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# Fascin Induces Epithelial-Mesenchymal Transition of Cholangiocarcinoma Cells by Regulating Wnt/ $\beta$ -Catenin Signaling

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

A **Xianhai Mao\***  
A **Xiaohui Duan\***  
A **Bo Jiang**

Department of Hepatobiliary Surgery, Hunan Provincial People's Hospital, Changsha, Hunan, P.R. China

**Corresponding Author:**  
**Source of support:**

\* Xianhai Mao and Xiaohui Duan contributed equally

Bo Jiang, e-mail: [jiangbodoctor@126.com](mailto:jiangbodoctor@126.com)

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**Background:** Our preliminary study suggested that the expression of Fascin was increased in cholangiocarcinoma, which indicating poor prognosis. The present study aimed to explore the roles and mechanisms of Fascin during the progression of cholangiocarcinoma.

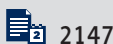
**Material/Methods:** We evaluated the knockdown effect of endogenous Fascin expression by Short hairpin RNA (shRNA) in QBC939 cells. Cell proliferation was confirmed by MTS assay. Migration and invasion assay was used to examine the cell invasive ability. Tumorigenesis abilities *in vivo* were analyzed with a xenograft tumor model. Western blot analysis was used to test epithelial-mesenchymal transition (EMT) biomarkers and critical proteins in the Wnt/ $\beta$ -catenin signaling pathway.

**Results:** shRNA-mediated gene knockdown of Fascin significantly inhibited cell proliferation, invasion, and EMT, and shRNA-Fascin markedly inhibited the xenograft tumor volume. Silencing of Fascin up-regulated phosphorylation of  $\beta$ -catenin and decreased its nuclear localization. Additionally, knockdown of Fascin led to the upregulation of  $\beta$ -catenin and E-cadherin expression in plasma membrane fraction of QBC939 cells.

**Conclusions:** Our data indicate a key role of Fascin in cell proliferation, migration, and invasion in cholangiocarcinoma. Fascin promotes EMT of cholangiocarcinoma cells, in part through regulating Wnt/ $\beta$ -catenin signaling.

**MeSH Keywords:** **Cholangiocarcinoma • Epithelial-Mesenchymal Transition • RNA, Small Interfering**

**Full-text PDF:** <http://www.medscimonit.com/abstract/index/idArt/897258>



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## Background

Cholangiocarcinoma, the second most common primary hepatobiliary malignancy, is an epithelial cell malignancy originating from the bile ducts [1]. Cholangiocarcinoma is characterized by a progressive increase in incidence and prevalence, and is associated with poor prognosis. The treatment of cholangiocarcinoma remains a challenge because of the aggressive nature of the disease [2,3]. Invasion and metastasis are the leading causes of cancer-related deaths in cholangiocarcinoma patients [4]. Therefore, determining the molecular mechanisms of invasiveness and metastasis is urgent for development of effective therapy for cholangiocarcinoma patients.

Epithelial-mesenchymal transition (EMT) is a process whereby epithelial cells lose ability to engage in cell-cell interaction and obtain new characteristics of mesenchyme [5]. EMT plays a critical role in this process, including embryonic development, fibrosis, and cancer progression in multiple organs [6,7]. Many recent reports support the idea that EMT is essential in progression of cancer [8–10]. In cancer, EMT plays a critical role in initiating epithelial cancer metastasis by disrupting the epithelial integrity and promoting the intravasation step of metastasis [11]. Multiple signaling pathways, such as TGF- $\beta$ , TNF- $\alpha$ , and Notch, are involved in the EMT process [12]. The latest study suggests that aberrant activation of the Wnt/ $\beta$ -catenin signaling pathway promotes EMT and progression of tumors [13,14].

Fascin, also known as Fascin-1, is a 55-kDa globular actin-bundling protein that contributes to the organization of cell protrusions that mediate cell interactions and migration, and cytoplasmic microfilament bundles that contribute to cell architecture, intracellular movement, and cell motility and metastasis [15]. Fascin is widely expressed in normal mesenchymal tissues and in the nervous system, but not in normal epithelial cells. Fascin is increased in several types of cancers, such as breast, colon, esophagus, and gastric cancers [16–19]. More recently, it was reported that Fascin overexpression leads to EMT, which plays a central role in carcinoma progression and metastasis [20]. Our preliminary data demonstrated that Fascin protein was highly expressed in cholangiocarcinoma, which was associated with tumor differentiation, vascular invasion, lymph node or distant metastasis, and prognosis, suggesting that overexpression of fascin protein may be a biomarker for poor prognosis of cholangiocarcinoma patients. In addition, we found that there was a reverse association between Fascin and E-cadherin expression, but there was a positive association between Fascin and vimentin expression [21]. Therefore, we postulated that Fascin may also be associated with EMT of cholangiocarcinoma cells. However, the mechanism by which this may occur needs to be further studied.

The present research mainly explored the effects of Fascin during the proliferation, invasion, and EMT of cholangiocarcinoma cells. In addition, we studied whether Fascin induces EMT of cholangiocarcinoma through Wnt/ $\beta$ -catenin signaling.

## Material and Methods

### Cell culture

The QBC939 cell line used in our study was obtained from the laboratory of Xiangya Hospital of Central South University, China. The cells were cultured in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich), 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin, and were maintained at 37°C with 5% CO<sub>2</sub> in a humidified atmosphere. The experiments were carried out on logarithmically growing cells.

### RNA silencing

The human QBC939 cells were genetically knocked-down for Fascin by using short hairpin RNA (shRNA) (sc-35359-sh or control shRNA sc-108060) constructs from Santa Cruz Biotechnology (Dallas, TX) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). The transfections were performed according to the manufacturer's protocols.

### Cell proliferation

MTS assay was used to assess cell proliferation, following the manufacturer's (Promega) instructions. At 24 h after transfection, cells were collected and approximately  $1 \times 10^3$  cells were plated into 96-well plates, which were treated for 24 h, 48 h, 72 h, 96 h, and 120 h, and then 20  $\mu$ l of MTS solution was added to each well. Plates were incubated at 37°C for 4 h. The absorbance of the samples was measured at 490 nm on a scanning multi-well spectrophotometer. The assay was repeated 3 times and the data are presented as the mean  $\pm$  standard error of the mean.

### Cell migration and invasion assay

We assessed migration of QBC939 cells using Millicell chambers (Millipore). At 24 h after transfection,  $5 \times 10^4$  cells were seeded into serum-free medium on the upper chambers of an insert. Media containing 10% FBS were added to the lower chamber. The chamber was cultivated in 5% CO<sub>2</sub> at 37°C for 24 h. Then, the cells in the upper chamber were removed with cotton wool, whereas the attached cells that had migrated or invaded into the lower section were not. Subsequently, cell counting was performed in 10 randomly selected fields under a fluorescence microscope after 0.1% crystal violet staining. The

invasion assay was the same as the migration assay except that matrigel (Sigma) was used in the transwell chambers and the cell suspension for the upper chambers were  $1 \times 10^5$  cells.

### Xenograft tumor model

All procedures involving mice were approved by the College Committee on Use and Care of Animals at the Hunan Provincial People's Hospital and conformed to the relevant regulatory standards. In these studies, tumor xenografts were established by standard techniques in 8-week-old male BALBc nu/nu nude mice. At 24 h after transfection,  $4 \times 10^6$  cells were implanted in nude mice subcutaneously. Quantitative measurements of tumor volume were taken every 7 days for a period of 42 days using calipers. The tumor volume was calculated using the following formula: Tumor volume ( $\text{mm}^3$ ) =  $[(\text{width})^2 \times \text{length}] / 2$ . All of the mice were sacrificed on day 42.

### Western blotting

Whole cell lysates, cytoplasm, and nuclear lysates were prepared with a protein extraction kit (Millipore Corporation, USA), and protein concentrations were quantified by BCA assay (Pierce, Rockford, IL, USA). SDS-PAGE (10%) was used to separate the protein samples. Then, Western blot analysis was carried out using monoclonal (rabbit) anti-Fascin, anti-E-cadherin, anti-vimentin, anti-GSK-3 $\beta$ , anti-phospho- $\beta$ -catenin (Ser33/37), anti- $\beta$ -catenin, anti-Histone H1, and anti- $\beta$ -actin antibody (Santa Cruz Biotechnology, USA). Goat anti-rabbit IgG (Pierce, Rockford, IL, USA) secondary antibody conjugated to horseradish peroxidase and ECL detection systems (SuperSignal West Femto, Pierce) were used for detection. Band densities were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The relative quantity of protein was determined by normalizing the densitometry value of interest to that of the internal loading control.

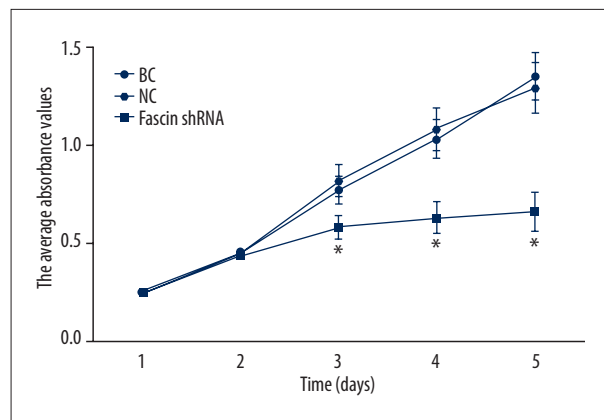
### Statistical analysis

Results are shown as mean  $\pm$  SEM. Continuous variables were compared using the *t* test or ANOVA if normally distributed, and the Wilcoxon rank sum test if distributions were non-parametric.  $P < 0.05$  (two-tailed) was considered to indicate a statistically significant difference. The calculations were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

## Results

### Effects of Fascin silencing on proliferation of QBC939 cells

With the purpose of elucidating the possible role of Fascin during cholangiocarcinoma tumorigenesis, we assessed the



**Figure 1.** Effects of Fascin knockdown on cell proliferation *in vitro*. In QBC939 cells in which Fascin was silenced, the cell proliferation rate was higher than that in the blank control (BC) and negative control (NC) cells. (\*  $P < 0.05$  vs. controls).

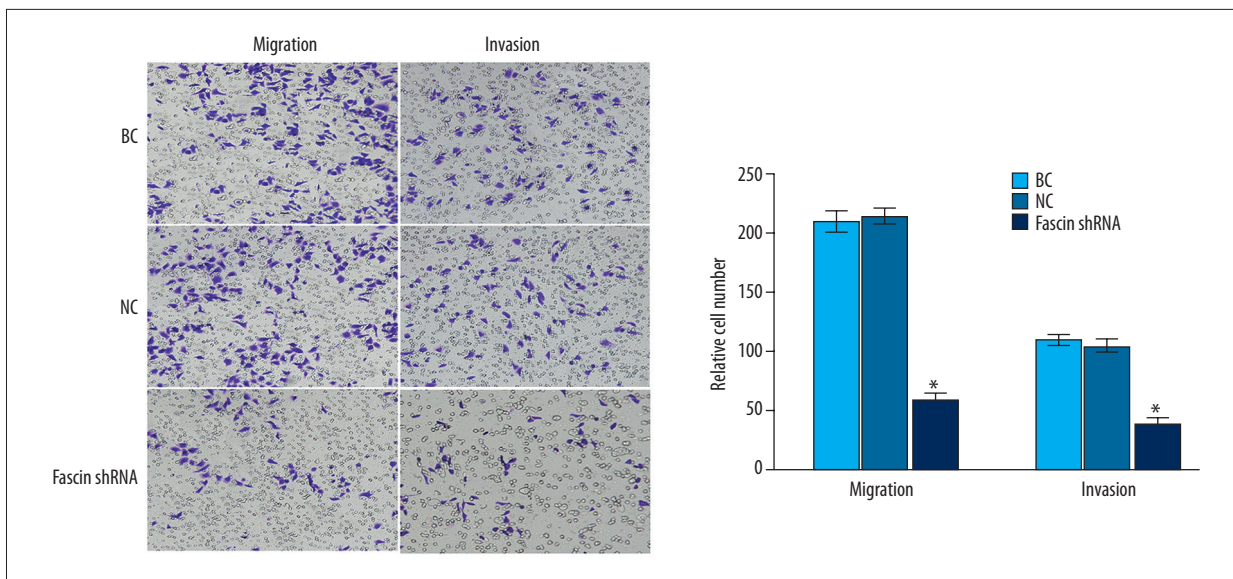
effect of Fascin in QBC939 cells growth by means of proliferation assay. QBC939 cells were transfected with Fascin-shRNA or negative control shRNA and stably transfected cells were established. The efficacy of Fascin knockdown was confirmed by Western blot (results not shown). MTS assay results show that knockdown of Fascin significantly inhibited cell proliferation with prolonged culture time, as compared with the control group (Figure 1). Taken together, these results indicated that Fascin knockdown suppresses cholangiocarcinoma cancer cell proliferation.

### Effects of Fascin silencing on migration and invasion of QBC939 cells

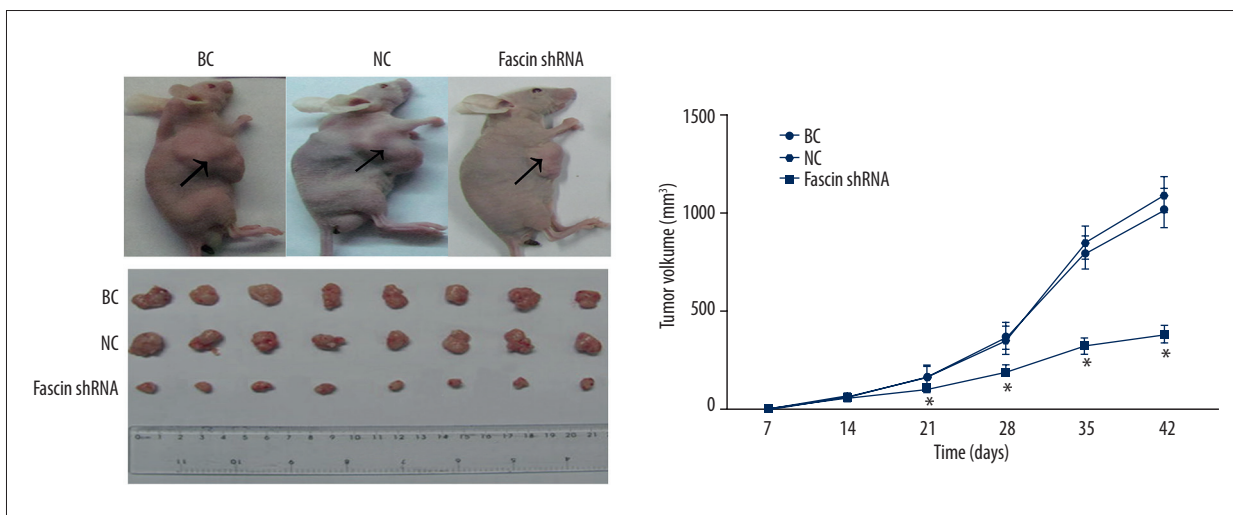
We silenced Fascin expression in QBC939 cells with transfection of Fascin-shRNA so as to clarify the effect of Fascin on cholangiocarcinoma. Fascin-shRNA significantly silenced Fascin expression in QBC939 cells in comparison with the blank control and the negative control. As shown in Figure 2, Fascin-silenced QBC939 cells exhibited significantly lower potential of migration and invasion in comparison with the control cells. These results clearly demonstrate that Fascin is crucial in mediating migration and invasion in QBC939 cells, which is a key determinant of cholangiocarcinoma malignant progression and metastasis.

### Effects of Fascin knockdown on tumor formation *in vivo*

For purpose of elucidating whether Fascin-shRNA affects tumor formation *in vivo*, we performed a subcutaneous tumor formation assay in nude mice. We evaluated tumor volume at 42 days after inoculation. The tumor formation caused by Fascin-shRNA-transfected cells were evidently smaller than those in the control group (Figure 3).



**Figure 2.** Effects of Fascin knockdown on cell migration and invasion *in vitro*. In QBC939 cells in which Fascin was silenced, the migratory and invasion capacities were lower than in the blank control (BC) and negative control (NC) cells. (\* P<0.05 vs. controls).



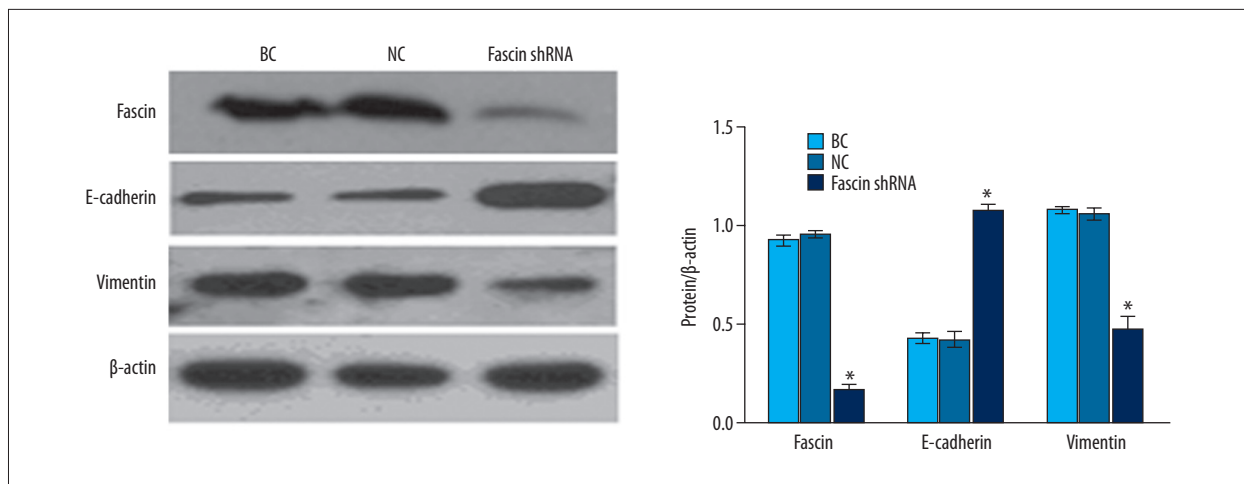
**Figure 3.** Effects of Fascin knockdown on tumor growth in nude mice. Representative xenograft tumors are shown. The tumor volume was monitored every 7 days. The average tumor volume was significantly lower in the Fascin-shRNA group compared to the blank control (BC) and negative control (NC) group. (\* P<0.05 vs. controls).

**Fascin mediates EMT in QBC939 cells**

To evaluate whether Fascin affects the EMT-related protein in QBC939 cells, Western blot analysis was performed to test E-cadherin and vimentin. Western blot analysis indicated that Fascin-knockdown markedly up-regulated E-cadherin expression and obviously down-regulating vimentin expression. All the results showed that Fascin is involved in EMT-related gene regulation and may contribute to the conversion of biliary tract tumors into invasive malignancies (Figure 4).

**Fascin induces EMT via Wnt/ $\beta$ -catenin signaling in QBC939 cells**

To assess how Fascin promotes EMT in QBC939 cells, we hypothesized that the Wnt/ $\beta$ -catenin signaling pathways would be activated by Fascin. We silenced Fascin expression in QBC939 cells with transfection of Fascin-shRNA or negative control shRNA. Western blot analysis showed that silencing of Fascin in QBC939 cells dramatically increased GSK-3 $\beta$  and phosphorylated  $\beta$ -catenin expression compared to controls, and nuclear  $\beta$ -catenin significantly decreased in Fascin silencing QBC939



**Figure 4.** Fascin knockdown can suppress EMT. The results revealed that Fascin silencing can suppress the EMT of QBC939 cells, which increased E-cadherin expression and inhibited vimentin expression compared to the blank control (BC) and negative control (NC) group. (\*  $P < 0.05$  vs. controls).

cells compared to control cells. These data strongly suggest that Fascin knockdown in QBC939 cells leads to inhibition of Wnt/ $\beta$ -catenin signaling. However, compared with negative controls, the expression of  $\beta$ -catenin and E-cadherin in plasma membrane fractions was increased in the Fascin knockdown QBC939 cells. Silencing of Fascin led to recruitment of  $\beta$ -catenin and E-cadherin to the plasma membrane of QBC939 cells (Figure 5).

## Discussion

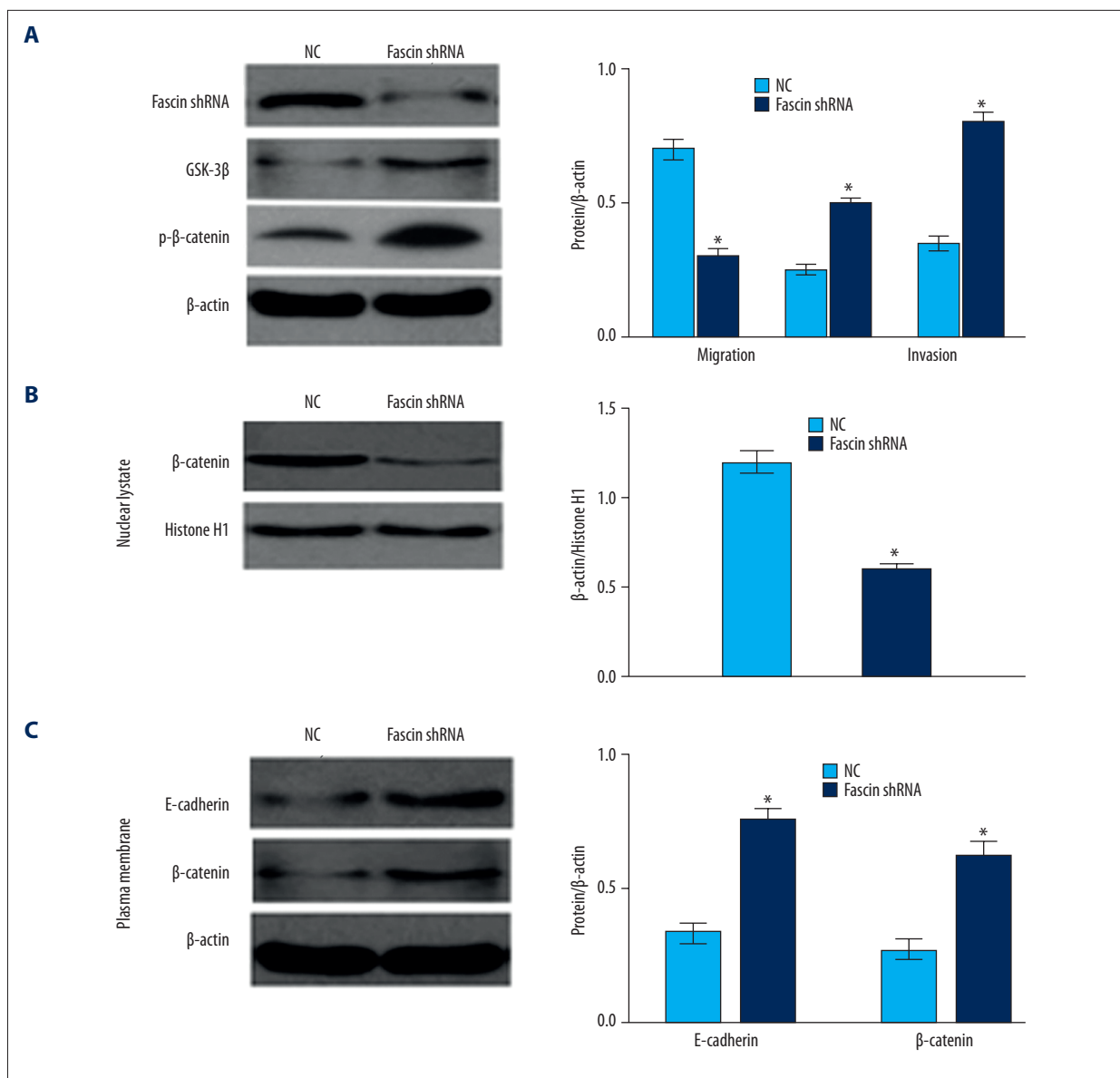
Fascin is a cytoskeletal protein crucial for cell adhesion and motility. Downregulation of Fascin can decrease the capacity of migration and amount of filopodia [15]. Fascin has emerged as an interesting potential biomarker due to its low or absent expression in the majority of normal adult epithelia. Fascin potentiates migratory and invasive behavior in neoplastic cells and has been shown to be upregulated in various malignancies [22]. There are only a few recent studies on the expression of Fascin in cholangiocarcinoma [23–25]. Onodera et al. reported that overexpression of Fascin may have an important function in the progression of cholangiocarcinoma [25]. In agreement with previous studies, our previous study reported that Fascin was significantly over-expressed in cholangiocarcinoma, and its expression was associated with poor survival, suggesting that Fascin may be an important marker for risk stratification of cholangiocarcinoma patients. However, its role and underlying mechanisms in cholangiocarcinoma are still unknown.

Recently, Fascin has drawn much attention due to its association with tumorigenesis [26].

The effect of Fascin *in vivo* and *in vitro* on tumorigenicity of QBC939 cells was studied in the present research. In MTS, Fascin knockdown inhibits the growth of QBC939 cells. *In vivo* results from nude mice models supported the experimental outcomes *in vitro*. Enforced expression of Fascin in cholangiocarcinoma cells significantly promoted the proliferation rate, whereas Fascin knockdown suppressed cancer cell growth, suggesting that Fascin acts as a positive regulator of growth in cholangiocarcinoma cells.

Our previous clinical findings suggest Fascin may participate in cholangiocarcinoma progression. Hence, we speculated that Fascin might be associated with the migration and invasion of QBC939 cells. To investigate the potential effect of Fascin on motility and invasiveness, the migration and invasive transwell assays were performed in QBC939 cells. The cell migration assay showed that the knockdown of Fascin significantly inhibited the migration capacity in QBC939 cells. The transwell invasive assay demonstrated that the knockdown of Fascin significantly repressed the invasiveness of QBC939 cells. These results prove that Fascin is a promoter of migration and invasion in cholangiocarcinoma. Since Fascin is essential for the migration and invasion of cholangiocarcinoma cells, we further examined the effect of Fascin on EMT in QBC939 cells. In this work, we found that Fascin knockdown up-regulated epithelial markers such as E-cadherin and down-regulated mesenchymal marker such as vimentin. Therefore, our results show that Fascin can promote EMT of cholangiocarcinoma cells and promotes invasion and metastasis.

The roles of several transcription factors as EMT regulators have been extensively reported [27]. But to our best knowledge, the underlying mechanisms of EMT in cholangiocarcinoma remains unclear [28]. In the present study, we speculated



**Figure 5.** Fascin-induced EMT is dependent on activation of the Wnt/β-catenin signaling pathway. (A). Expression of GSK-3β and phosphorylated β-catenin (Ser33/37) in Fascin knockdown QBC939 cells were detected by Western blot analysis. (B). Levels of β-catenin of nuclear fractions in Fascin knockdown QBC939 cells was determined by Western blotting. Histone H1 served as loading controls. (C). Expression levels of β-catenin and E-cadherin from plasma membrane fractions of Fascin silencing QBC939 cells were processed for Western blotting analysis. \* P<0.05 vs. negative control (NC).

that Fascin promotes EMT, in part through Wnt/β-catenin signaling. It was reported that Wnt/β-catenin is a crucial signalling pathway of malignant biological behaviors in many cancers, and it may influence E-cadherin expression [29]. The current data show that knockdown of Fascin in QBC939 cells activated GSK3β, increased the phosphorylation of β-catenin, and decreased its nuclear localization by targeting the β-catenin destruction complex. These data suggest that Wnt/β-catenin signaling is strongly suppressed by silencing of Fascin. In Fascin-knockdown QBC939 cells, expression of β-catenin and

E-cadherin was increased in the plasma membrane fraction. We finally concluded that the effects of Fascin on EMT in cholangiocarcinoma cells were partially mediated by the Wnt/β-catenin pathway.

### Conclusions

These results suggest that Fascin is critical for malignant proliferation and invasion of QBC939 cells. Further research results

suggest that Fascin can induce EMT of cholangiocarcinoma cells, and this process likely occurs via the Wnt/ $\beta$ -catenin pathway. Therefore, it is reasonable to speculate that Fascin may be a potential target for cancer prevention and treatment.

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## Conflict of interest

The authors disclose no potential conflicts of interest.