Knockdown of GOLM1 inhibits growth of laryngeal cancer cells via the Wnt/β-catenin pathway

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Abstract

Background Laryngeal squamous cell carcinoma(LSCC) is a common type of malignant tumors of the head and neck. At present, the early biological markers of laryngeal cancer are still the focus of attention by otolaryngologists.Golgi membrane protein 1 (GOLM1) is a transmembrane glycoprotein present in the cis-Golgi complex.In recent years, successive findings have revealed the carcinogenic effects of GOLM1.However, the biological impact of GOLM1 on LSCC has not been clarified.

Results In this study, GOLM1 expression was found to be significantly correlated with the progression and prognosis of LSCC. By silencing GOLM1, the Wnt/β-catenin pathway was downregulated, thereby inhibiting cell proliferation, migration, and invasion, as well as the progress of EMT, and promoting TU686 cell apoptosis.

Conclusions Our study revealed the influence of GOLM1 through the Wnt/β-catenin pathway on the biological behavior of LSCC. GOLM1 is thus a potential target for the diagnosis and treatment of LSCC.

Background

As the most common pathological type of laryngeal cancer[1], laryngeal squamous cell carcinoma has a very high incidence and is the second most common type of head and neck malignancy[2]. Although clinical research on LSCC has made some progress, the survival rate of this disease has not been significantly improved[3]. There is thus an urgent need to find specific molecular markers that can predict the clinical outcome of LSCC and act as therapeutic targets.

Golgi membrane protein 1 (GOLM1), also known as GOLPH2 or GP73, is a transmembrane glycoprotein present in the cis-Golgi complex. It is mainly involved in modifying endoplasmic reticulum proteins after their synthesis and assists in the completion of Golgi protein transport and secretion[4]. In recent years, successive findings have revealed the carcinogenic effects of GOLM1. Specifically, studies[5, 6] have shown that GOLM1 is involved in the progression and metastasis of a variety of malignant tumors by participating in immune regulation, activation, and degradation of intracellular signal transduction factors, and epithelial-mesenchymal transition (EMT). However, the biological impact of GOLM1 on LSCC has not been clarified.

The classic Wnt pathway, the Wnt/β-catenin pathway, is one of the key cascades regulating development and cell stemness[7]. Its key component, β-catenin, participates in the occurrence and development of cancer by regulating intracellular signal transduction. The Wnt/β-catenin pathway is regulated by many factors. Our previous study based on the GEPIA system found that the expression of GOLM1 mRNA in head and neck squamous cell carcinoma (HNSCC) was significantly higher than that in normal tissues, that there was a significant positive correlation between the expression of GOLM1 and WNT1, and that the latter was a key ligand for activation of the Wnt/β-catenin pathway. Against this background, here we attempted to provide new insights into the pathogenesis of LSCC and strategies to treat it by exploring the relationship between GOLM1 and the Wnt/β-catenin pathway.

In this study, GOLM1 expression was found to be significantly correlated with the progression and prognosis of LSCC. By silencing GOLM1, the Wnt/β-catenin pathway was downregulated, thereby inhibiting cell proliferation, migration, and invasion, as well as the progress of EMT, and promoting cell apoptosis. This revealed the influence of GOLM1 through the Wnt/β-catenin pathway on the biological behavior of LSCC. GOLM1 is thus a potential target for the diagnosis and treatment of LSCC.

Result

GOLM1 is overexpressed in HNSCC

We analyzed GOLM1 mRNA in 519 cases of HNSCC and 44 cases of normal tissues based on GEPIA. The expression of GOLM1 was significantly higher in the HNSCC group than in normal tissues (Fig. 1A, $P<0.01$). In HNSCC cases, higher expression of GOLM1 was significantly associated with poorer prognosis (Fig. 1B, $P<0.01$). We also found that, in HNSCC tissue, the expression levels of GOLM1 and WNT1 were significantly positively correlated (Fig. 1C, $P<0.01$, $r=0.019$).

GOLM1 is overexpressed in LSCC

The results of immunohistochemistry showed that the rate of positivity for GOLM1 protein expression was significantly higher in the LSCC group (31/60) than in the normal group (5/30) (Fig. 2A, $c^2=10.208$, $P<0.01$). Meanwhile, the rate of GOLM1 positivity in poorly differentiated tumor tissues was significantly higher than that in the well-differentiated group ($c^2=5.884$, $P<0.05$). Moreover, the rate of GOLM1 positivity increased with the clinical stage, with the differences between groups being significant (Fig. 2B, $c^2=19.882$, $P<0.001$; Table 3). Kaplan-Meier survival analysis showed that the median survival times of the GOLM1-positive and -negative groups were 22.3months (SE=1.27, 95% CI=19.838–24.824) and 26.4months (SE=0.27, 95% CI=25.863–26.925), which were significantly different ($P<0.05$, log-rank test; Fig. 2C). Cox regression analysis showed that GOLM1 was an independent factor affecting the prognosis of LSCC ($P<0.05$). The prognosis of GOLM1-positive patients was significantly lower than that of GOLM1-negative patients (Exp=8.633, 95% CI=1.083–69.333).

The qRT-PCR results showed that the expression level of GOLM1 mRNA in TU686 cells was significantly higher than that in NOK cells (Fig. 3A, $P<0.001$). Meanwhile, Western blotting results showed that the GOLM1 protein expression level in TU686 cells was higher than that in NOK cells (Fig. 3B, C).

Cell transfection efficiency

We screened two sets of siRNA duplexes (siRNA1 and siRNA2) for their efficacy in inhibiting GOLM1 expression, and used siNC as a control. qRT-PCR and Western blot assays were used to evaluate the efficiency of siRNA transfection. Compared with the levels in cells transfected with nonspecific control siNC, GOLM1 mRNA and protein levels in TU686 cells were significantly reduced after transfection of GOLM1-specific siRNA1 and siRNA2 ($P<0.01$, Fig. 3D–F). In addition, siRNA1 showed higher silencing

efficiency than siRNA2 at the mRNA and protein levels. Therefore, we chose siRNA1 for follow-up experiments, hereinafter referred to as siRNA.

GOLM1 silencing inhibits the proliferation of LSCC cells

The CCK-8 experiment showed that the downregulation of GOLM1 significantly inhibited the proliferative ability of TU686 cells in the siRNA group after 24, 48, and 72 h of culture ($P<0.05$, $P<0.01$, and $P<0.01$, respectively; Fig. 4A).

GOLM1 silencing inhibits the migration and invasion of LSCC cells

The results of the wound healing assay showed that GOLM1 silencing significantly reduced the migration rate after 24 h in TU686 cells compared with the level in the siNC group ($P<0.01$, Fig. 4B, C). GOLM1 silencing also significantly inhibited the invasiveness of TU686 cells. The number of TU686 cells that invaded the Transwell chamber in the siRNA group was significantly lower than that in the siNC group ($P<0.01$, Fig. 4D, E).

GOLM1 silencing increases apoptosis and induces G2/M cell cycle arrest in LSCC cells

GOLM1 silencing induced G2/M cell cycle arrest in TU686 cells ($P<0.01$), and the proportion of TU686 cells in G0/G1 phase was also significantly reduced ($P<0.01$, Fig. 4F, G). In addition, the apoptosis rate of TU686 cells with downregulated GOLM1 was significantly higher than that in the siNC group ($P<0.01$, Fig. 4H, I).

GOLM1 silencing downregulates the Wnt/β-catenin pathway in LSCC

We analyzed the mRNA and protein levels of key factors in the Wnt/β-catenin pathway after inhibiting the expression of the GOLM1 gene in LSCC cells. qRT-PCR results showed that the expression levels of Wnt1 and nuclear β-catenin mRNA in TU686 cells were significantly decreased after silencing GOLM1 compared with those in the siNC group ($P<0.05$ and $P<0.01$, respectively; Fig. 5A). The expression levels of Wnt1, nuclear β-catenin, and p-GSK3β protein in TU686 cells in the siRNA group were also significantly lower than those in the siNC group, while the expression level of p-β-catenin was significantly increased compared with that in cells in the siNC group (Fig. 5B, C).

GOLM1 silencing inhibits the process of EMT

We analyzed the protein levels of EMT-related markers after inhibition of the GOLM1 gene in LSCC cells. The expression level of the epithelial marker E-cadherin in TU686 cells was clearly increased after GOLM1 silencing, while the expression levels of mesenchymal markers such as N-cadherin, Vimentin, and Twist1 were significantly decreased (Fig. 5D).

Discussion

Laryngeal cancer is a common malignant tumor of the head and neck that has high morbidity and mortality. Owing to the lack of effective markers for its early diagnosis, most patients with laryngeal cancer have already reached an advanced stage when they are diagnosed, by which time the optimal period for treatment has passed[11]. Epidemiological data show that the 5-year survival rate of patients with laryngeal cancer is less than 50%[12]. LSCC is the most common pathological type of laryngeal cancer, accounting for 90% of cases[1]. At present, surgery is often performed for LSCC patients, combined with radiotherapy or chemotherapy depending on the clinical stage. Owing to the characteristics of the laryngeal anatomy, the above treatments consistently lead to speech disorders, dysphagia, and other adverse outcomes, which seriously affect the quality of life and mental health of patients. Against this background, there is a need to find targets for early diagnosis and treatment.

GOLM1 is expressed in a variety of human epithelial tissues and plays an important role in maintaining the normal function of cells. Its abnormal secretion is closely related to diseases such as cancer and viral infection[13]. However, the specific mechanism behind GOLM1's involvement in cancer has not been definitively clarified. Studies[14] have shown that GOLM1 selectively interacts with EGFR to activate EGFR/RTK pathway recirculation and downstream MMP9 expression, thereby promoting the growth and metastasis of hepatocellular carcinoma (HCC). The GOLM1 level in serum is important for guiding diagnosis, treatment, and prognostic assessment of liver cancer. In glioblastoma (GBM)[9], GOLM1 promotes the proliferation and migration of GBM cells by activating the PDGFA/PDGFRα pathway. However, the study of GOLM1 in LSCC is still in a blank period.

Our study found that GOLM1 is significantly highly expressed in HNSCC, and its higher expression is correlated with poorer prognosis. Subsequently, we confirmed by immunochemistry that GOLM1 has significantly high expression in LSCC and that cases with a lower degree of tumor differentiation or at a later clinical stage have a higher rate of GOLM1 positivity. We also found that GOLM1-positive patients have a worse prognosis. After silencing GOLM1, the ability of TU686 cells to proliferate, migrate, and invade was significantly inhibited, the cell cycle was arrested in the G1 phase, and the proportion of cells in the G2 phase was significantly reduced. At the same time, the apoptosis rate was significantly increased. The above results indicate that GOLM1 is an important regulatory factor that promotes the occurrence and development of LSCC.

When the Wnt/β-catenin signaling pathway is blocked, β-catenin mainly relies on glycogen synthase kinase (GSK)3β-mediated phosphorylation and forms complexes with GSK3β and AXIN1, among others, and is finally degraded by ubiquitination[15]. When the Wnt ligand is present, it binds to Frizzled (Fzd) protein and lipoprotein-related receptor protein (LRP)5/6, and simultaneously recruits Disheveled (Dvl) proteins in the cytoplasm, inducing the binding of Dvl to degradation complex of β-catenin. This in turn causes β-catenin to dissociate from GSK3β and AXIN1 complex, accumulate and translocate into the nucleus, and initiate the transcription of downstream target genes. Therefore, the Wnt/β-catenin pathway is a strong potential target in cancer treatment[16].

The Wnt/β-catenin pathway plays a role in the development of laryngeal cancer[17]. Interestingly, in our research, we found that GOLM1 and Wnt1, a key ligand of the Wnt pathway, have significant synergistic expression. Another research[18] indicated that GOLM1-rich exosomes activate the GSK-3β/MMP signal axis of recipient cells and accelerate cell proliferation and migration. These results support our conjecture about GOLM1 acting to regulate the Wnt/β-catenin pathway.

In our study, the expression of Wnt1 and p-GSK-3β was significantly downregulated after silencing GOLM1, the ability of GSK-3β to bind β-catenin was enhanced, and the expression of p-β-catenin was upregulated, indicating that the silencing of GOLM1 can negatively regulate the Wnt/β-catenin pathway and inhibit the development of LSCC. This in turn suggests that GOLM1 may activate WNT1 expression and promote GSK-3β phosphorylation to free β-catenin.

EMT is a vital process by which epithelial cells acquire mesenchymal characteristics, which is regulated by many factors.E-cadherin conversion is usually characteristic performance of EMT[19]. EMT can enhance the invasiveness of cancer cells, produce circulating tumor cells and cancer stem cells, and enhance their resistance to anticancer drugs. EMT is also an important mechanism for the process of LSCC metastasis[20]. In this study, after silencing GOLM1, the expression level of the epithelial marker E-cadherin was significantly increased, while the expression levels of the mesenchymal markers N-cadherin, Vimentin, and Twist1 were significantly decreased, suggesting that GOLM1 can regulate the EMT process through the Wnt/β-catenin pathway.

Conclusion

In summary, GOLM1 promotes the development of LSCC through the Wnt/β-catenin pathway. The key steps in this may be WNT1 ligand activation and GSK-3β phosphorylation. Silencing GOLM1 can inhibit WNT1 ligand activation and GSK-3β phosphorylation. Blocking the Wnt/β-catenin pathway, thereby inhibiting the process of EMT, impedes the progression of LSCC. The mechanism of interaction between GOLM1 and proteins in the Wnt/β-catenin pathway requires further elucidation, and the significance of GOLM1 in guiding the diagnosis and treatment of LSCC is expected to be further clarified by increasing the sample size and extending the follow-up time.

Methods

Gene expression profile

The GOLM1 expression data in HNSCC used in this study were derived from the gene expression interactive analysis website GEPIA (http://gepia.cancer-pku.cn/), while the RNA sequencing data obtained by GEPIA for analysis were from the TCGA and GTEx databases[8].

Immunohistochemical staining of LSCC tissues

We collected 60 samples of LSCC tissues surgically removed from LSCC patients who were hospitalized in the First Affiliated Hospital of China Medical University, and collected 30 samples of normal tissues adjacent to the cancerous tissue as controls. All specimens were obtained after the provision of informed consent from the patients before resection, and this study was reviewed by the Ethics Committee of China Medical University.

All specimens were fixed in formalin, embedded in paraffin, serially sectioned at a thickness of 4 μm, and subjected to SP immunohistochemical staining. Strictly following the instructions of the SP kit (ZSGB Bio, Beijing, China), the sections were deparaffinized and hydrated, exposed to 3% H2O2 to eliminate endogenous enzyme activity, and subjected to a high-temperature and high-pressure method for antigen retrieval. They were exposed to the primary anti-GOLM1 monoclonal antibody (BOSTER,Wuhan, China; working concentration 1:200) overnight at 4°C, incubated with secondary antibody, and exposed to DAB(3,3'-Diaminobenzidine, DAB)(BOSTER) for color development, followed by dehydration, transparency, and mounting.

GOLM1 was found to be expressed centrally around the nucleus[9]. Five high-powered fields of cancer nests (malignant tumors) or cell aggregation areas (non-malignant tumors) were randomly selected under a microscope (Nikon DS-F12). The average value of the proportion of GOLM1-positive cells in each section was used as the positive cell index (labeling index, LI). The results are expressed as mean±standard deviation, with LI ≥0.25 being considered positive.

Cell line culture

The human laryngeal squamous cell line TU686 (Fenghui Bio, Changsha, China) and human oral normal squamous epithelial cell line NOK (Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were used in this study. The cells were seeded in RPMI-1640 medium (Solarbio, China) containing 10% FBS and cultured in an incubator at 37°C and 5% CO2.

siRNA and overexpression vector construction

siRNA targeting GOLM1, negative control siRNA, GOLM1 overexpression vectors, and their negative controls were designed and synthesized by Wanleibio (Shenyang, China). TU868 cells in logarithmic growth phase were transfected with LipofectamineTM 3000 (Invitrogen, USA) when the cell density reached 60%–70%. After transfection for 48 h, qRT-PCR and Western blotting were used to determine the transfection efficiency. GOLM1-overexpressing cells were treated with Wnt/β-catenin inhibitor (20 μM ICG001) halfway through the 48 h of transfection[10], and again 24 h after that treatment.

Real-time PCR

Total cell RNA or nuclear RNA was extracted using the UV spectrophotometer NANO 2000 (Thermo Scientific, USA) to determine the concentration of RNA in each sample. Each RNA sample was reverse-transcribed with Super M-MLV reverse transcriptase (BioTeke, Beijing, China) to the corresponding cDNA under reaction conditions of 37℃ for 15 min and then 85℃ for 5 s, 4℃ +∞. Then, cDNA samples were

subjected to qRT-PCR using the SYBR qPCR Master Mix system (BioTeke) in an Exicycler 96 fluorescence quantitative PCR instrument (Bioneer, South Korea). The reaction conditions were 95℃ for 30 s, followed by 40 cycles of 95℃ for 5 s and 60℃ for 34 s, and then melting curve analysis at 65–95℃. The results were calculated by the 2ΔΔCt method and expressed as relative levels using GAPDH as an internal reference gene. See Table 1 for the real-time PCR primer sequences.

Western blotting

Total protein or nuclear protein was extracted using the Nuclear and Cytoplasmic Protein Extraction Kit (Wanleibio). Then, the protein concentration in each cell lysate sample was determined by the BCA (Bicinchoninic acid, BCA) method. Proteins were heated at 100℃ for 10 min to denature them and then electrophoresed on SDS-PAGE gels. Proteins were subsequently transferred to a membrane at 4℃ with 200 mA constant current for 1 h, followed by blocking with 5% skimmed milk at room temperature for 1 h. After the primary antibody had been incubated overnight at 4°C and the secondary antibody had been incubated at 37°C for 45 min, an ultrasensitive ECL chemiluminescence kit (Wanleibio) was used for luminescence development, the film was scanned, and a gel image processing system (Gel-Pro- Analyzer software) was used for analysis with the optical density value of the target band. The antibodies are shown in Table 2.

CCK-8 detection of cell proliferation

The cells were digested with trypsin and resuspended as a cell suspension at a concentration of 5×104/mL. This suspension was seeded in a 96-well plate at 100 μL per well, with five replicate wells being set in each group. After regular culture for a certain period of time (24, 48, or 72 h), 10 μL of CCK-8 (Wanleibio) solution was added to each well, followed by incubation for 2 h, after which the optical density (OD) value at 450 nm was measured with a microplate reader (Bioteke).

Transwell assay

A Transwell chamber (Corning, USA) was placed in a 24-well plate, precoated with Matrigel (Corning) to prepare a single cell suspension (1×105/ml), 800 μl of culture medium containing 10% FBS was added to the lower chamber, and 200 μl of cell suspension was added to the upper chamber, giving cell density of 3×104/well. After culturing in groups for 24 h the cells were fixed with 4% paraformaldehyde (Aladdin, China) for 20 min, stained with 0.5% crystal violet (Amresco, USA) for 5 min, rinsed with distilled water, and the cells in the lower layer of the microporous membrane were counted under a microscope (OLYMPUS, Japan; 200×).

Wound healing assay

The cells in each group were scratched with a 200 μl pipette tip and photographed under a microscope (Olympus; 100×) after the transfected cells had reached confluence . After adding serum-free and antibiotic-free medium and continuing the culture at 37℃ for 24 h, another image of each wound was taken and the cell migration distance was calculated.

Flow cytometry

The cells were centrifuged at 300g for 5 min and washed with phosphate buffered saline(PBS). Next, the cells were incubated with ice-cold 75% alcohol at 20℃ overnight for fixation. Then, the cells were centrifuged and incubated with RNase A at 37℃ for 30 min, followed by staining with propidium iodide at 4℃ for 30 min. After this staining, the cells were analyzed using a NovoCyte flow cytometer (ACEA Biosciences Inc., USA).

Statistical analysis

The statistical software programs GraphPad Prism 7.0 and SPSS 22.0 were used for statistical analysis, and the data are expressed as mean ± SD. Chi-squared test or Fisher's exact probability method was used for counted data. Kaplan-Meier survival curve and Cox regression analysis were used for survival analysis, while the log-rank test was used to determine significance. Spearman's rank method was used for correlation analysis. The measured data were compared between two groups by t-test, while comparisons between multiple groups were performed by one-way analysis of variance. All differences were considered statistically significant at $P<0.05$, and the experiments was replicated more than three times.

Declarations

Ethics approval and consent to participate: All the informed consent obtained from study participants was written. Our research has been reviewed by the Ethics Committee of Medical Science Research of the First Affiliated Hospital of China Medical University [2021332].

Consent to publish: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:Not applicable

Funding: Not applicable

Authors' contributions: JW performed histological experiments as well as cytological experimentsand was a major contributor in writing the manuscript.SW and XD searched the relevant literature and conducted a feasibility analysis of the study.YH collected case information.

AY provided experimental site and experimental equipment and guided the writing of the thesis. All authors read and approved the final manuscript.

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Conflict of interest statement

None.

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Tables

Table1. Primer in this study

Gene	Forward	Reverse
GOLM1	GAAGGAGGTGAAGGAACAG	TGTCTGGGACTTGCTGTTA
GSK3β	CAACTGCCCGACTAACAC	GAGGAGGAATAAGGATGGTA
Wnt1	TTATCTTCGCTATCACCTCCG	GGCCTGCCTCGTTGTTG
β-catenin	CAAGTGGGTGGTATAGAGG	GGATGGTGGGTGTAAGAG
GAPDH	GACCTGACCTGCCGTCTAG	AGGAGTGGGTGTCGCTGT

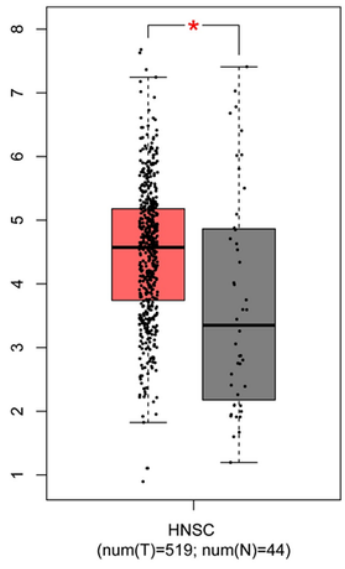
Table2. Antibody in this study

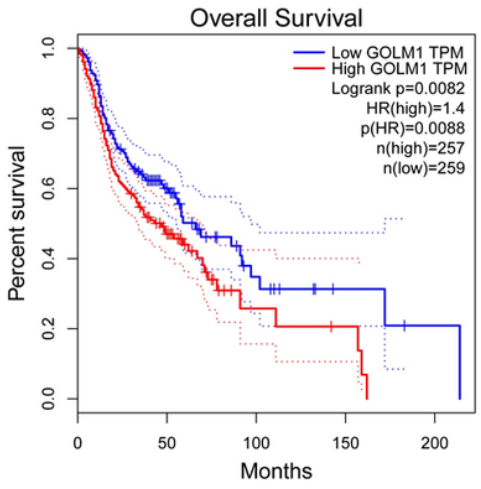
Antibody	Brand	Ratio	Incubation conditions
WB			
GOLM1 antibody	Abcam	1 500	4℃ overnight
Wnt1 antibody	Abcam	1 500	4℃ overnight
p-β-catenin antibody	Abcam	1 500	4℃ overnight
β-catenin antibody	Abcam	1 1000	4℃ overnight
p-GSK3β antibody	Abcam	1 500	4℃ overnight
GSK3β antibody	Abcam	1 500	4℃ overnight
E-cadherin antibody	Abcam	1 400	4℃ overnight
N-cadherin antibody	Abcam	1 400	4℃ overnight
Vimentin antibody	Abcam	1 1000	4℃ overnight
Twist1 antibody	Abcam	1 500	4℃ overnight
GAPDH antibody	Abcam	1 400	4℃ overnight
IHC			
GOLM1 antibody	Boster	1:200	4℃ overnight

Table3.Golm1 expression in the clinical characteristics of LSCC

Clinical features	Cases	GOLM1			
		GOLM1(+)	GOLM1(-)	c^2	*p*
Total	60	31	29	10.208	0.001
Differentiation					
Highly differentiated	36	14	12	5.884	0.015
Poorly differentiated	24	17	7		
Clinical stage					
	10	2	8		
	21	6	15	19.882	0.000
	14	9	5		
	15	14	1		
Sex					
Males	56	30	26	-	0.346
Females	4	1	3		
Age					
≥60	35	19	16	0.231	0.631
<60	25	12	13		

Figures





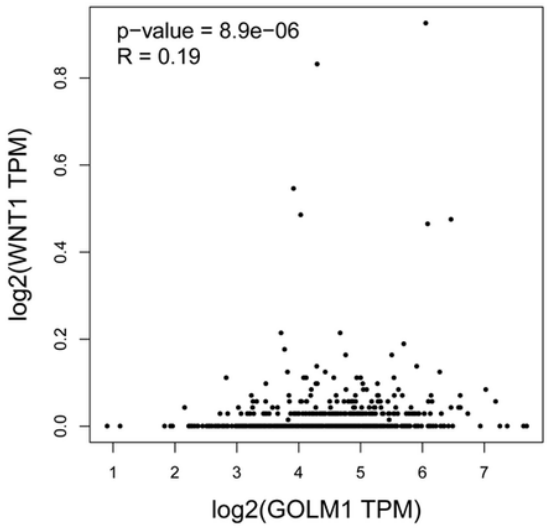


Figure 1

GOLM1 is up-regulated in HNSCC

1A. Analysis of 519 cases of HNSCC and 44 cases of normal tissues in the TCGA and GTEx database based on the GEPIA , the expression of GOLM1 mRNA in HNSCC tissues was significantly higher than that of normal tissues

1B. In HNSCC cases, the survival time of GOLM1 high expression patients was significantly lower than that of GOLM1 low expression patients

1C. GOLM1 mRNA was significantly positively correlated with the expression of WNT1 mRNA in HNSCC tissues

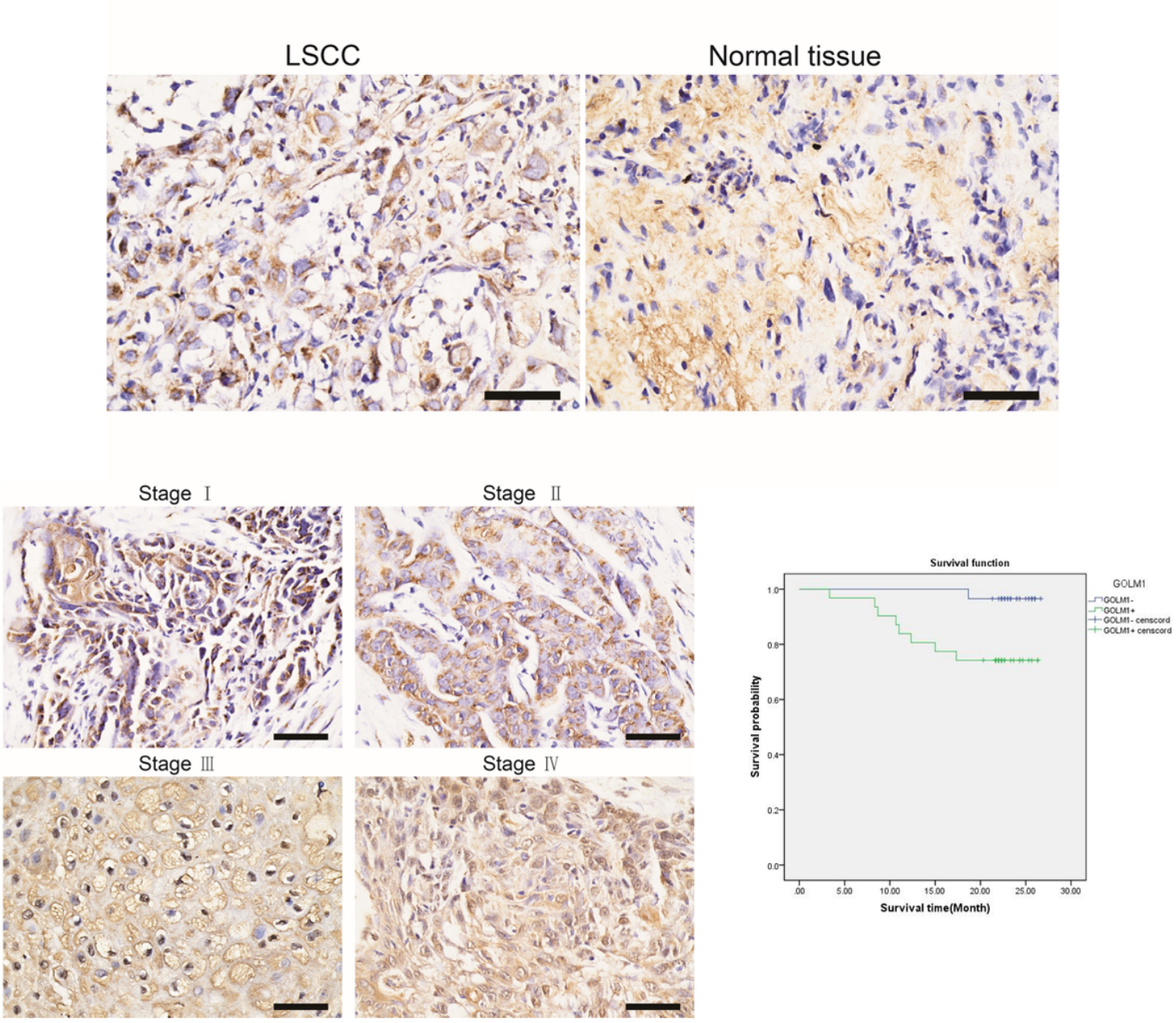


Figure 2

GOLM1 overexpression in LSCC

2A. Immunohistochemistry staining for GOLM1 in LSCC tissue and normal tissues. Cytoplasmic staining was considered to be positive for GOLM1.The positive rate of GOLM1 in the LSCC group (31/60) was significantly higher than that of normal tissues(5/30), 400X

2B. GOLM1 immunohistochemical staining in different clinical stages

2C. Kaplan-Meier survival analysis the median survival time of GOLM1 positive expression and negative expression groups were 22.331 months and 26.394 months

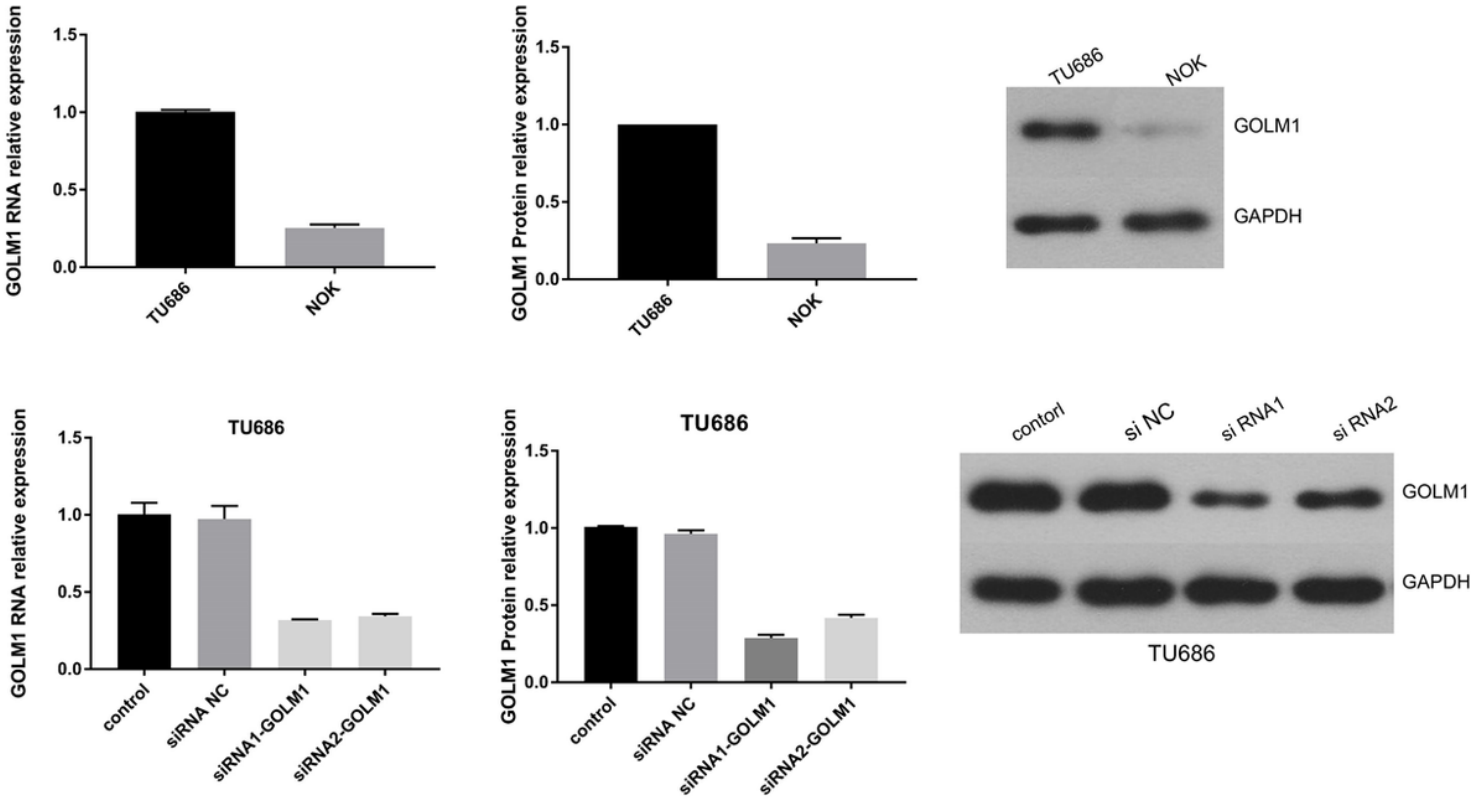


Figure 3

GOLM1 overexpression in TU686 cells and Silence of GOLM1 in TU686 cells

3A. qRT-PCR analysis of the GOLM1 mRNA expression level in TU686 cells and NOK cells (P<0.0001)

3B. Western blot assay was used to detect GOLM1 protein expression in TU686 cells and NOK cells

3C. The level trend of each protein after grayscale analysis in TU686 and NOK was showed by Histogram (P<0.05)

3D. GOLM1 knockdown significantly reduces GOLM1 mRNA expression level in TU686 cells

3E~3F. GOLM1 knockdown significantly reduces GOLM1 protein expression level in TU686 cells

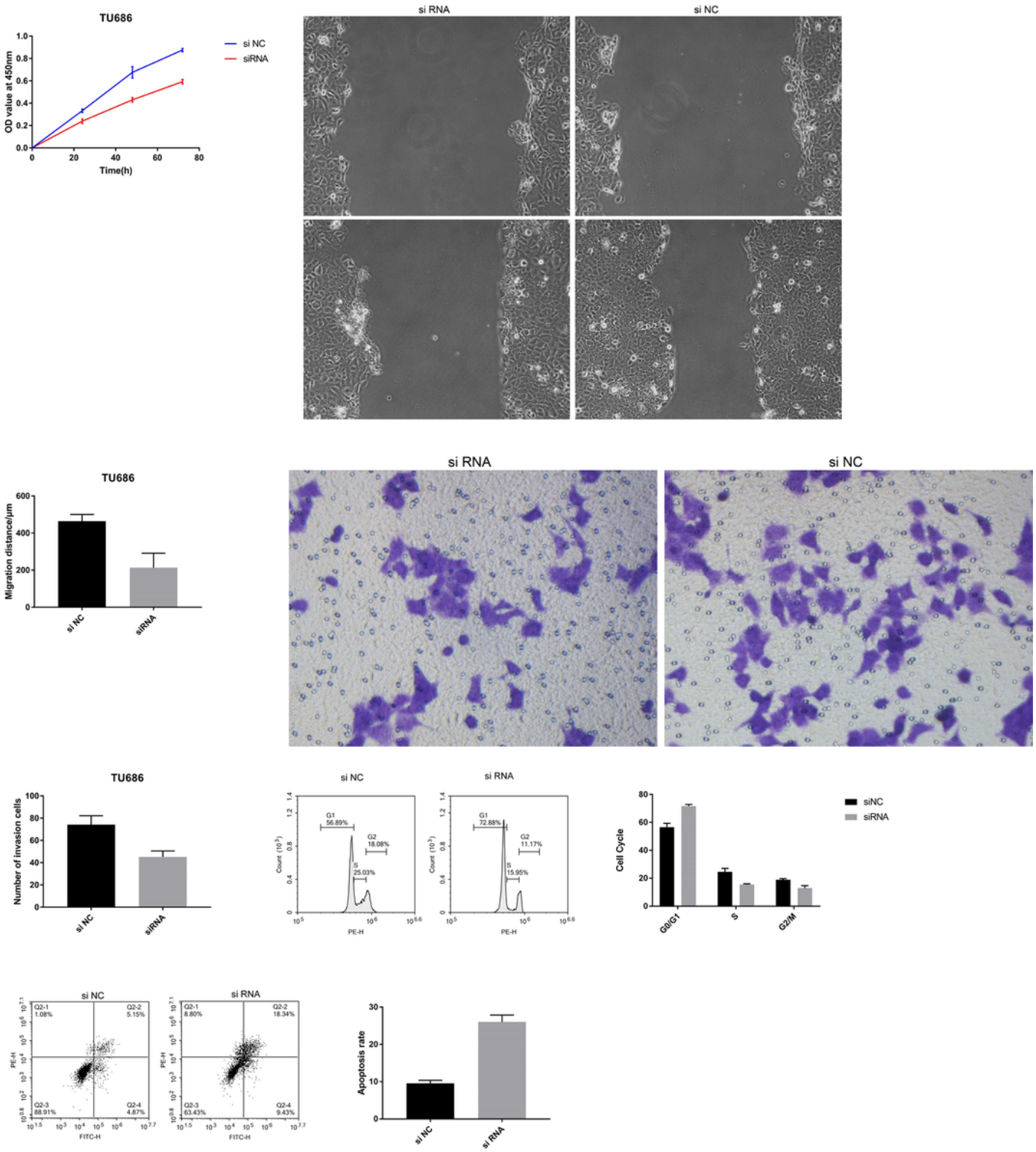


Figure 4

The effect of silencing GOLM1 on the proliferation and migration ability of TU686

4A. CCK-8 assay was used to detect the OD value of TU686 cells at 450nm for 24h, 48h and 72h ($P<0.05$), respectively for si RNA group and si NC group ($P<0.05$)

4B~4C. Wound healing assays were performed to explore the migration capability of TU686 cells. Images are captured at 0 and 24 hours after the scratch is made. The solid line is the original scratch edge (100X).The cell migration distance was calculated as described in the Materials and Methods(si RNA and si NC)

4D~E.The number of TU686 cells that invaded the Transwell chamber in the siRNA group was significantly lower than that in the siNC group .

4F~4G. Compared with the si-NC group, GOLM1 silencing can cause cell G2/M phase arrest,while the percentage of cells in G1 phase increased significantly .

4H~4I. After GOLM1 silencing, the apoptosis rate of cells was significantly increased compared with Si-NC group

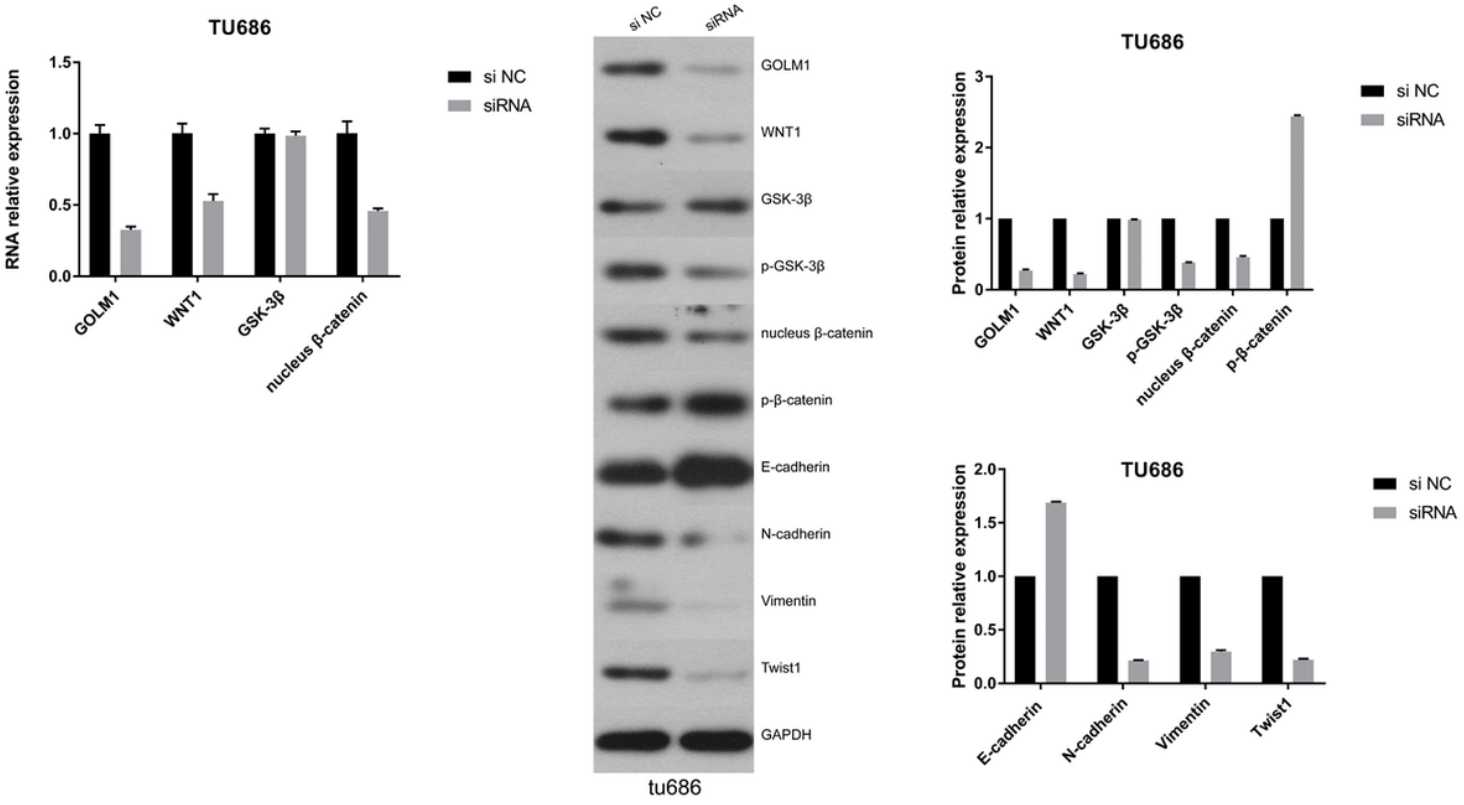


Figure 5

The effect of silencing GOLM1 on the Wnt/β-catenin pathway in LSCC

5A. qRT-PCR detects the mRNA expression levels of Wnt1, nuclear β-catenin, and GSK3β in TU686 cells after GOLM1 silencing

5B~C. Western blot assay detection of the protein expression of Wnt1, nuclear β-catenin, p-β-catenin, GSK3β and p-GSK3β in TU686 cells after GOLM1 silencing

5D. Western blot assay was used to detect the expression level of E-cadherin, N-cadherin, Vimentin and Twist1 proteins in TU686 cells after GOLM1 was silenced