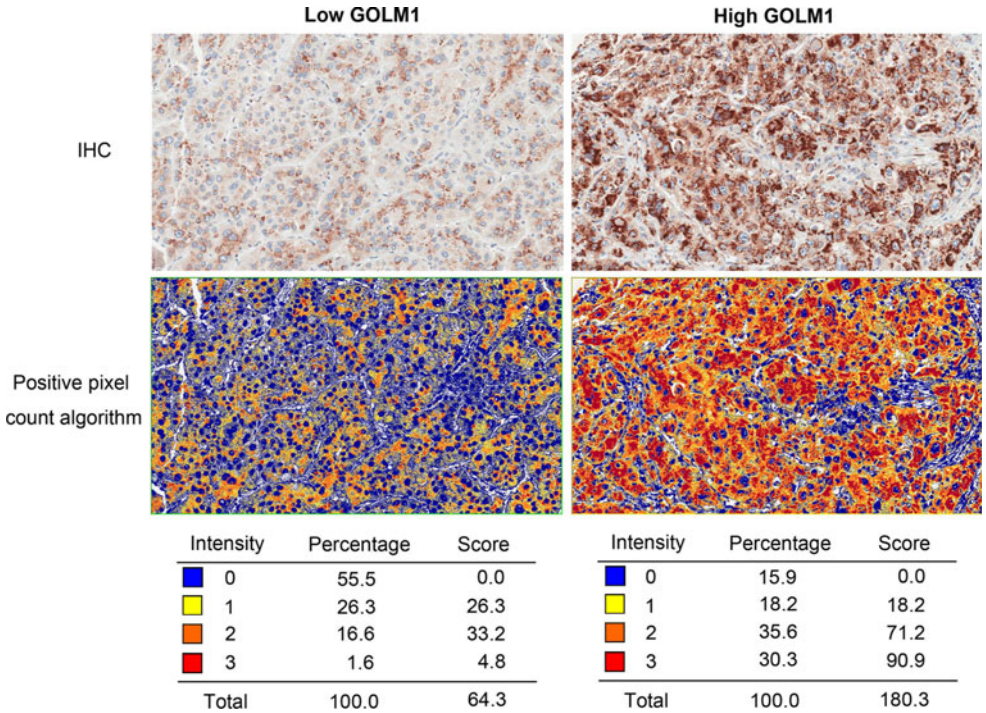

FIG. 1 GOLM1 expression levels in representative HCC tissues by the *color* deconvolution algorithm. Positive pixel count algorithm from Aperio ScanScope software was used to quantify the staining intensity and percentage. The GOLM1 expression was dichotomized into low-(score <150, *left panel*) and high-level (score ≥150, *right panel*) groups. The representative images and quantitative results for low- and high-level of GOLM1 expression are shown in this figure

TABLE 1 Relationship between GOLM1 expression and clinicopathological features in 193 HCC patients

Characteristics	GOLM1 expression		*p* value*
	Low ($n = 123$)	High ($n = 70$)	
Age			0.64
Years (mean ± SD)	61.5 ± 12.0	60.6 ± 12.6	
Gender			0.17
Male	104	64	
Female	19	6	
AFP (ng/mL)			0.197
<400	101	52	
≥400	22	18	
Stage			0.52
I + II	88	47	
III	35	23	
HBV			0.75
Negative	45	24	
Positive	78	46	
HCV			0.61
Negative	89	53	
Positive	34	17	
Recurrence status			0.65
No	28	14	
Yes	95	56	
Liver cirrhosis			0.366
No	68	34	
Yes	50	33	
Unknown	5	3	
Microvessel invasion			0.097
No	46	18	
Yes	77	52	
Tumor size (cm)			0.464
<3	41	27	
>3	82	43	
Tumor numbers			0.266
Solitary	76	37	
Multiple	47	33	
Hepatic vein invasion			0.307
No	113	66	
Yes	6	4	
Unknown	4	0	
Portal vein invasion			0.610
No	111	60	
Yes	9	8	
Unknown	3	2	

SD standard deviation

* *p* value for age was derived using a 2-tailed *t* test; other *p* values were derived with a 2-tailed Pearson chi-square test

left); 70 of 193 (36.3 %) resected HCC tumors were strongly positive for GOLM1 expression. GOLM1 expression scores were significantly higher in HCC tumor tissues than in corresponding nontumor tissues ($p < 0.01$; Fig. 3, right).

GOLM1 Expression is an Independent Prognostic Factor in HCC

Univariate survival analysis was performed to test the prognostic significance of clinicopathologic variables in

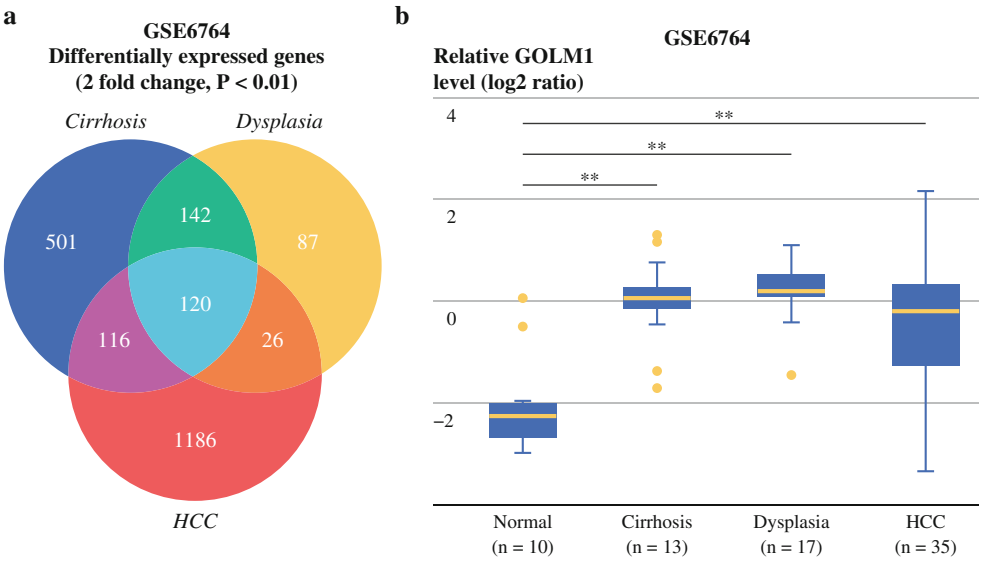

FIG. 2 Gene overexpression of GOLM1 in liver cirrhosis, liver dysplasia, and HCC. **a** Venn diagram analysis of differentially expressed genes in liver cirrhosis, liver dysplasia, and HCC from GSE6764 microarray datasets. **b** Relative GOLM1 mRNA level (217771_at) in 10 normal liver samples, 13 cirrhosis samples, 17 dysplasia samples, and 35 HCC samples from GSE6764 microarray datasets. Expression data were normalized to the median intensity of all probes and scaled to $\log_2$ transformation

HCC. GOLM1 expression ($p = 0.003$, Fig. 4, right), staging ($p = 0.016$), tumor size ($p = 0.007$), hepatic vein invasion ($p < 0.001$), and portal vein invasion ($p = 0.004$) were shown to have an adverse impact on DFS (Table 2). Cox multivariate analysis showed that both GOLM1 expression and hepatic vein invasion were independent prognostic factors of DFS (Table 2). OS was also adversely affected by GOLM1 expression ($p = 0.004$, Fig. 4, left), staging ($p = 0.035$), tumor size ($p = 0.002$), hepatic vein invasion ($p = 0.001$), and portal vein invasion ($p < 0.001$) (Table 3). Both GOLM1 expression and portal vein invasion were significant predictors of OS in the multivariate analysis (hazard ratio, 1.696 and 1.515; 95 % CI, 1.160–2.479 and 1.008–2.277; $p = 0.006$ and 0.046) (Table 3).

GOLM1 Expression is also a Prognostic Factor in Validation Cohort

To validate the prognostic significant of GOLM1, we used another independent HCC cohort from the National Health Research Institute (NHRI) database that included 120 HCC patients with HBV and HCV history and performed IHC analysis. By applying the same staining condition and scoring criteria, it was possible to stratify

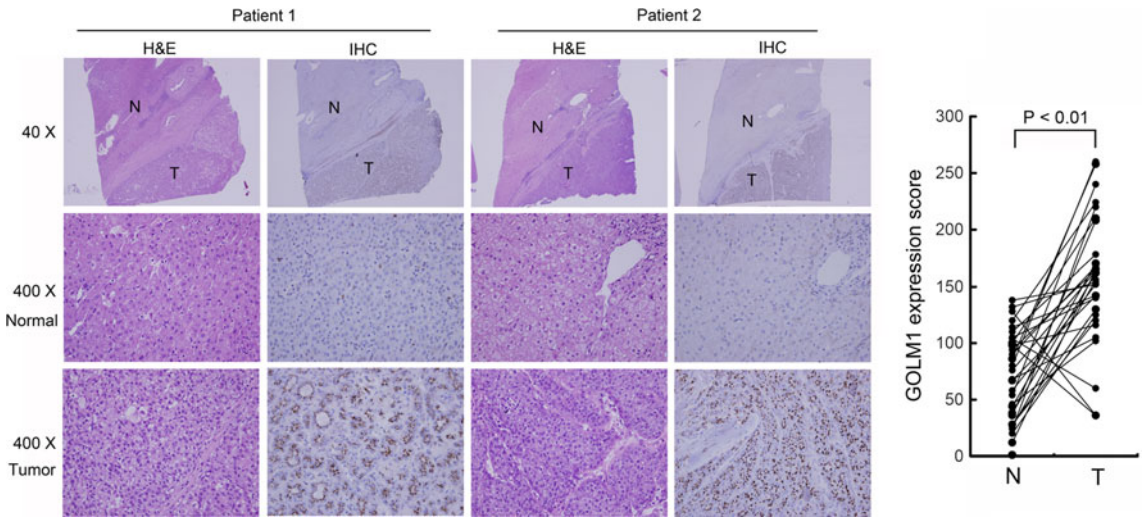

FIG. 3 Overexpression of GOLM1 in human HCC tumors. The hematoxylin and eosin stains and immunohistochemical stain from two HCC patients. In low-power field (×40), tumor and adjacent nontumor tissue were clearly differentiated (*left*, *top panel*). The immunohistochemical staining in high-power field showed overexpression of GOLM1 in tumor tissue, but not in nontumor tissue (*left*, *middle*, and *lower panels*). GOLM1 expression scores were significantly higher in tumor than nontumor tissue ($p < 0.01$) (*right*). *H&E* hematoxylin and eosin stain, *IHC* immunohistochemical stain, *N* nontumor tissue, *T* tumor tissue

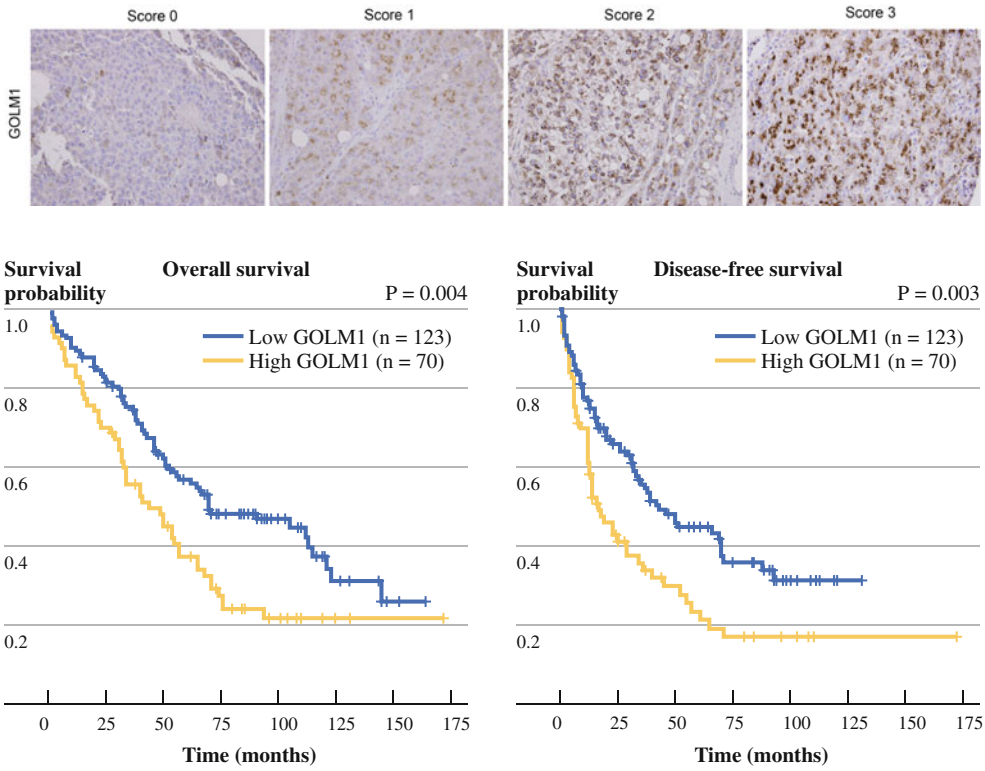

FIG. 4 GOLM1 expression correlated with survival in resectable HCC patients. Immunohistochemical stains of the different intensity scores for GOLM1 expression (*upper panel*); The high GOLM1 group showed significantly worse overall survival ($p = 0.004$) and disease-free survival ($p = 0.003$) than the low GOLM1 group (*lower panels*)

TABLE 2 Cox univariate and multivariate regression analysis of prognostic factors and GOLM1 expression for disease-free survival in 193 HCC patients

Variables	Comparison	HR (95 % CI)	*p* value
Cox univariate analysis (DFS)			
Age	<61 years; ≥61 years	1.060 (0.733–1.532)	0.758
Gender	Female; male	1.337 (0.778–2.298)	0.293
Stage	I; II; III	1.377 (1.061–1.787)	0.016*
AFP	<400 ng/mL; ≥400 ng/mL	1.358 (0.917–2.012)	0.127
HBV	Negative; positive	1.114 (0.766–1.619)	0.572
HCV	Negative; positive	0.949 (0.632–1.424)	0.800
Tumor size	≤5 cm; >5 cm	1.551 (1.129–2.129)	0.007*
Cut margin	<1 cm; > 1 cm	1.308 (0.983–1.832)	0.114
PVI	No; yes	1.672 (1.173–2.384)	0.004*
HVI	No; yes	3.259 (2.137–4.970)	0.000*
GOLM1	Low; high	1.747 (1.215–2.511)	0.003*
Cox multivariate analysis (DFS)			
Stage	I; II; III	1.056 (0.776–1.437)	0.731
PVI	No; yes	1.242 (0.808–1.910)	0.323
HVI	No; yes	2.886 (1.764–4.724)	0.000*
Tumor size	≤5 cm; >5 cm	1.242 (0.808–1.910)	0.323
GOLM1	Low; high	1.765 (1.100–2.026)	0.003*

DFS disease-free survival, *PVI* portal vein invasion, *HVI* hepatic vein invasion, *HR* hazard ratio, *95 % CI* 95 % confidence interval

* $p < 0.05$

TABLE 3 Cox univariate and multivariate regression analysis of prognostic factors and GOLM1 expression for overall survival in 193 HCC patients

Variables	Comparison	HR (95 % CI)	*p* value
Cox univariate analysis (OS)			
Age	<61 years; ≥61 years	1.107 (0.766–1.600)	0.589
Gender	Female; male	1.277 (0.743–2.194)	0.376
Stage	I; II; III	1.333 (1.021–1.739)	0.035*
AFP	<400 ng/mL; ≥400 ng/mL	1.478 (0.980–2.230)	0.063
HBV	Negative; positive	1.145 (0.788–1.664)	0.477
HCV	negative; positive	0.892 (0.595–1.337)	0.579
Tumor size	≤5 cm; >5 cm	1.725 (1.217–2.444)	0.002*
Cut margin	<1 cm; >1 cm	1.303 (0.901–1.884)	0.159
PVI	No; yes	1.897 (1.335–2.694)	0.000*
HVI	No; yes	1.803 (1.269–2.562)	0.001*
GOLM1	Low; high	1.710 (1.190–2.458)	0.004*
Cox multivariate analysis (OS)			
Stage	I–II; III	1.063 (0.786–1.436)	0.691
PVI	No; yes	1.515 (1.008–2.277)	0.046*
HVI	No; yes	1.539 (0.968–2.449)	0.068
Tumor size	≤5 cm; >5 cm	1.233 (0.805–1.888)	0.336
GOLM1	Low; high	1.696 (1.160–2.479)	0.006*

OS overall survival, *PVI* portal vein invasion, *HVI* hepatic vein invasion, *HR* hazard ratio, *95 % CI* 95 % confidence interval

* $p < 0.05$

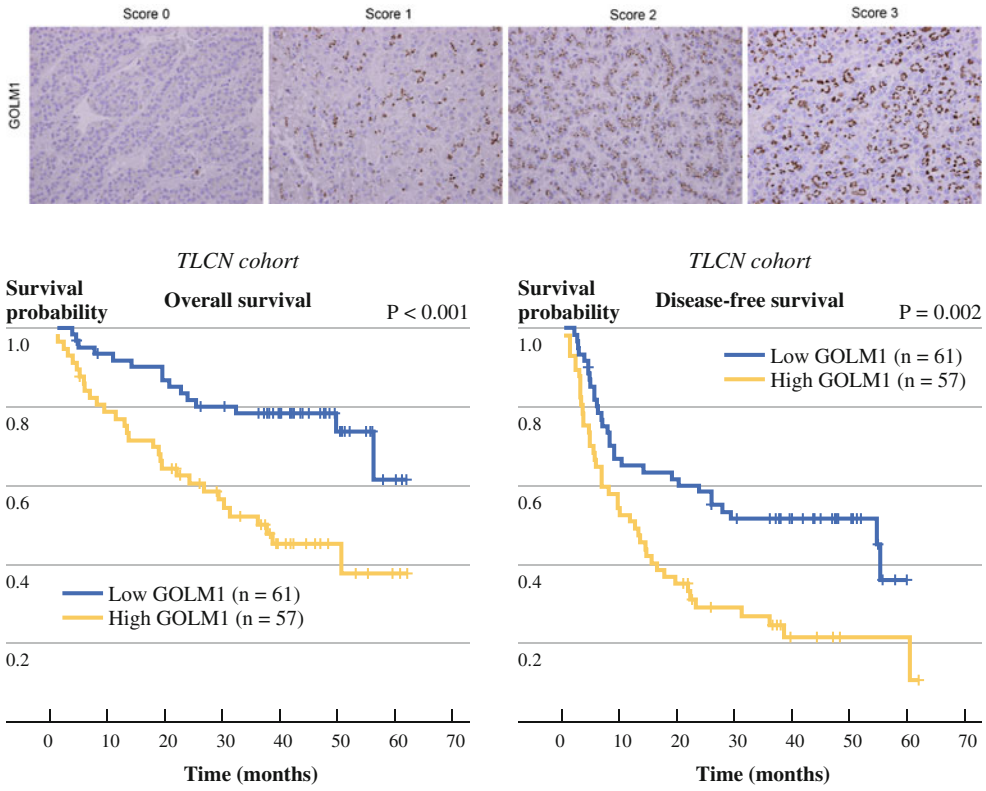

FIG. 5 GOLM1 expression correlated with survival in HCC patients in validation cohort. Immunohistochemical stains of the different intensity scores for GOLM1 expression (*upper panel*); The high GOLM1 group showed significantly worse overall survival ($p < 0.001$) and disease-free survival ($p = 0.002$) than the low GOLM1 group (lower panels)

patients into high GOLM1 group and low GOLM1 group, and high levels of GOLM1 still significantly correlated with worse overall survival as well as DFS in this validation cohort (Fig. 5, $p < 0.001$ and $p = 0.002$).

DISCUSSION

GOLM1 (also known as GP73 and GOLPH2), a 73-kDa Golgi membrane glycoprotein encoded by the *GOLM1* gene, is expressed in cells of epithelial lineage, including prostate, bronchial, intestinal, renal, and biliary epithelial cells.[13] In normal liver tissue, GOLM1 expression in the hepatocytes is either absent or weak and is limited to scattered cells in the periportal area.[13] Upregulation of GOLM1 was observed in several liver diseases, including acute and chronic hepatitis, liver cirrhosis, and HCC (Fig. 2); however, the biological function and mechanism of this upregulation remains unclear.[19,20] We found that GOLM1 expression was significantly higher in HCC tumor tissues than in adjacent nontumor tissues. Furthermore, high GOLM1 protein expression was an independent poor prognostic factor for DFS and OS in HCC.

Recently, high GOLM1 expression in HCC was strongly associated with higher tumor grade, larger tumor size, and increased vein invasion, suggesting that GOLM1 augments tumor invasion and metastasis.[19,21] In previous studies, GOLM1 tissue expression was not significantly associated with patient survival. Sun et al. [21] reported that GOLM1 overexpression was significantly associated with aggressive behavior of HCC but not with OS in 36 HCC patients. Riener et al. [19] also reported that high GOLM1 expression was significantly correlated with a high tumor grade but not survival in 170 HCC patients. The small patient population and lack of consistent follow-up may account for the differences in the association of GOLM1 expression with tumor behavior and prognosis between our study and previous studies.[19,21]

Data on the efficacy of surveillance strategies for predicting prognostic outcomes in resectable HCC are limited, and surveillance is primarily recommended for high-risk patients.[22] AFP has been widely used for predicting post-hepatectomy outcome; however, it has not been correlated with survival.[23] Because we have identified the GOLM1 expression as an important prognostic marker in HCC, serum GOLM1 may further complement current surveillance methods in predicting the outcome in HCC patients after resection. Further studies are warranted to confirm the prognostic significance of GOLM1 expression in HCC.

In summary, GOLM1 expression is significantly elevated in HCC and is an independent prognostic marker for DFS and OS in HCC. To our knowledge, this is first report that shows the prognostic role of GOLM1 expression in HCC.

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