Neoplasma 2022; 70(1): 136–144

doi:10.4149/neo_2023_220816N835

GOLM1 facilitates human colorectal cancer progression and metastasis via activating the AKT/GSK3β/EMT axis

Zonglei MAO[1,2,#], Yong WU[2,#], Pingan YAO[3,#], Chungen XING[2,*]

[1]Department of Gastroenterological Surgery, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, China; [2]Department of General Surgery, The Second Affiliated Hospital of Soochow University, Suzhou, China; [3]Department of Gastrointestinal Surgery, Shanghai East Hospital Affiliated to Tongji University, Shanghai, China

**Correspondence: xingcg@suda.edu.cn*
[#]Contributed equally to this work.

Received August 16, 2022 / Accepted January 23, 2023

GOLM1 (Golgi membrane protein 1), a key tumor progression- and metastasis-related marker, is highly expressed in a variety of epithelium-derived human cancers. However, its expression and functions in human colorectal cancer (CRC) have been rarely explored. The present study verified the high expression of GOLM1 within CRC tissues and cell lines. GOLM1 was positively correlated with vascular invasion, TNM stage, and lymph node metastasis among CRC cases. *In vitro* experiments showed that GOLM1 downregulation inhibited the growth, migration, and invasion of Caco-2 and HCT116 cells, while the overexpression of GOLM1 facilitated the growth, migration, and invasion of SW480 cells. *In vivo* experiments revealed that the knockdown of GOLM1 reduced the growth of nude mouse xenografts and lung metastasis of HCT116 cells. Furthermore, GOLM1 was found to be a motivator for the epithelial-mesenchymal transition (EMT) phenotype and the AKT/GSK3β pathway in CRC cells. Finally, MK2206, an AKT inhibitor, could markedly reverse GOLM1-elicited proliferation, migration, invasion, and EMT phenotype by inhibiting the AKT/GSK3β pathway. Collectively, our data indicate that GOLM1 facilitates human CRC progression and metastasis via activating the AKT/GSK3β/EMT axis. Most importantly, our study makes substantial support for the clinical translation of GOLM1 in CRC target therapy.

Key words: GOLM1; AKT/GSK3β; EMT; CRC; metastasis

Colorectal cancer (CRC) ranks as the 3rd most frequently occurring malignant tumor and is the second leading cause of cancer-associated mortality worldwide [1]. Conventional remedies, including curative surgery, chemotherapy, and radiotherapy, dramatically improve the prognosis of primary CRC. However, the above-mentioned therapeutic options for cases with postoperative recurrence and metastasis at initial diagnosis remain unsatisfactory [2]. As a result, an exhaustive investigation of metastasis-related molecular mechanisms and developing potential therapeutic options for metastatic CRC (mCRC) is highly significant.

Epithelial-mesenchymal transition (EMT), a prevalent biological process in tumor progression and metastasis, occurs in cells that gradually lose epithelial phenotypes and acquire mesenchymal features [3]. Numerous studies have indicated that EMT is the primary contributor to CRC progression and metastasis [4-6]. In CRC, particularly mCRC, co-expression of epithelial- and mesenchymal-marker is an ordinary hybrid state. While various studies have investigated the molecular mechanisms and signaling pathways, which regulate EMT [7–10], fully reversing EMT and thereby reducing CRC metastasis have still not been achieved. Thus, it is crucial to explore other factors or mechanisms responsible for EMT-induced tumor progression and metastasis.

GOLM1 (Golgi membrane protein 1, also named GP73 and GOLPH2), a type II cis-Golgi transmembrane glycoprotein, was first reported in the year 2000. It was shown that viral infection is one of the important inducers of its expression [11, 12]. Two teams have demonstrated that GOLM1 plays an important function in normal tissue development [13, 14]. Subsequent studies demonstrate that GOLM1 acts as an oncogene and a prognostic marker in human cancers. A series of studies have indicated that GOLM1 is highly expressed in hepatocellular cancer [15], lung cancer [16], prostate cancer [17], pancreatic cancer, and glioma [18, 19]. The above-mentioned studies also uncover that highly expressed GOLM1 promotes cancer progression and metas-

tasis via activating the EMT phenotype. However, there are only sporadic studies in CRC. A handful of existing research uncovered that GOLM1 restricts the development and facilitates metastasis in a certain stage of CRC [20, 21]. One prior study further demonstrates that GOLM1 can be specifically targeted to inhibit the progression of CRC-PDX [22]. The study also suggests that GOLM1 exerts high clinical translational prospects in CRC target therapy. However, the detailed biological behaviors and underlying mechanisms of GOLM1 in CRC remain largely unclear.

Herein, we explored the precise function of GOLM1 in CRC and attempted to unravel the possible mechanisms. This study is a vital attempt in the area and is expected to provide a novel potent intervention target for CRC.

Materials and methods

Cell culture. Human CRC cells (Caco-2, HCT116, LoVo, RKO, SW480, and HCT8) together with human normal intestinal crypt cells (HIEC) were provided by the Chinese Academy of Sciences (Shanghai, China). Caco-2, HCT116, RKO, SW480, HCT8, and HIEC were cultured in DMEM, and LoVo was cultured in Ham's F-12K. The above two mediums contained 10% fetal bovine serum (FBS), and cells were incubated in a humidified incubator with 5% CO_2 and 37 °C.

Cell transfection and establishment of GOLM1 stable expression in CRC cells. Caco-2 and HCT116 cells were transfected by lentivirus-mediated short hairpin RNAs (shGOLM1-1, -2, -3, GeneChem Technologies), and SW480 cells were transfected with lentivirus-mediated full-length GOLM1 (LV-GOLM1, GeneChem Technologies). After 72 h, the cells were transferred to Petri dishes with a medium containing 8 μg/ml puromycin for screening out the GOLM1 stable knockdown or overexpressing CRC cells. The targeting sequences were as follows: shNC: 5'-TTCTCCGAACGTGTCACGT-3'; shGOLM1-1: 5'-GCAGGGAATGACAGAAACATA-3'; shGOLM1-2: 5'-CGAATAGAAGAGGTCACCAAA-3'; shGOLM1-3: 5'-GAAGGGAAACGTGCTTGGTAA-3'.

CCK-8 assay. Cells (2500/well) were inoculated into the 96-well plates and incubated in the 37 °C thermostat incubator. Subsequently, to every well 90 μl DMEM plus 10 μl CCK-8 mixed solution was added at indicated time points (24 h, 48 h, 72 h, and 96 h) and incubated for 2 h at 37 °C in accordance with specific protocols. Specifically, the concentration of MK2206 (Selleck) used in our experiments was 1 nM (dissolved in DMSO). A microplate reader was used to measure cell viability at 450 nm.

Colony formation assay. Cells (80–200/well) were inoculated into 6-well plates and cultured in DMEM. During the period of colony culture, we replaced the medium at three days intervals. Fourteen days later, colonies were washed thrice with PBS and fixed with 4% formaldehyde for 15 min. After washing again, colonies were stained with 0.1% crystal violet for 10 min. Colonies were counted, and results represented the average from 3 independent experiments.

Migration and invasion assays. The variation in migration (Matrigel-uncoated) and invasion (Matrigel-coated) capacity of GOLM1 knockdown or overexpression in CRC cells was examined. CRC cells (2×10^4 cells) were suspended in 200 μl DMEM containing 1% FBS and plated in the upper chamber (8.0 μm pores), and 600 μl DMEM containing 20% FBS was added into the lower chamber. After 24 h of incubation for migration and 48 h for invasion, the residual cells on the upper chamber were cleared, while those on the opposite side of the upper chamber were fixed by 4% formaldehyde for 15 min, followed by 10 min of 0.1% crystal violet staining. Then, migrated and invaded cells were calculated microscopically (200× magnification) in five randomly chosen fields.

Quantitative real-time PCR (qRT-PCR). Total cellular RNA was extracted using TRIzol reagent (Invitrogen, USA), and 400 ng of the above total RNA was used to prepare cDNA using an RT Reagent Kit (Takara, Japan) through reverse transcription following manufacturer's protocols. Then, qRT-PCR was conducted using SYBR Premix Ex Taq (Takara, Japan) in Roche light cycler 480II Real-time PCR system (Roche, USA). Data were analyzed using the $2^{-\Delta\Delta CT}$ method and normalized to 36B4 (Gene ID: 6175-RPLP0). The primers were as below: GOLM1 Forward, 5'-CCGGAGCCTCGAAAAGAGATT-3', Reverse, 5'-ATGATCCGTGTCTGGAGGTC-3'; 36B4 Forward, 5'-GCAGACAACGTGGGCTCCAAGCAGAT-3', Reverse, 5'-GGTCCTCCTTGGTGAACACGAAGCCC-3'.

Western blot analysis. The CRC tissues and pretreated cells were lysed by RIPA (Beyotime Biotechnology, China) containing protein inhibitor phenylmethylsulphonyl fluoride (Beyotime Biotechnology, China). Then, total protein content was detected using a BCA protein kit (Beyotime Biotechnology, China). Then, sodium dodecyl sulfate-polyacrylamide gel electrophoresis was conducted to separate 30 μg protein and transferred to PVDF membranes (Millipore, USA). Proteins on the PVDF membranes were detected using the following primary antibodies: GOLM1 (Abcam, ab109628); E-cadherin (Proteintech, 20874-1-AP); N-cadherin (Abcam, ab76011); Snail1 (Proteintech, 13099-1-AP); AKT (CST, #4691); p-AKT (ser473, CST, #4060); GSK3β (CST, #12456); p-GSK3β (ser9, CST, #5558); β-actin (Proteintech, 20536-1-AP); GAPDH (Proteintech, 10494-1-AP). The experiments were repeated thrice.

Animal studies. Four-week-old BALB/C male nude mice were provided by the SLAC laboratory animal center (Shanghai, China) and raised in a barrier facility. For the proliferation model, nude mice were injected subcutaneously with 4×10^6/100 μl GOLM1 stable transfected HCT116 cells (shNC and shGOLM1) into bilateral flanks (N=5/group). Tumor diameter was monitored at intervals of four days, and tumor volume (mm^3) was determined using the formula: (length × width^2)/2. For the lung metastasis model, nude mice were injected with 1×10^6 of the above-mentioned cells

in the tail vein. Mice were sacrificed after 28 days of injection for the proliferation model and after 40 days for the lung metastasis model. The isolated transplanted tumor tissues in the proliferation model and lung tissues in the metastasis model were photographed and then identified by H&E staining. All *in vivo* experiments were approved by the ethics committee of Soochow University.

Immunohistochemistry (IHC) analysis. Tumor specimens of 125 CRC cases undergoing curative surgical treatment were obtained from the Second Affiliated Hospital of Soochow University from 2009 to 2018. All patients had not received other treatments before specimen collection. For IHC analysis, the blocked sections were incubated using a primary antibody against GOLM1 (Abcam, ab109628). Each section was assessed by two pathologists, and staining signals were designated as: 0 (no staining); 1 (weak staining, light brown); 2 (moderate staining, brown); 3 (strong staining, dark brown). Staining signal ≥2 was counted as a high expression; <2 was counted as a low expression.

Statistical analysis. All results were expressed as mean ± SD. The Student's t-test was applied in experiments conducted *in vitro* and *in vivo*. Mann-Whitney U test was used to assess the results of GOLM1 expression in 11 pairs of CRC and non-cancerous tissues. The relationship between GOLM1 and the clinical pathologic features of CRC was determined by the chi-square test. A p-value <0.05 stood for statistical significance. GraphPad Prism 6.0 software (GraphPad Inc., USA) was used to analyze all statistical data.

Results

GOLM1 was highly expressed in human CRC samples. For exploring GOLM1 expression, GEPIA (public database) was adopted for analysis and comparison of mRNA expression in CRC and normal tissues. Compared to normal samples (N=667), GOLM1 mRNA was highly expressed in CRC samples (N = 349, Figure 1A) [23]. Subsequently, the protein levels within CRC and non-cancerous intestinal tissues were further investigated. Western blot analysis revealed that GOLM1 protein was highly expressed in CRC samples (N=11) compared with that in matched non-cancerous tissues (Figures 1B, 1C). IHC analysis further revealed that GOLM1 was expressed in both CRC and non-cancerous intestinal tissues; besides, the positive expression was higher in CRC tissues (N=125, positive rate: 68.8%) compared to that in normal intestinal tissues (N=43, positive rate: 44.19%) (Figures 1D, 1E). As many studies have suggested that GOLM1 plays a vital role in cancer progression and therapy, we speculated that a higher GOLM1 expression also facili-

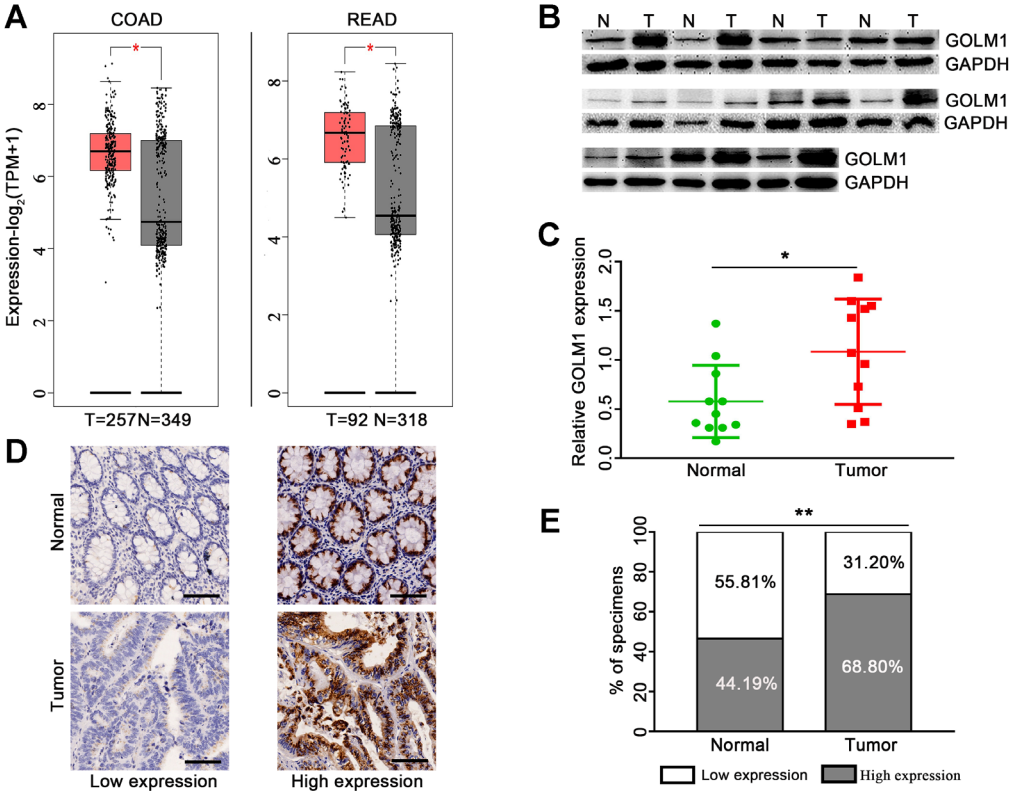

Figure 1. The expression of GOLM1 in CRC. A) GEPIA analysis of GOLM1 mRNA. B) Western blot analysis and C) quantification of GOLM1 expression in 11 pairs of matched CRC and adjacent tissues. D) IHC analysis of GOLM1 in CRC and normal intestinal tissues (scale bar = 100 μm, 200x magnification). E) Quantification of GOLM1 protein expression in 43 normal intestinal tissues and 125 CRC tissues. *p<0.05, **p<0.01

tates CRC progression and may be a promising clinical target in CRC therapy.

GOLM1 was positively correlated with CRC TNM stage, lymph node metastasis, and vascular invasion. To investigate the relationship between GOLM1 and CRC clinical features, the expression of GOLM1 was examined in 125 CRC specimens by IHC analysis. The results showed that a higher GOLM1 expression had a positive relation with the TNM stage (p = 0.0148), lymph node metastasis (p=0.0273), and vascular invasion (p=0.0079, Table 1). Collectively, our data indicate that GOLM1 may favor CRC progression and metastasis.

Table 1. Correlations between GOLM1 expression and clinicopathological characteristics in CRC patients.

Variables All cases		GOLM1 expression		p-values
		High (%)	Low (%)	
Colorectal cancer	125	86 (68.8)	39 (31.2)	
Age (years)				
≥60	86	59 (47.2)	27 (21.6)	0.9442
<60	39	27 (21.6)	12 (9.6)	
Gender				
Male	70	52 (41.6)	18 (14.4)	0.1738
Female	55	34 (27.2)	21 (16.8)	
Tumor size (cm)				
≥5	53	34 (27.2)	19 (15.2)	0.3358
<5	72	52 (41.6)	20 (16.0)	
T stage				
Tis/T1+T2	26	17 (13.6)	9 (7.2)	0.6728
T3+T4	99	69 (55.2)	30 (24.0)	
N stage				
N0	75	46 (36.8)	29 (23.2)	0.0273*
N1+N2	50	40 (32.0)	10 (8.0)	
Nerve invasion				
Yes	32	20 (16.0)	12 (9.6)	0.3725
No	93	66 (52.8)	27 (21.6)	
Vascular invasion				
Yes	47	39 (31.2)	8 (6.4)	0.0079*
No	78	47 (37.6)	31 (24.8)	
AJCC-TNM stage				
I+II	73	44 (35.2)	29 (23.2)	0.0148*
III+IV	52	42 (33.6)	10 (8.0)	

Note: *p<0.05 was considered statistically significant

Successful establishment of GOLM1 stable knockdown and overexpressing cell lines. The differential expression of GOLM1 was evaluated in six human CRC cell lines and human normal intestinal crypt cell line HIEC. Western blot analysis verified higher expression of GOLM1 in five cell lines (Caco-2, HCT116, LoVo, RKO, and HCT8) than in the HIEC cell line (Figures 2A, 2B). Next, Caco-2 and HCT116 (two GOLM1 highest expressing cell lines) were used for the knockdown, and SW480 (a GOLM1 low expressing cell line) was used for overexpression. Our study revealed that two sequences (shGOLM1-2 and -3) for GOLM1-knockdown were efficient, and shGOLM1–3 was the most efficient, while SW480 cells were markedly upregulated by LV-GOLM1, as detected by PCR and western blot analysis (Figures 2C, 2D).

GOLM1 promoted *in vitro* and *in vivo* CRC cell proliferation. On evaluating the effect of GOLM1 on CRC cell growth, CCK-8 and colony formation assays displayed that GOLM1 knockdown suppressed the growth and colony formation ability of Caco-2 and HCT116 cells while overexpression of GOLM1 enhanced the growth and colony formation ability of SW480 cells (Figure 3A–3C). Next, we further explored GOLM1-regulated CRC cell proliferation *in vivo*. Consistent with *in vitro* experiments, GOLM1 knockdown significantly inhibited the expression of Ki-67, weight, and volume of CRC cell xenografts (Figures 3D–3H). Thus, GOLM1 acts as a promoter in CRC cell proliferation.

GOLM1 facilitated CRC cell migration, invasion, and lung metastasis. GOLM1 is positively correlated with CRC TNM stage, vascular invasion, and lymph node metastasis;

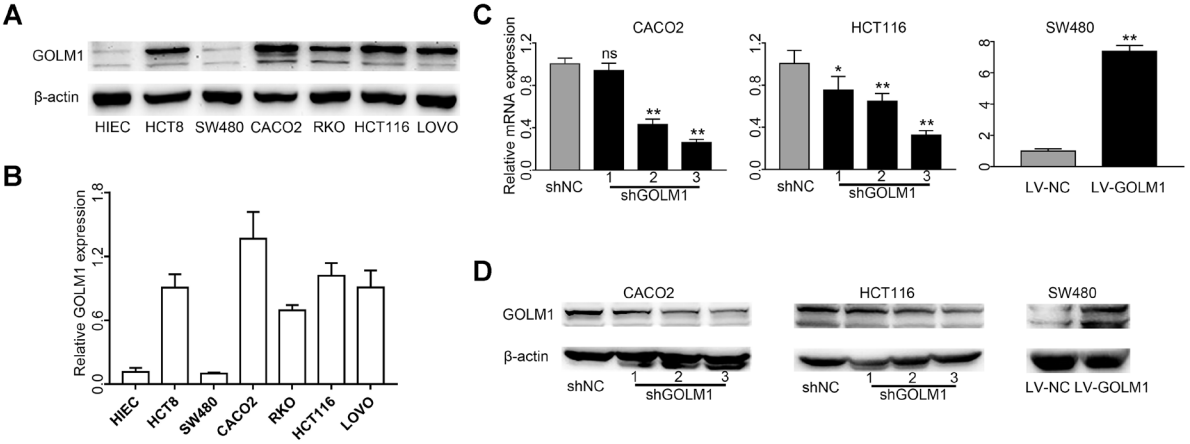

Figure 2. Detection of GOLM1 expression in the normal intestinal crypt and CRC cell lines. A, B) Western blot analysis and quantification of GOLM1 protein expression in HIEC cells and six CRC cell lines. C, D) mRNA and protein of GOLM1 expression in Caco-2 and HCT116 cell lines targeted by shRNAs as well as in SW480 transfected by LV-GOLM1. All data compared to shNC or LV-NC. *p<0.05, **p<0.01; Abbreviation: ns-not significant

therefore, our study investigated the influence of GOLM1 on CRC cell metastasis *in vivo*, as well as *in vitro* invasion and migration. According to our results, GOLM1 knockdown remarkably suppressed the invasion and migration of Caco-2 and HCT116 cells while its overexpression conspicuously enhanced the invasion and migration of SW480 cells (Figures 4A–4D). Moreover, our *in vivo* investigation revealed that GOLM1 knockdown decreased lung metastasis of HCT116 cells (Figures 4E–4G). These data, therefore, show that GOLM1 is an important driving factor in CRC cell migration, invasion, and metastasis.

GOLM1 promoted CRC progression and metastasis via activating the AKT/GSK3β/EMT axis. EMT, a key driver of tumor metastasis, has also been confirmed to play an important role in GOLM1-elicited metastasis. Herein, we evaluated the changes in EMT in GOLM1-regulated CRC progression and metastasis. The data revealed that GOLM1 knockdown upregulated the expression of E-cadherin and downregulated N-cadherin and Snail1 in HCT116 cells. Meanwhile, overexpression of GOLM1 elicited opposite results in SW480 cells (Figure 5A). AKT signaling pathway, a vital downstream component of GOLM1 in hepatocellular cancer, prostate cancer, and glioma, was also verified to relate positively in CRC by GSEA analysis upon on TGCA and GEO database, respectively (Figure 5B). Hence, we evaluated the change in AKT and its key effectors (GSK3β and mTOR) in GOLM1-regulated CRC cells. The data revealed that GOLM1 knockdown downregulated AKT/GSK3β in HCT116 cells. Meanwhile, overexpression of GOLM1 elicited opposite results in SW480 cells. Both GOLM1-knockdown and -overexpression experiments showed no change in mTOR (data not shown) (Figure 5C). To further identify the association of EMT and the AKT/GSK3β pathway, GOLM1-overexpressing SW480 cells were treated with MK2206, an AKT inhibitor. MK2206 could significantly reverse GOLM1-enhanced proliferation, migration, invasion, and EMT phenotype in the above-mentioned SW480 cells (Figures 5D–5J). According to the above results, the AKT/

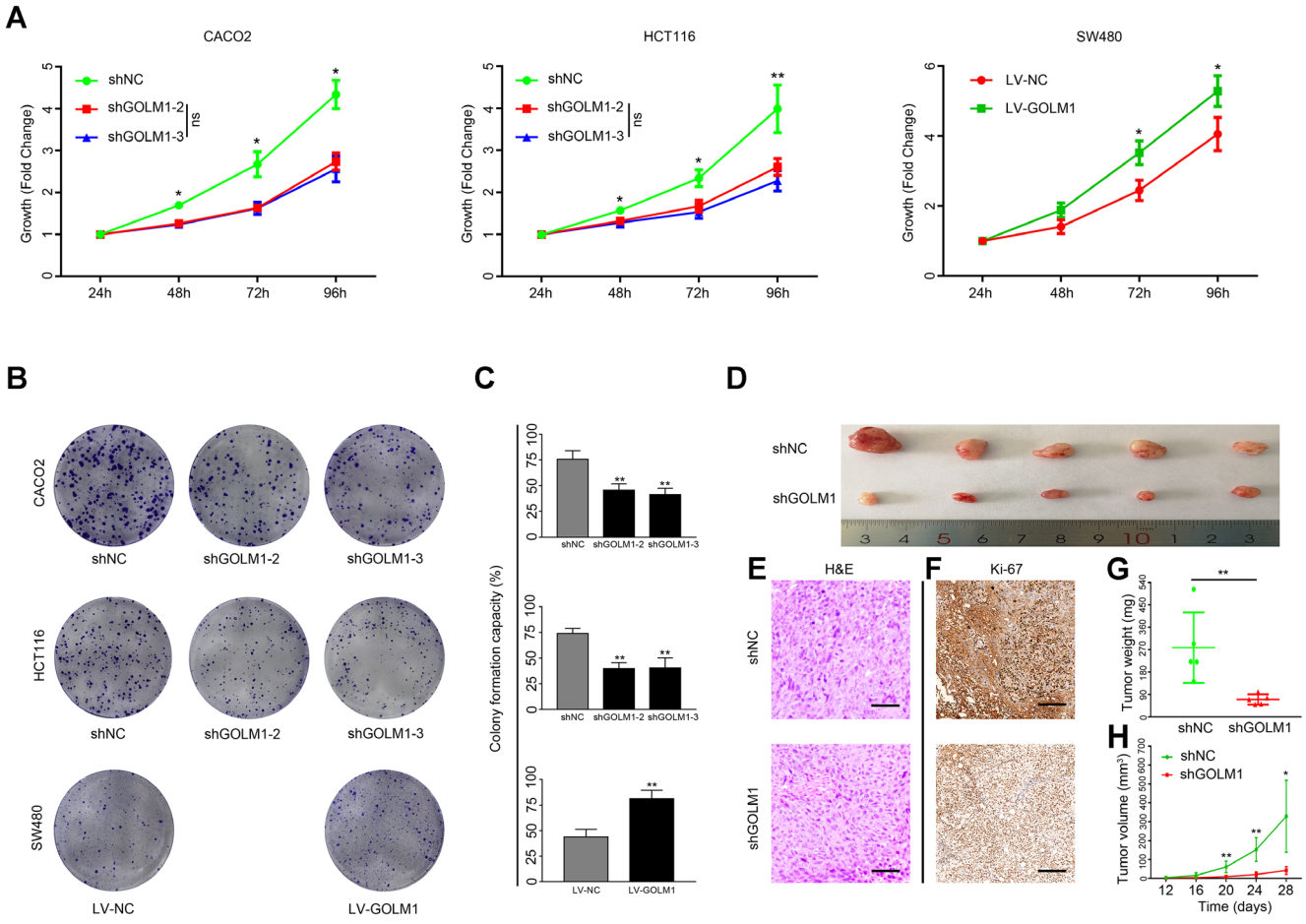

Figure 3. GOLM1 facilitates CRC cell proliferation *in vitro* and *in vivo*. A) CCK-8 and B, C) colony formation analysis of CRC cells with GOLM1-knockdown and -overexpression *in vitro*. D, E) Representative subcutaneous tumor and H&E staining images of HCT116-shNC and HCT116-shGOLM1 (scale bar = 100 μm, 100× magnification). F–H) Knockdown of GOLM1 markedly inhibits the expression of Ki-67, weight, and volume of HCT116 nude mouse xenografts. All data compared to shNC or LV-NC. *p<0.05, **p<0.01

GSK3β pathway-mediated EMT is an important requirement for GOLM1-elicited CRC progression and metastasis.

Discussion

GOLM1, a Golgi-related protein, is highly expressed within various human cancers and promotes tumor progression and metastasis. Existing studies reveal that GOLM1 is highly expressed in CRC tissues, and GOLM1-targeted antibodies remarkably inhibit the progression of CRC-PDX models [22]. Nevertheless, its role and mode of action in CRC are still largely obscure. Thus, in this study, we focused on GOLM1-mediated CRC progression and metastasis and evaluated the possible underlying mechanisms. The results revealed that the expression of GOLM1 was higher in CRC tissues than in non-cancerous intestinal tissues. Further, GOLM1 was positively associated with the TNM stage, vascular invasion, and lymph node metastasis of CRC cases. Subsequently, *in vitro* experiments suggested that GOLM1 knockdown attenuated the growth, invasion, and migration of Caco-2 and HCT116 cells, while its overexpression enhanced the growth, invasion, and migration of SW480 cells. *In vivo* experiments revealed that GOLM1 knockdown inhibited the growth of xenografts and reduced lung metastasis of HCT116 cells. These findings indicate that GOLM1 is a novel biomarker and facilitator of CRC progression and metastasis.

EMT is identified to be a critical precondition for tumor progression and metastasis. Studies have reported that a hybrid state of EMT (co-expression of epithelial and mesenchymal markers) exists in cancer cell lines, PDX models, and cancer tissues [24, 25], and it increases tumor cell migration, invasion, and metastasis in CRC [5, 6]. Consistently, in this study, we observed that GOLM1 knockdown enhanced E-cadherin but downregulated N-cadherin levels in HCT116 cells, whereas GOLM1 overexpression had opposite effects in SW480 cells. Snail1, an important EMT transcriptional factor, is a master regulator of EMT [26]. It is expressed in diverse human cancers and is associated with tumor migration, metastasis, and invasion [27]. Snail1 expression is extremely high in CRC and positively correlates with the latter's migratory and invasive properties [28, 29]. A clinical study reveals that high expression of Snail1 is predictive of a high risk of CRC metastasis [29]. Therefore, we examined the variation of Snail1 expression after GOLM1 silencing or overexpression and observed that GOLM1 silencing downregulated Snail1 expression in HCT116 cells while its overexpression upregulated Snail1 expression in SW480 cells. These findings suggest that GOLM1 evidently impacts the EMT process, thereby favoring metastasis of CRC cells.

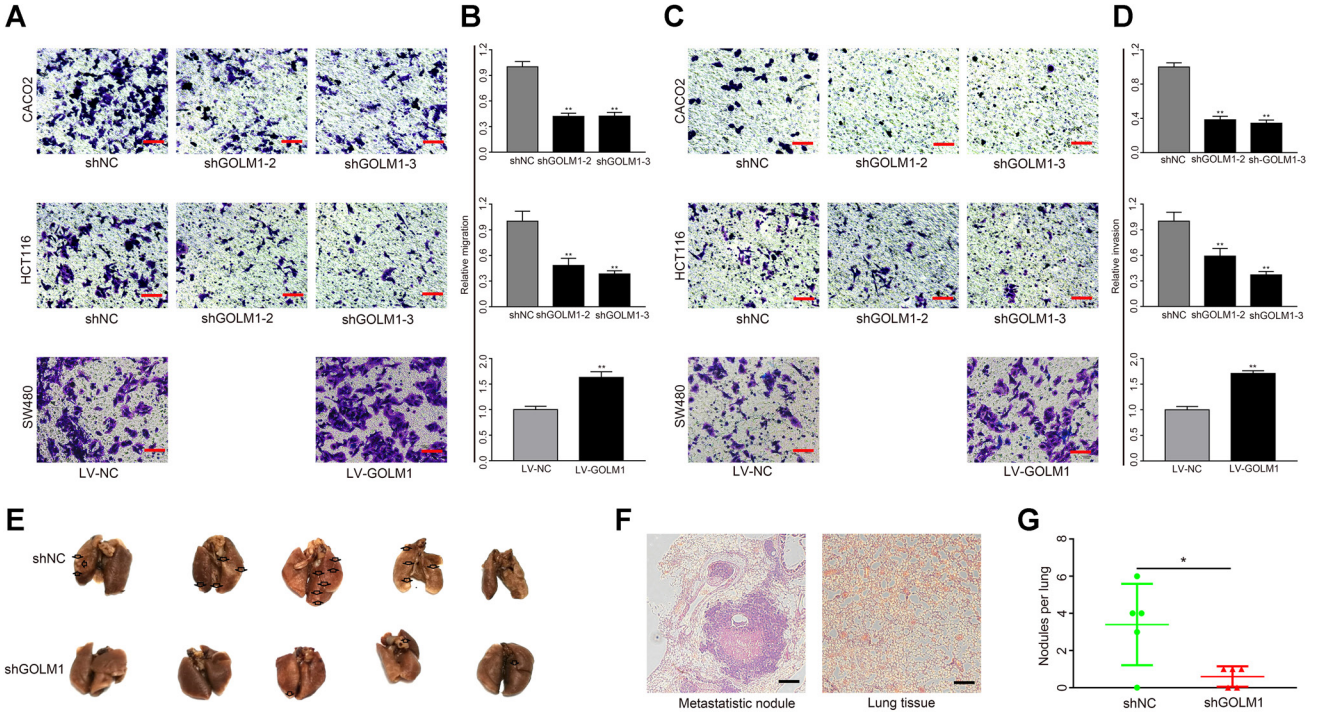

Figure 4. GOLM1 facilitates CRC cell migration, invasion, and lung metastasis. A, B) Representative images and quantification of migrated Caco-2 and HCT116 cells with or without GOLM1-knockdown and SW480 cells with or without GOLM1-overexpression by Transwell assay (scale bar = 100 μm, 200× magnification). C, D) Representative images and quantification of invaded Caco-2 and HCT116 cells with GOLM1-knockdown or not as well as SW480 cells with or without GOLM1-overexpression by Transwell assay (scale bar = 100 μm 200× magnification). E, F) Representative lung metastatic nodules and H&E staining images (scale bar = 100 μm, 100× magnification). G) Knockdown of GOLM1 significantly reduces lung metastatic nodules in HCT116 cells. All data compared to shNC or LV-NC. *p<0.05, **p<0.01

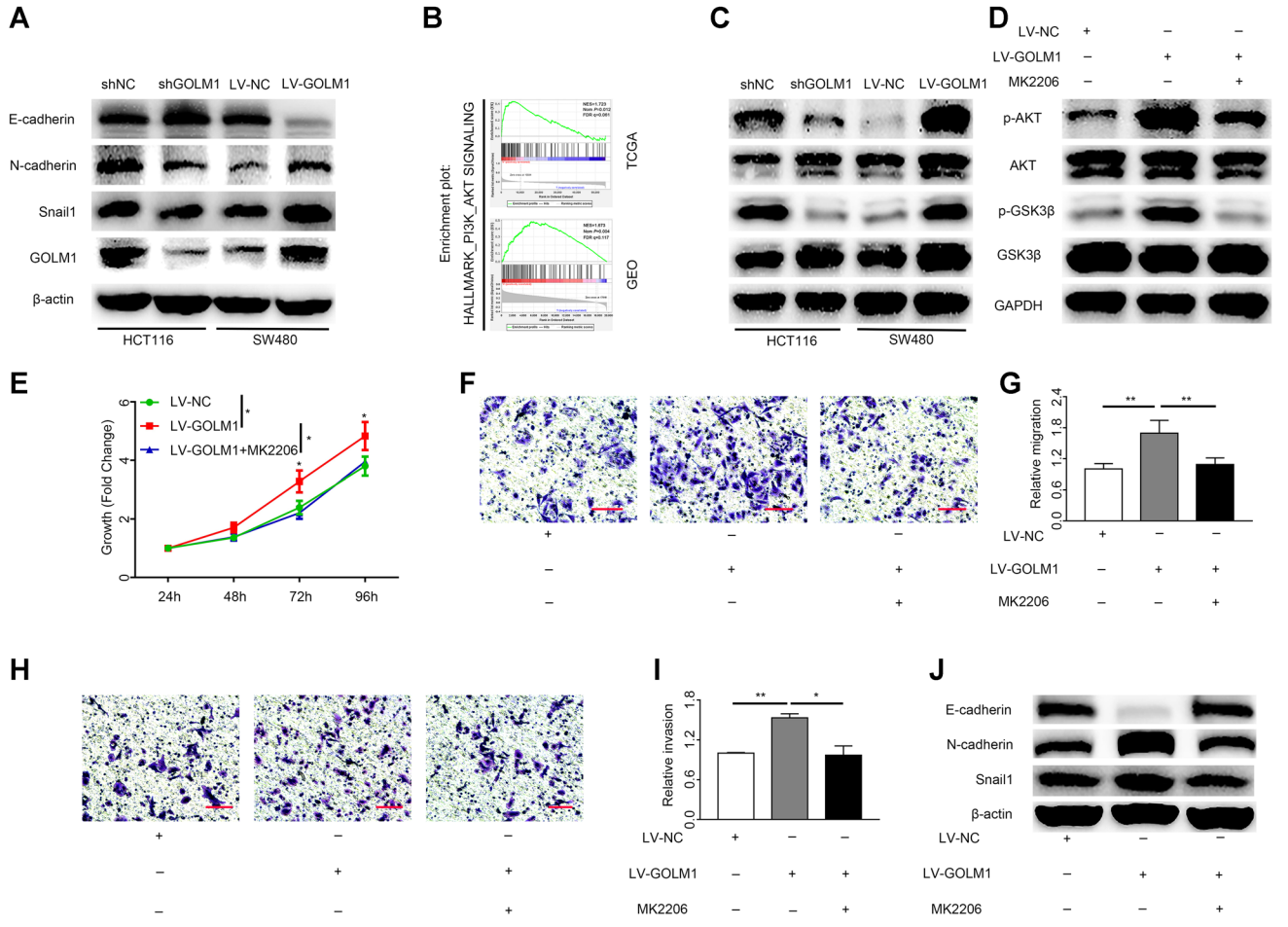

Figure 5. GOLM1 facilitates CRC progression and metastasis by activating the AKT/GSK3β/EMT axis. Western blot analysis of the expression of A) EMT-related proteins E-cadherin, N-cadherin, and EMT transcriptional factor Snail1. B) GSEA analysis and C) AKT/GSK3β pathway-related molecules p-AKT (ser473) and p-GSK3β (ser9) in indicated CRC cells. The variation of D) p-AKT (ser473) and p-GSK3β (ser9), E) proliferation, F, G) migration, H, I) invasion, J) E-cadherin, N-cadherin, and Snail1 in GOLM1-overexpression SW480 cells using AKT inhibitor MK2206 (scale bar = 100 μm, 200× magnification). *p<0.05

On observing that GOLM1 facilitates CRC cell proliferation, migration, invasion, and metastasis by modulating EMT phenotype, elucidating the pivotal signaling mechanisms between GOLM1 and EMT may further facilitate the development of a promising therapeutic option for CRC patients. An earlier study reports that GOLM1 interacts with EGFR/RTKs, thereby activating AKT phosphorylation; thus, leading to the progression and metastasis of hepatocellular cancer [15]. GOLM1 is also found to promote human glioma progression by activating the AKT signaling pathway [19]. Moreover, activation of the AKT pathway can inhibit GSK3β activity by reducing Snail1 degradation and promoting the EMT process [30, 31]. We evaluated changes in the AKT/GSK3β pathway after GOLM1 knockdown and overexpression following our previous results. Knockdown of GOLM1 could repress AKT/GSK3β phosphorylation in HCT116 cells, while its overexpression enhanced AKT/GSK3β phosphorylation in SW480 cells. To verify whether the AKT/GSK3β pathway is upstream of GOLM1-regulated EMT in CRC cells, we used MK2206 to inactivate AKT in GOLM1-overexpressing SW480 cells. We observed that MK2206 substantially reversed GOLM1-elicited proliferation, migration, and invasion, as well as inhibited the AKT/GSK3β pathway, thereby repressing the EMT phenotype. These data provide strong evidence that GOLM1 facilitates CRC progression and metastasis by activating the AKT/GSK3β/EMT axis. In the future study, we will focus on the mechanisms of GOLM1-regulated AKT/GSK3β activity, with the most important direction being Ser/Thr and RTKs (such as EGFR, VEGFR, etc.) following the findings of earlier studies.

Taken together, our research verifies that GOLM1 is highly expressed in CRC tissues and acts as a critical facilitator of CRC progression and metastasis, concretely embodying CRC proliferation, migration, invasion, metastasis, and EMT phenotype. Furthermore, an investigation of underlying mechanisms reveals that the AKT/GSK3β/EMT axis plays

a crucial role in GOLM1-regulated CRC progression and metastasis. GOLM1 may become a novel metastatic marker and a treatment target; besides, using inhibitors targeting GOLM1 or a combination of GOLM1 and AKT inhibitors are two promising options to control CRC progression and metastasis.

Acknowledgments: This work was supported by The Key Medical Project of Jiangsu Commission of Health (ZD2021045), the Health and Family Planning Commission Fund Project of Jiangsu Province (No CXTDA2017016), Open Projects of State Key Laboratory of Radiation Medicine and Protection (No GZK1202010), Postgraduate Research & Practice Innovation Program of Jiangsu Province (No KYCX20_2679).

References

[1] BRAY F, FERLAY J, SOERJOMATARAM I, SIEGEL RL, TORRE LA et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394–424. https://doi.org/10.3322/caac.21492

[2] LEE JJ, SUN W. Options for Second-Line Treatment in Metastatic Colorectal Cancer. Clin Adv Hematol Oncol 2016; 14: 46–54.

[3] PASTUSHENKO I, BLANPAIN C. EMT Transition States during Tumor Progression and Metastasis. Trends Cell Biol 2019; 29: 212–226. https://doi.org/10.1016/j.tcb.2018.12.001

[4] MI L, ZHU F, YANG X, LU J, ZHENG Y et al. The metastatic suppressor NDRG1 inhibits EMT, migration and invasion through interaction and promotion of caveolin-1 ubiquitylation in human colorectal cancer cells. Oncogene 2017; 36: 4323–4335. https://doi.org/10.1038/onc.2017.74

[5] GU Y, WANG Q, GUO K, QIN W, LIAO W et al. TUSC3 promotes colorectal cancer progression and epithelial-mesenchymal transition (EMT) through WNT/β-catenin and MAPK signalling. J Pathol 2016; 239: 60–71. https://doi.org/10.1002/path.4697

[6] SUN J, ZHANG T, CHENG M, HONG L, ZHANG C et al. TRIM29 facilitates the epithelial-to-mesenchymal transition and the progression of colorectal cancer via the activation of the Wnt/β-catenin signaling pathway. J Exp Clin Cancer Res 2019; 38: 104. https://doi.org/10.1186/s13046-019-1098-y

[7] ZAVADIL J, BÖTTINGER EP. TGF-beta and epithelial-to-mesenchymal transitions. Oncogene 2005; 24: 5764–5774. https://doi.org/10.1038/sj.onc.1208927

[8] SPADERNA S, SCHMALHOFER O, HLUBEK F, BERX G, EGER A et al. A transient, EMT-linked loss of basement membranes indicates metastasis and poor survival in colorectal cancer. Gastroenterology 2006; 131: 830–840. https://doi.org/10.1053/j.gastro.2006.06.016

[9] KALLURI R, WEINBERG RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009; 119: 1420–1428. https://doi.org/10.1172/jci39104

[10] ZHOU BP, DENG J, XIA W, XU J, LI YM et al. Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol 2004; 6: 931–940. https://doi.org/10.1038/ncb1173

[11] KLADNEY RD, BULLA GA, GUO L, MASON AL, TOLLEFSON AE et al. GP73, a novel Golgi-localized protein upregulated by viral infection. Gene 2000; 249: 53–65. https://doi.org/10.1016/s0378-1119(00)00136-0

[12] LIU Y, HU X, LIU S, ZHOU S, CHEN Z et al. Golgi Phosphoprotein 73: The Driver of Epithelial-Mesenchymal Transition in Cancer. Front Oncol 2021; 11: 783860. https://doi.org/10.3389/fonc.2021.783860

[13] WRIGHT LM, YONG S, PICKEN MM, ROCKEY D, FIMMEL CJ. Decreased survival and hepato-renal pathology in mice with C-terminally truncated GP73 (GOLPH2). Int J Clin Exp Pathol 2009; 2: 34–47.

[14] LI L, WEN L, GONG Y, MEI G, LIU J et al. Xenopus as a model system for the study of GOLPH2/GP73 function: Xenopus GOLPH2 is required for pronephros development. PLoS One 2012; 7: e38939. https://doi.org/10.1371/journal.pone.0038939

[15] Ye QH, Zhu WW, Zhang JB, Qin Y, Lu M et al. GOLM1 Modulates EGFR/RTK Cell-Surface Recycling to Drive Hepatocellular Carcinoma Metastasis. Cancer Cell 2016; 30: 444–458. https://doi.org/10.1016/j.ccell.2016.07.017

[16] ARUNA, LI LM. Overexpression of golgi membrane protein 1 promotes non-small-cell carcinoma aggressiveness by regulating the matrix metallopeptidase 13. Am J Cancer Res 2018; 8: 551–565.

[17] YAN G, RU Y, WU K, YAN F, WANG Q et al. GOLM1 promotes prostate cancer progression through activating PI3K-AKT-mTOR signaling. Prostate 2018; 78: 166–177. https://doi.org/10.1002/pros.23461

[18 SONG YX, XU ZC, LI HL, YANG PL, DU JK et al. Overexpression of GP73 promotes cell invasion, migration and metastasis by inducing epithelial-mesenchymal transition in pancreatic cancer. Pancreatology 2018; 18: 812–821. https://doi.org/10.1016/j.pan.2018.08.009

[19] XU R, JI J, ZHANG X, HAN M, ZHANG C et al. PDGFA/PDGFRα-regulated GOLM1 promotes human glioma progression through activation of AKT. J Exp Clin Cancer Res 2017; 36: 193. https://doi.org/10.1186/s13046-017-0665-3

[20] PU Y, SONG Y, ZHANG M, LONG C, LI J et al. GOLM1 restricts colitis and colon tumorigenesis by ensuring Notch signaling equilibrium in intestinal homeostasis. Signal Transduct Target Ther 2021; 6: 148. https://doi.org/10.1038/s41392-021-00535-1

[21] DANG Y, YU J, ZHAO S, JIN L, CAO X et al. GOLM1 Drives Colorectal Cancer Metastasis by Regulating Myeloid-derived Suppressor Cells. J Cancer 2021; 12: 7158–7166. https://doi.org/10.7150/jca.61567

[22] LIEWEN H, MARKULY N, LÄUBLI H, LIU Y, MATTER MS et al. Therapeutic Targeting of Golgi Phosphoprotein 2 (GOLPH2) with Armed Antibodies: A Preclinical Study of Anti-GOLPH2 Antibody Drug Conjugates in Lung and Colorectal Cancer Models of Patient Derived Xenografts (PDX). Target Oncol 2019; 14: 577–590. https://doi.org/10.1007/s11523-019-00667-z

[23] TANG Z, LI C, KANG B, GAO G, LI C et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98–W102. https://doi.org/10.1093/nar/gkx247

[24] PASTUSHENKO I, BRISEBARRE A, SIFRIMC A, FIORAMONTI M, REVENCO T et al. Identification of the tumour transition states occurring during EMT. Nature 2018; 556: 463–468. https://doi.org/10.1038/s41586-018-0040-3

[25] GEORGE JT, JOLLY MK, XU S, SOMARELLI JA, LEVINE H. Survival Outcomes in Cancer Patients Predicted by a Partial EMT Gene Expression Scoring Metric. Cancer Res 2017; 77: 6415–6428. https://doi.org/10.1158/0008-5472.can-16-3521

[26] KAUFHOLD S, BONAVIDA B. Central role of Snail1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. J Exp Clin Cancer Res 2014; 33: 62. https://doi.org/10.1186/s13046-014-0062-0

[27] BARRALLO-GIMENO A, NIETO MA. The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development 2005; 132: 3151–3161. https://doi.org/10.1242/dev.01907

[28] ROY HK, SMYRK TC, KOETSIER J, VICTOR TA, WALI RK. The transcriptional repressor SNAIL is overexpressed in human colon cancer. Dig Dis Sci 2005; 50: 42–46. https://doi.org/10.1007/s10620-005-1275-z

[29] FAN F, SAMUEL S, EVANS KW, LU J, XIA L et al. Overexpression of snail induces epithelial-mesenchymal transition and a cancer stem cell-like phenotype in human colorectal cancer cells. Cancer Med 2012; 1: 5–16. https://doi.org/10.1002/cam4.4

[30] ZHANG J, LIN X, WU L, HUANG JJ, JIANG WQ et al. Aurora B induces epithelial-mesenchymal transition by stabilizing Snail1 to promote basal-like breast cancer metastasis. Oncogene 2020; 39: 2550–2567. https://doi.org/10.1038/s41388-020-1165-z

[31] LI P, CAO G, ZHANG Y, SHI J, CAI K et al. FHL3 promotes pancreatic cancer invasion and metastasis through preventing the ubiquitination degradation of EMT associated transcription factors. Aging (Albany NY) 2020; 12: 53–69. https://doi.org/10.18632/aging.102564