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Downregulation of desmoglein 2 promotes EMT progression in gallbladder cancer

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Summary. Objective. To explore the correlation between the expression level of Desmoglein 2 (DSG2) and the epithelial-mesenchymal transition (EMT) progression in gallbladder cancer (GBC).

Method. 106 GBC tissue specimens and corresponding clinical information were collected to make a tissue microarray. Immunohistochemical method was used to test the expression level of DSG2 in GBC tissues. DSG2 was knocked down in the GBC cell line GBC-SD to detect the change of its invasion and metastasis ability. Then RT-qPCR and Western Blot were applied on the DSG2-knocked down GBC-SD cells to detect the expression level change of genes associated with EMT.

Result. The high expression rate of DSG2 was significantly correlated with the N, M and TNM staging of patients ($P<0.05$). Survival analysis identified that GBC patients with high DSG2 expression level had significantly better survival ($P<0.05$). To further investigate the potential mechanism of DSG2 on regulating GBC tumor progression, we used knockdown DSG2 on GBC-SD cell lines. The results showed that GBC-SD cell lines with DSG2 knockdown showed a promotion of cell invasion and metastatic ability. The mRNA levels of EMT-related genes E-Cadherin, Snail, Twist, ZEB1, and β-catenin, which is a key protein in the Wnt signaling pathway, were also significantly altered. Besides, protein levels of E-cadherin and Snail showed consistent results.

Conclusion. The downregulation of DSG2 in gallbladder cancer is hypothesized to be associated with the invasion and metastasis progression of gallbladder cancer cells by regulating EMT-related pathways. Its expression level can be a novel biomarker for gallbladder cancer, providing new perspectives for diagnosis and treatment strategies.

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Introduction

Gallbladder cancer (GBC) has a low incidence across the world, and is a kind of relatively rare but highly lethal malignant tumor (Lv et al., 2019). GBC is recognized to be the most common malignancy in the biliary tract, for which surgical resection is the only effective treatment method (Zhu and Luo, 2019). However, due to lack of specific clinical symptoms and sufficient indication of surgical operations, patients in early GBC stage cannot be treated correctly, more than 85% of GBC patients were in advanced stage when they were clearly diagnosed. High recurrence rate and poor prognosis were discovered even after complete surgical removal of the tumor was conducted (Hundal and Shaffer, 2014). Therefore, exploring and identifying early markers of GBC and screening patients for these markers may be a potential approach for clinical diagnosis and prevention of gallbladder cancer.

Intercellular adhesive junctions such as desmosomes, are essential for maintaining epithelial homeostasis and integrity (Takeichi, 2014), and their functional dysregulation is tightly correlated with tumor progression. Previous research reported that the absence of desmosome adhesion is one of the induction conditions for the occurrence and promotion of epithelial-mesenchymal transition (EMT) (Overmiller et al., 2016; Chang et al., 2021). Dysregulated expression of the component proteins in desmosomes can disrupt the intercellular adhesive junction structure, resulting in a series of changes intracellularly and intercellularly, thus engaging in tumorigenesis and tumor promotion. Desmoglein 2 (DSG2) is a cell adhesion protein belonging to the cadherin superfamily, and is a major component of intercellular desmosomes playing essential roles in cell adhesion and proper function (Fuchs et al., 2022).

Previous studies have indicated the potential roles of

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DSG2 in various cancers (Hütz et al., 2017). Anaplastic thyroid cancer (ATC) patients with low DSG2 expression levels presented with distant metastasis and Dsg2 depletion significantly increased cell migration, invasion and motility (Lee et al., 2020). Loss of DSG2 promotes gallbladder carcinoma progression and resistance to EGFR-targeted therapy through Src kinase activation (Lee et al., 2021). Low expression level of DSG2 was recognized as an independent prognostic factor in aggressive prostate cancer (Barber et al., 2014). DSG2 expression is also an independent predictor of poor prognosis patients with multiple myeloma (Ebert et al., 2022). Additionally, low DSG2 expression is associated with differentiation, lymph node metastasis, distant metastasis and AJCC stage for colon cancer patients (Yang et al., 2021). These studies have shown that DSG2 affects the functions of a number of cancer cells, indicating a regulatory role of DSG2 in the early development and prognosis of cancers. However, the condition and mechanism of DSG2 in GBC remain elusive. Therefore, it has certain clinical significance to study whether DSG2 may be a biomarker for the early diagnosis of GBC.

This study aims to investigate the expression of DGS2 in gallbladder cancer tissues and paracancerous tissues, analyze the relationship between DSG2 expression and clinical characteristics of patients, as well as survival rates. Besides, we explored the relationship between DSG2 expression levels and EMT pathway-related genes, to assess the clinical significance of DSG2 in gallbladder cancer tissues. Thus, our study tries to explain how DSG2 can influence the occurrence and development of cancer by regulating the process of EMT.

Materials and methods

Sample collection and cinical data acquisition

106 gallbladder cancer patients whose tumor tissues were assembled on tissue arrays after surgical removal between January 2007 to December 2012 was purchased from Shanghai Outdo Biotech Co., Ltd. Consent was acquired from all the patients and this study was approved by the Human Ethics committee and the Research Ethics committees of Affiliated Hospital of Yanbian University in China (No: 2015044). Among the 106 patients, 33 were male and 73 were female; 56 were ≤65 years old and 50 were >65 years old. For pathological grading, 5 were diagnosed as grade 1 patients, 39 were grade 2 patients, 62 were grade 3 patients. All tumor pathology grading is based on 'Malignant epithelial tumors' section of 2020 WHO gallbladder with extrahepatic bile duct tumor histological classification. For TNM stage, 12 were stage I patients, 27 were stage II, 34 were stage III, 27 were stage IV, and 6 were unspecified stage patients. All clinical staging was performed according to the gallbladder cancer section of AJCC 7th edition. The criteria for the patients in this study were: 1) Ages were between 45 to 75 years old; 2) They were first diagnosis; 3) All were diagnosed as GBC; 4) All were with normal liver and kidney function. Patients excluded in this study were: 1) received radiotherapy; 2) diagnosed with other kinds of tumors; 3) diagnosed with severe cardio, hepatic and renal basal disease; 4) patients who refused to be engaged in this study. The lesioned tumor tissues and the normal paracancerous tissues were collected freshly within 1h after cholecystectomy. All patients involved in this study were followed up via re-examination or through telephone after 80 months, the patients' overall survival time was defined from the timepoint at the end of the initial treatment to the timepoint of death due to the disease or the follow-up cut-off date. All patients received chemotherapy or radiotherapy after surgery.

Immunohistochemistry

The collected tissues were fixed using formalin and embedded in paraffin to manufacture the tissue paraffin wax. For the paraffin wax for each tissue sample, sections were cut with a microtome and stained with hematoxylin-eosin (HE), then representative tumor regions were selected and marked on the wax by an experienced pathologist based on the staining results. Next, the selected tissue region was punched out from the original wax and arranged on an empty white wax block according to the design using a tissue microarray spotter to finally make a tissue microarray wax, which was then serially sectioned using a microtome to prepare tissue microarray (TMA) slides. Afterwards the TMA was firstly baked under 63°C in an oven for 1h and deparaffinized in xylene, then rehydrated in concentration-graded ethanol solutions. After antigen retrieval, slides were washed in PBST buffer and incubated under 4°C overnight with the primary antibody for DSG2 which had been diluted in working concentration. Next the slides were incubated with the secondary antibody for 1h the following day. Chromogenic agent was applied after washing, and slides were counterstained with hematoxylin for 1min. Finally, the slide was dried in room temperature and sealed with resin. The stained TMA sections were placed under a 200X light microscope for observation and photography to confirm the DSG2-positive expression of cells. The staining results of sections were determined by staining intensity score: score 0 indicated "negative", score 1 referred to "pale yellow", score 2 meant "yellow" and score 3 was "brown". The staining positivity score of sections was based on the interpretation of positive staining rates: 0 referred to "negative"; 1 indicated the rate was below 70%, 2 meant the rate was between 71% and 80%, 3 indicated that the rate was from 81% to 90%, and 4 showed the rate was 91% to 100%. The sum of "staining intensity score" and "staining positivity score" was performed for the grouping. Samples with the sum of scores less than or

equal to 3 were classified as "low DSG2 expression", while samples with total score greater than 3 were classified as "high DSG2 expression".

DSG2 knockdown experiment

The LV-DSG2-RNAi lentiviruses and corresponding nonspecific control lentiviruses were constructed by cloning into the vector in accordance with the manufacturer's protocol. Next, the LV-DSG2-RNAi lentiviruses or control lentiviruses were applied to infect the GBC-SD cells at the multiplicity of infection (MOI) of 5 with 5 μg/ml polybrene when they reached 20% confluency. Cells with stable transfection were next cultured with fresh conventional medium then selected and harvested for RT-qPCR to validate the DSG2 knockdown efficiency.

RT-qPCR

Total RNA was extracted from gallbladder cancer cell line GBC-SD with TRIzol reagent according to the standard protocol of the manufacturer. SYBR Green PCR kit was applied for quantitative real-time PCR (RT-qPCR), which was performed on cDNA reverse-transcribed from cell RNAs. The primer list for RT-qPCR is shown in Table 1. The resulting data were analyzed and normalized using GAPDH as internal control.

Transwell Assays

The GBC-SD cell invasion ability change after DSG2 knockdown was measured with Matrigel-coated transwell chambers. Cells in si-DGS2 group and si-NC group were seeded onto the upper chambers with serum-free medium, whereas the lower chambers were supplemented with fresh medium with 10% serum. The invading cells that could secret extracellular proteases to degrade the Matrigel then invaded into the lower chambers were detected with 0.1% crystal violet and recorded using a 100x light microscope after 24h incubation.

In addition, the migration ability changes of GBC-SD cells upon DSG2 knockdown were detected also with the transwell chambers in which the upper and lower chambers were separated by polycarbonate membranes. Cells in si-DGS2 group and si-NC group were cultured in the upper chambers with serum-free medium, while the lower chambers were filled with serum-contained medium. Migrated cells were stained using Giemsa dye and recorded with a 100X light microscope for cell number counting.

Western blot

Cells were collected by centrifugation for protein extraction, and the protein concentration was measured using BCA Protein Assay Kit. Proteins were separated with SDS-PAGE gel electrophoresis and transferred onto PVDF membranes, then blocked in TBST buffer for 1h. The membrane was next incubated with specific antibodies overnight at 4°C. The working concentration for specific antibodies were as following: DSG2 (1:500), E-Cadherin (1:500), Snail (1:400), and GAPDH (1:2000). Next the membrane was washed on the second day and then incubated with the secondary antibody (Mouse, 1:2000) or (Rabbit, 1:2000) at 25°C for 1.5 hours. The protein band images were observed and captured with the Pierce™ ECL Western Blotting Substrate imaging system.

Statistical analysis

The software SPSS 22.0 was used for statistical analysis of the experimental data. Differences in the DSG2 staining results from gallbladder cancer tissues and paracancerous tissues were tested with paired t-Test, and χ^2 test was used for the comparison between groups. Correlation analyses of staining intensity, as well as

Table 1. Sequences of primers in this study.

Gene	Upstream Primer Sequences	Downstream Primer Sequences	Length of replicons (bp)
GAPDH	TGACTTCAACAGCGACACCCA	CACCCTGTTGCTGTAGCCAAA	121
ADA	GCGAGACTTCGGGGTCAAGG	GTACGGTGAATGCCGCTCTT	213
SNAI1	CCCCAATCGGAAGCCTAA	CCTTTCCCACTGTCCTCAT	261
CDH1	AACGCATTGCCACATACA	CGGGCTTGTTGTCATTC	114
PTEN	TGGATTCGACTTAGACTTGACCT	TTTGGCGGTGTCATAATGTCTT	184
VIM	AATGGCTCGTCACCTTCG	CTAGTTTCAACCGTCTTAATCAG	225
SNAI2	CAAGGACACATTAGAACTCACAC	CTACACAGCAGCCAGATTCC	199
JUP	TCGCCATCTTCAAGTCGGG	AGGGGCACCATCTTTTGCAG	169
NFKB1	AGGATTTCGTTTCCGTTATGT	CCTGAGGGTAAGACTTCTTGTTC	92
ZEB1	CAGGCAGATGAAGCAGGATG	CAGCAGTGTCTTGTTGTTGTAG	115
CTNNB1	GGTCTCCTTGGGACTCTTGT	CAGGCTCAGTGATGTCTTCC	193
TWIST1	GTCCGCAGTCTTACGAGGAG	GCTTGAGGGTCTGAATCTTGCT	156
ST14	GCCTCCCCTGTCTCTAAGGA	ACACACACTGAAGTCCACCC	197
PIK3CA	TGAAGCACCTGAATAGGCAAGTCG	AGAAAGCCCTGTAGAGCATCCATG	160
STAT1	GGCACCAGAACGAATGAGG	CCACAACGGGCAGAGAGG	124

clinical data from gallbladder cancer patients were performed utilizing the Spearman correlation method. Univariate analysis of patient survival was conducted using Kaplan-Meier survival analysis and log-rank statistical test, variables that were statistically significant in the univariate analysis were then included in the Cox multi-factor survival regression analysis. P value<0.05 was considered statistically significant.

Results

DSG2 expression showed slight differences in gallbladder cancer and paracancerous tissues

It has been reported that DSG2 in different cancer types has different roles in cancer progression and its functional mechanisms (Lee et al., 2021). In our study, Immunohistochemical results indicated that DSG2 was strongly stained on the cell membrane and cytoplasm in the paracancerous tissues (Fig. 1A). By comparison, DSG2 staining showed differences among GBC tissues, with some tissues strongly stained while others weakly stained (Fig. 1B,C). Among the patients diagnosed with GBC and who provided clear clinical information, the rate of high DSG2 expression in tumor tissues was 39.6% (42/106), slightly higher than the 35% (7/20) in normal paracancerous tissues adjacent to the tumor. For the 20 paired GBC tumor tissues and corresponding paracancerous tissues collected, 7 tumor tissues showed high DSG2 expression while 13 were low expressed, and there were also 7 paracancerous tissues defined with high DSG2 expression while 13 indicated low, which revealed that the differences were not statistically significant (Chi-square Value=0, P=1). Therefore, the results suggest that DSG2 expression cannot be used as a significant differentiating factor between cancer and paracancerous tissues.

DSG2 expression level correlates with clinical characteristics of patients diagnosed with gallbladder cancer

To determine whether the differences in DSG2 expression in gallbladder carcinoma tumor tissues were associated with clinical features, we analyzed the clinical information of all GBC patients. The results showed that there were significantly correlations between high DSG2 expression and N stage, M stage, and TNM stage, respectively (P<0.05) (Table 2). However, no

Table 2. Expression of DSG2 in gallbladder cancer tissues of patients with different clinical characteristics.

variables	DSG2 expression		total	rs
	high	low		
Sex				-0.003
Female	29	44	73	
Male	13	20	33	
Age(year)				-0.070
≤65	24	32	56	
>65	18	32	50	
Grade				0.121
1	0	5	5	
2	15	24	39	
3	27	35	62	
T stage				-0.102
T1	7	5	12	
T2	14	21	35	
T3	16	32	48	
T4	2	1	3	
null			8	
N stage				-0.314
N0	35	37	72	
N1	1	9	10	
N2	2	12	14	
Null			10	
M stage				-0.259
M0	41	51	92	
M1	1	13	14	
TNM stage				-0.276
I	7	5	12	
II	13	14	27	
III	14	20	34	
IV	5	22	27	
null			6	

rs: To explain the closeness and correlation direction of linear correlation between variables. The range of rs value is between -1 and 1. rs value is positive/negative means positive/negative correlation, rs value is zero means no correlation. |rs|=0.8-1.0, means an extremely strong correlation; |rs|=0.6-0.8, means a strong correlation; |rs|=0.4-0.6, means a moderate correlation; |rs|=0.2-0.4, means a weak correlation; |rs|=0.0-0.2, means a very weak or no correlation.

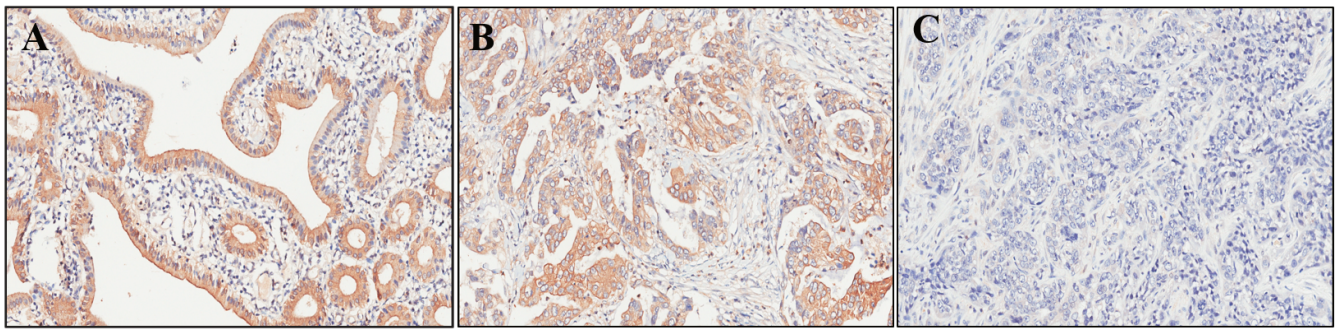

Fig. 1. Immunohistochemical staining of DSG2 in gallbladder cancer tissue and normal paracancerous tissues. **A.** Staining of normal gallbladder tissue. **B.** Strongly stained GBC tissue. **C.** Weakly stained GBC tissue. x 200.

statistically significant differences with other clinical features, including gender, age, histological grade, and the tumor T stage (P>0.05).

DSG2 can affect overall survival of gallbladder cancer patients

Univariate analysis showed no significant difference in the comparison of overall survival time among GBC patients with different gender, histological grade, and age, which indicated no statistical significance (P>0.05). In contrast, the comparison of overall survival time among GBC patients with different DSG2 expression levels, T-stage, N-stage, M-stage, and TNM-stage were all significantly different and showed statistical significance (P<0.05). Multivariate analysis revealed that DSG2 could not be regarded as an independent prognostic factor for gallbladder cancer (P>0.05) (Table 3).

Gallbladder cancer patients with hgh DSG2 expression showed significantly longer survival tme than those with low expression

The Kaplan-Meier survival analysis was applied to examine the correlation between DSG2 expression levels and patients' postoperative survival. GBC patients with high DSG2 expression level had a median postoperative survival time of 48.013 months, while patients with low DSG2 expression showed a median postoperative survival time of 30.793 months. Based on Log Rank (Mantel-Cox) test, GBC patients with DSG2 high expression level survived significantly longer than those with low expression level, and the difference was statistically significant (P<0.01) (Fig. 2).

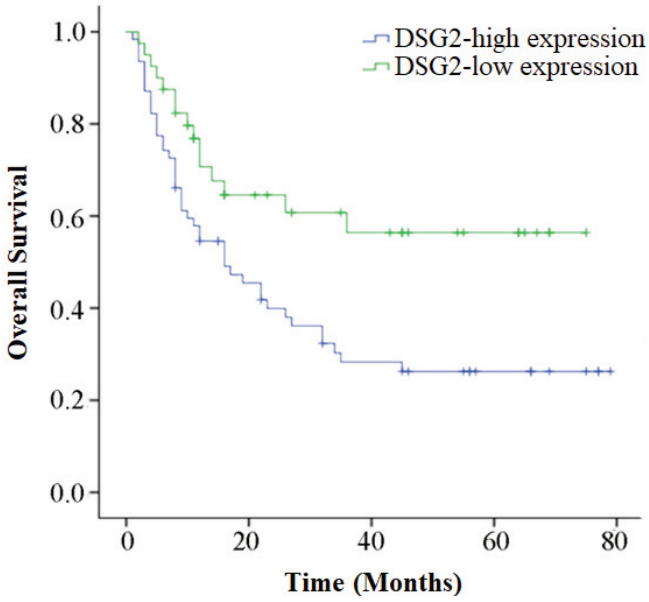

Fig. 2. Survival curves for GBC patients with high DSG2 expression (n=40) versus GBC patients with low DSG2 expression (n=62).

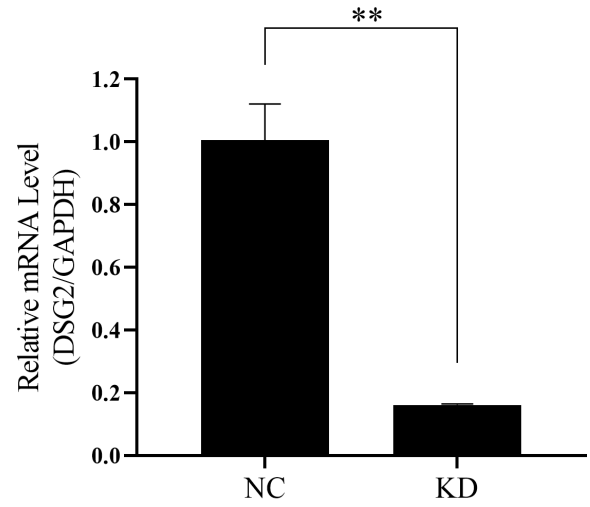

Fig. 3. RT-qPCR results show the knockdown efficiency of DSG2 in GBC-SD cells. Cells were treated with siRNA si-DSG2 for 72h. DSG2 mRNA level was significantly decreased upon the siRNA si-DSG2 treatment. **P<0.01. NC, negative control; KD, knockdown.

Table 3. Analysis of factors affecting the overall survival time for patients diagnosed with gallbladder cancer.

variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	p value	HR	95%CI	p value
expression	0.467	0.259-0.841	0.011	0.636	0.334-1.211	0.168
sex	0.776	0.431-1.398	0.399			
Grade	1.344	0.841-2.148	0.217			
Age	0.761	0.451-1.285	0.307			
T stage	2.004	1.419-2.832	0.000*	0.534	0.074-3.836	0.533
N stage	1.636	1.218-2.197	0.001*	0.655	0.225-1.911	0.439
M stage	2.546	1.254-5.169	0.010*	0.500	0.064-3.910	0.509
TNM stage	2.018	1.515-2.686	0.000*	3.933	0.523-29.549	0.183

Knockdown of DSG2 protein in gallbladder cancer cell line GBC-SD promotes cell invasion and metastasis

The siRNA si-DSG2 group used for DSG2 knockdown, 54396-11, 54395-2, 54397-2, as well as the negative control for transfection (si-NC), were transfected into the gallbladder cancer cell line GBC-SD, respectively, thereby knocking down the expression level of DSG2 protein. The RT-qPCR results for the RNAs extracted from the cells showed that the

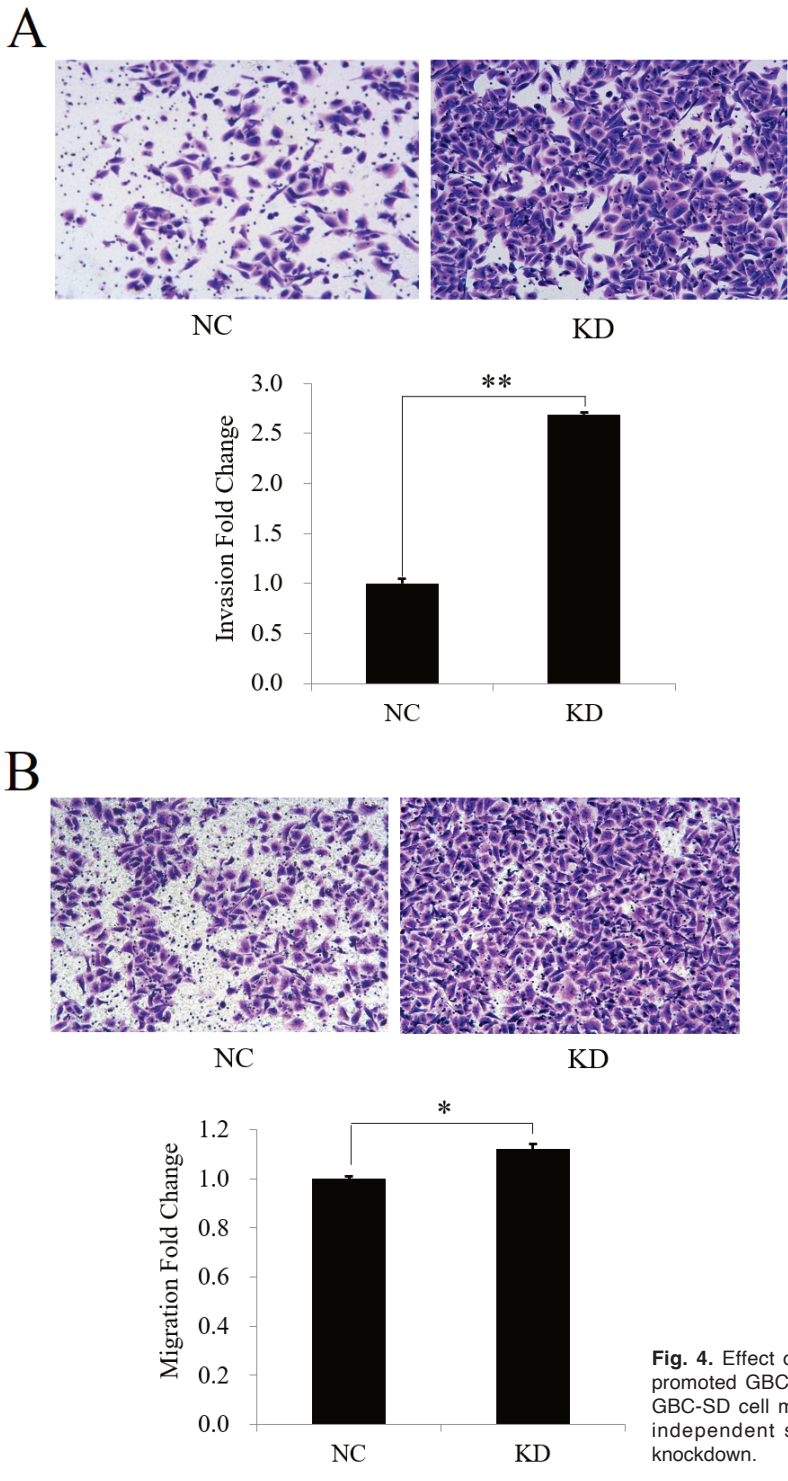

Fig. 4. Effect of DSG2 knockdown in GBC-SD cells. A) DSG2 knockdown promoted GBC-SD cell invasion ability; B) DSG2 knockdown increased the GBC-SD cell metastasis ability. Experimental results were derived from ≥3 independent samples. **P<0.01, *P<0.05. NC, negative control; KD, knockdown.

expression level of DSG2 was significantly decreased in each of the three si-DGS2 groups, all with knockdown efficiency over 80%, and si-54396-11 was chosen as the representative with knockdown efficiency at 83.9%, while the DSG2 mRNA level change was not significantly different between the si-NC group and the normal culture control (Fig. 3).

The abilities of GBC-SD cell invasion and migration after DSG2 knockdown were measured by transwell and wound healing assays, respectively. Compared with the normal culture control group, there was no significant change in the GBC-SD cell invading and metastatic abilities for the si-NC group. However, GBC-SD cells in the si-DGS2 group showed a promotion of cell invasion and metastatic capabilities, i.e., the number of invaded and migrated cell was significantly increased (Fig. 4).

Knockdown of DSG2 regulates the EMT-associated gene expression level in GBC-SD cells

RT-qPCR results for the six EMT-associated genes showed that compared with the si-NC group, the intracellular mRNA expression level of Slug and Vimentin in the si-DSG2 group were not statistically significantly changed, however, the level of E-Cadherin was significantly downregulated, while the expression of Snail, Twist, and ZEB1 were significantly upregulated; meanwhile, the RNA level of β-catenin was also significantly upregulated, which differences were statistically significant. Western Blot results showed that the protein level of E-Cadherin was nearly three-fold lower in the si-DSG2 group compared to the si-NC group. In contrast, the protein level of Snail was nearly three-fold higher. All the differences were consistently statistically significant, indicating DSG2 played a role in regulating EMT progression (Fig. 5).

Discussion

The pathogenesis of gallbladder cancer is relatively insidious, and there are no obvious clinical symptoms for the early stage, which usually makes this disease hard notice and be detected in time, thus delaying treatment (Branch of Biliary Surgery, Chinese Surgical Society and Chinese Committee of Biliary Surgeons, 2020). Consequently, identifying the dysregulated expressed genes and their regulatory roles in gallbladder cancer cells are essential for the study of molecular mechanisms associated with tumor progression. Among the desmogleins family, DSG2 has been reported to be abnormally expressed in a variety of tumor tissues, while the role of DSG2 in gallbladder cancer remains elusive. In this study, we tested the expression level of DSG2 in pathological tissues of patients diagnosed with GBC through immunohistochemistry, and found that the tumor cell membrane and cytoplasm showed evident yellow or brown positive staining for DSG2, while paracancerous tissues were strongly stained. There were no statistically significant differences in the high expression of DSG2 in gallbladder cancer tissues compared to normal paracancerous tissues adjacent. Statistical analysis indicated that DSG2 could not be used as an independent prognostic factor for GBC, but its high expression rate was significantly correlated with the N stage, M stage, and TNM stage of the tumor, and the overall survival time of GBC patients with high DSG2 expression level was significantly longer. These results suggest that the decrease of DSG2 expression level may be involved in gallbladder cancer promotion and progression.

Currently, there are few studies targeting DSG2 in GBC. Lee et al. (2021) reported that loss of DSG2 promotes gallbladder cancer progression and resistance

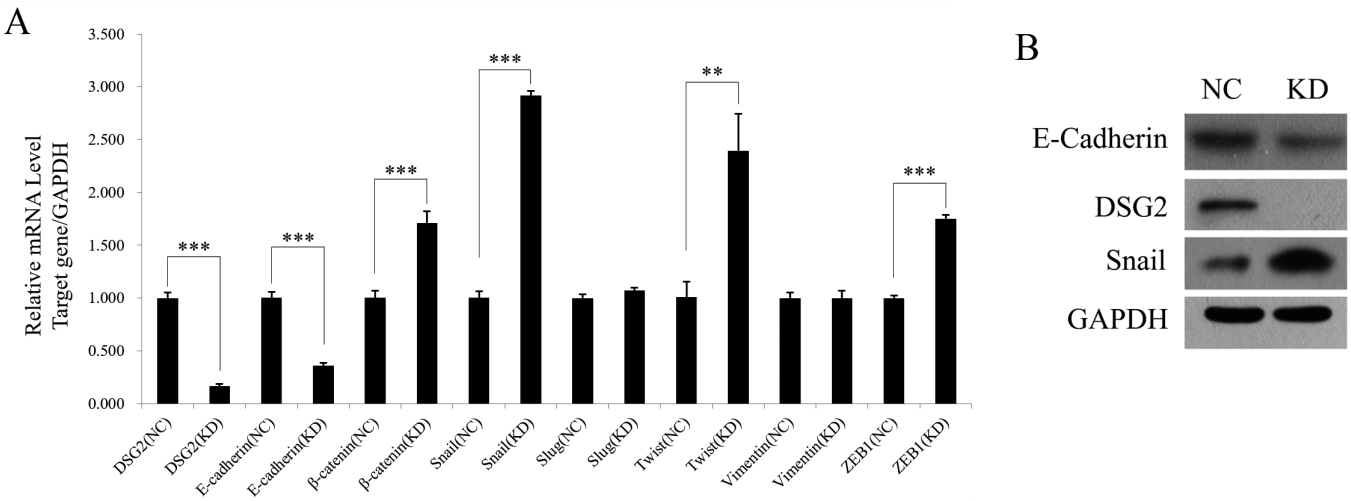

Fig. 5. Effect of DSG2 knockdown on the expression levels of EMT-associated genes in GBC-SD cells. **A.** RT-qPCR showed changes in the mRNA levels of related genes. **P<0.01, ***P<0.001. **B.** Western Blot indicated changes in protein levels of related genes. All experimental results were derived from ≥3 independent samples. NC, negative control; KD, knockdown.

to EGFR-targeted therapy by activating Src kinase. This study made a more in-depth elaboration on GBC resistance, but we paid more attention to the role of DSG2 in metastasis and found that DSG2 regulates the EMT process. Among them, the clinicopathological analysis of GBC patients in this study suggested that the reduction of DSG2 expression was highly correlated with lymphatic infiltration, which was basically consistent with our analysis results. In addition, this study also found that DSG2 exhibited significantly enhanced proliferation, migration and invasion in vitro and tumor growth and metastasis in vivo through Src-mediated signaling activation, which also corroborated with our main study on the effect of DSG2 on tumor metastasis. Both studies can corroborate and complement each other.

To detect the regulatory mechanism of DSG2 in GBC, we next applied DSG2 knockdown in the gallbladder cancer cell line GBC-SD. The results showed that decrease of DSG2 level promoted cell invasion and metastasis, which suggested that DSG2 can affect tumor growth and progression by regulating the biological characteristics of gallbladder cancer cells.

EMT is closely related to the acquisition of invasive and metastatic properties for tumor cells (Lamouille et al., 2014) and can directly affect the prognosis of patients. The mRNA expression level of E-Cadherin, which is an epithelial marker of the EMT pathway, was significantly decreased in DGS2 knockdown GBC-SD cells, in which the E-Cadherin protein levels showed consistent results. Both the mRNA and protein levels of a E-Cadherin suppressor, Snail, were significantly upregulated. The mRNA expression levels of Twist and ZEB1, where the latter is the most critical transducer of EMT, were significantly increased. Moreover, β-catenin as a key protein of Wnt signaling pathway, also showed significantly elevated mRNA expression. Consequently, the downregulation of DSG2 in gallbladder cancer cells might disrupt the intergranular junction structure, reduce the adhesion strength, and participate in the EMT process of tumor cells, thus promoting their invasion and metastasis. Therefore, we showed that the down-regulation of DSG2 in gallbladder cancer cells was correlated with poorer prognosis of patients, and DSG2 possessed clinical significance in gallbladder cancer promotion and progression.

Overall, this study reported that the downregulated expression level of DSG2 in gallbladder cancer tissues might be involved in the development of gallbladder cancer through regulating EMT-associated pathways, thus promoting tumor cell invasion and metastasis. The specific molecular mechanism by which DSG2 regulates EMT-associated pathways in gallbladder cancer is unclear, and needs to be further investigated in the future.

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