Int J Clin Exp Pathol 2018;11(3):1374-1382
www.ijcep.com /ISSN:1936-2625/IJCEP0071373

Original Article

JUB induces epithelial-mesenchymal transition via the Wnt/β-catenin signaling pathway in hepatocellular carcinoma cells

Hao Wang*, Jun Wang*, Xiaolei Shi, Yitao Ding

*Department of Hepatobiliary Surgery, Drum Tower Clinical Medical College of Nanjing Medical University, Nanjing, China. *Co-first authors.*

Received December 22, 2017; Accepted January 17, 2018; Epub March 1, 2018; Published March 15, 2018

Abstract: Epithelial-mesenchymal transition (EMT) is a key process involving acquisition of metastatic potential for cancer cells. Hepatocellular carcinoma (HCC) is a common cancer where metastasis is linked to patient survival. JUB has been reported to be involved in several physiological processes, however, pathological roles of JUB in tumor metastasis have been poorly studied. Here, we report that JUB is highly upregulated in clinical specimens and HCC cell lines. Through gain- and loss-of-function studies, JUB was demonstrated to facilitate HCC cell EMT. We found that JUB expression was upregulated in the HCC tissues compared to borderline or benign tissues. Experimental EMT induced by JUB plasmid transfection proved that JUB protein overexpression could enhance transwell migration ability and significantly upregulate mesenchymal marker proteins, N-cadherin and concurrently downregulate the epithelial marker E-cadherin. Subsequently, through Western blot assay, we found that JUB could activate the Wnt-signaling component β-catenin in the nuclei. Our findings revealed that JUB might be a key molecule regulating EMT through modulation of the Wnt/β-catenin signaling pathway.

Keywords: Liver cancer, Epithelial-mesenchymal transition, JUB, Wnt/β-catenin signaling

Introduction

Globally, hepatocellular carcinoma (HCC) is the fifth most common type of cancer. In the past few decades, molecular therapies targeting signaling cascades involved in HCC development and progression have been explored. Sorafenib has been used as a first-line therapy for advanced HCC patients [1, 2]. However, clinical trials have found that on average, these patients only experience three months of benefit from sorafenib treatment. Moreover, after sorafenib resistance, none of the tested drugs revealed positive responses as either first- or second-line treatment [3]. Therefore, it is important that new treatment strategies be developed.

A large number of liver cancer patients present with regional or distal metastatic cancer [4]. Epithelial-mesenchymal transition (EMT) is the key step to bestow metastatic potential to epithelial cancer cells enabling them to invade, migrate, and disseminate to distant metastases [5, 6]. EMT is the dominant program in human cancer, robust EMT biology is highly prognostic for cancer recurrence [7]. Consequently, illustration of the molecular mechanism underlying EMT may radically aid in the development of new therapeutic strategies against HCC. During EMT, epithelial cells lose their polarity, basement membrane and cell-cell adhesion, and acquire spindle like morphology providing greater ability for migration and invasion [8, 9]. EMT in carcinomas has been confirmed to generate cells with stem cell like properties and might be behind the generation of cancer stem cells [10, 11]. Corresponding to this theory, circulating tumor cells have been identified with the signatures of EMT. After attachment to the foreign site, the mesenchymal cells are transported back to its cancerous epithelial parental state through epithelial transition. Induction of EMT in transformed epithelial cells could induce cells with circulating tumor cell properties suggesting that EMT may

play a critical role in progression and metastases of cancer [12].

EMT changes will cause some special biomarkers could be detected and some signaling pathways activation. When EMT occurs, tumors are always accompanied by downregulation of epithelial cell markers, such as E-cadherin, and upregulation of mesenchymal cell markers such as N-cadherin [13, 14]. Aberrant activation of the Wnt signaling pathway is regarded as the initiation of tumor cell EMT [15, 16]. The downstream pathway known as the Wnt/β-catenin pathway could be activated by binding of Wnt proteins to their transmembrane cell receptors. The β-catenin protein is the key molecule in this pathway, and is stabilized by the molecule T-cell factor/lymphoid enhancer-binding factor-1 (TCF/LEF-1) to form a functional transcription factor that mediates metastasis [17]. Activation of the Wnt/β-catenin pathway can upregulate expression of the mesenchymal cell marker vimentin, which is also regard as an EMT marker [18]. Vimentin is normally expressed in migratory epithelial cells and in mesenchymal cells [19]. Vimentin is also overexpressed in some malignant diseases [20]. Because the promoter has been confirmed to be a target of the β-catenin-TCF/LEF-1 transcription factor, vimentin now is regarded as an EMT marker.

The LIM protein Ajuba family contains three members, JUB, LIMD1, and WTIP, and are characterized by the conservation of a unique pre-LIM region at the N-terminal, and the C-terminal with each domain containing of two zinc fingers [21-23]. Ajuba family proteins have been reported to regulate many events in cells, including meiotic maturation of oocytes, DNA damage response, and embryonal cell proliferation and differentiation [24]. Liang et al. found that upregulated JUB induces EMT by acting as a corepressor of Snail and JUB is also required for Snail-induced EMT [24]. However, the expression level, function and mechanisms of Ajuba family proteins in HCC remain largely unknown.

In this study, we report that the LIM protein JUB is overexpressed in HCC specimens and cell lines. Ectopic overexpression of JUB induces EMT and promotes migration and invasion in HCC cells. Silencing of JUB impairs EMT and inhibits migration and invasion in HCC cells. Furthermore, our findings indicate that JUB might be a key molecule regulating EMT involved in Wnt/β-catenin signaling pathway. JUB could provide a new theoretical basis for the treatment of HCC.

Materials and methods

Cell lines and clinical samples

The human HCC cell lines Hep3B, Huh-7 and HepG2 were purchased from the Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All of the cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco Life Technology, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Hyclone, Rockford, IL, USA), streptomycin (100 μg/mL), and penicillin (100 U/mL) at 37°C under 5% CO_2 in a humidified incubator (Heracell 150i; Thermo Fisher Scientific, Langenselbold, Germany). For experiments, cells were serum-starved 24 h at 70% confluence before treatment. Dickkopf-1 (DKK1, 300 ng/ml) was used to block the Wnt/β-catenin signaling pathway.

All 50 HCC cases from January 1, 2014 to December 31, 2016 were collected in this study and they were included according to the following criteria: i) first-time diagnosed HCC patients without any chemotherapy, radiotherapy or any other treatment prior to surgery; and ii) without other tumors. The age of the patients ranged from 51 to 87 years. All samples and date were obtained with prior written informed consents from the patients and approved by the ethics committees of hospital. The malignant and borderline tissues were obtained from the surgical pathology archives in the Nanjing Drum Tower Hospital. Follow-up data were conducted using hospital medical records.

Immunohistochemistry analyses for JUB

Tissue sections were deparaffinized and rehydrated. Antigen was retrieved using heat-induced epitopes in 10 mM citrate buffer (Ph= 6.0). Endogenous peroxidases were blocked for 5 min using 0.3% H2O2. The sections were incubated with mouse polyclonal primary anti-JUB antibody (diluted 1:500, Abcam, USA) overnight in a cool room. The next day, slides were treated with horseradish peroxidase-labeled secondary antibody after three PBS washes. To

evaluate JUB immunostaining, the percentage of positive cells and intensity of staining were analyzed. The intensity of immunostaining was estimated according to the following scores: 0, colorless; 1, buffer; 2, brownish yellow; and 3, dark yellow. The scores of the positive cell percentage were defined as follows: 0, ≤10%; 1, 10%-25%; 2, 25%-50%; 3, 50-75%; and 4, >75%. We defined the scores ≤4 as low expression of JUB and 5-12 as high expression of JUB.

Quantitative polymerase chain reaction (q-PCR)

Total RNA of the tissues or cells were extracted using the TRIzol total RNA extraction kit (Tiangen, China) according to the manufacturer's instructions. The cDNA was synthesized using reverse transcription PCR kit (Tiangen, China) according to the manufacturer's instruction. Gene expression at the mRNA level was evaluated by quantitative polymerase chain reaction (q-PCR) using Exicycler™ 96 Real-Time Quantitative Thermal Block (Bioneer, China). Amplification conditions were as follows: 95°C for 10 min; 40 cycles of 95°C for 10 s, 60°C for 20 s and 72°C for 30 s. All samples were run in triplicate. Germany). The primers used to amplify JUB were: forward, AGAGGCCAGGGAGGACTACT, and reverse, GAGCAGCAAACAAAGCACTG. β-actin served as an internal control. The primers used to amplify β-actin were: forward, 5'-ACCCACACTGTGCCCATCTA-3', and reverse, 5'-GCCACAGGATTCCATACCCA-3'. The relative changes in transcript levels were analyzed using ΔΔCt method by comparing Ct values of mRNA expression relative to the internal control.

Stable cell line establishment

For overexpression of JUB, the HCC cell line HepG2 was transfected with pSin-EF2-puro-retro-JUB. For silencing of JUB, the HCC cell line Huh-7 was transfected with pSuper-retro-JUB-RNAi. The target sequences were: RNAi: GGACCGGGATTATCACTTT. For stable cell line establishment, pSin-EF2-puro-retro-JUB and pSuper-puro-retro-JUB-RNAi were packed into retrovirus in 293T cells. The viruses were harvested and HepG2 and Huh-7 cells were infected. After infection for 2 days, the cells were selected with medium containing 0.5 μg/mL puromycin over 1 week.

Scratch wound-healing and transwell assays.

Scratch wound-healing and transwell assays were used to evaluate the cell motility. For the scratch wound-healing assay, cells were seeded in 6-well plates and grown to 90% confluence. After aspirating the growth medium, a scratch wound in the monolayer was made using a sterile pipette tip. The well plate was then washed with PBS to remove the cellular debris. The cells left were cultured in DMEM again. The width of the wound was measured in 10 randomly screened fields by an Olympus microscope at 0, 24, and 48 h after incubation. Each test was performed in triplicate.

For the transwell assay, cells were suspended in serum-free medium and seeded into the upper trans-well cell culture pre-coated with Matrigel (BD Biosciences, San Jose, CA, USA). The lower chamber was loaded with culture media containing 10% FBS. After incubation for 24 h at 37°C in 5% CO_2, cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet (Sigma, USA). Cells that migrated to the underside of the membrane were counted using light microscopy in five randomly selected areas. The results are representative of three independent experiments.

Western blotting

The cell lysates were collected and centrifuged at 20,000 rpm for 5 min at 4°C. The supernatant was transferred to a clean tube and proteins concentrations were measured using the BCA Kit (Pierce, Rockford, IL, USA). Proteins were separated by 10% SDS-PAGE using 5% stacking gel and gradient separating gel with 6, 8, 10, and 13%, then blotted onto nitrocellulose membranes. Membranes were blocked with 5% skim milk for 1 h and incubated overnight at 4°C with primary antibody. The primary antibodies used were anti-JUB 1:500; anti-vimentin 1:500; anti-E-cadherin 1:1,000; anti-N-cadherin 1:1,000; anti-β-catenin 1:1,000. All antibodies were purchased from Abcam (Cambridge, UK). Membranes were washed six times in TBST for 5 min and incubated with secondary antibodies, HRP-conjugated goat anti-mouse/rabbit antibody (1:5,000; Invitrogen) for 2 h at room temperature. After washing in TBST, the protein bands were visualized using ECL reagents (EMD Millipore). The optical density of

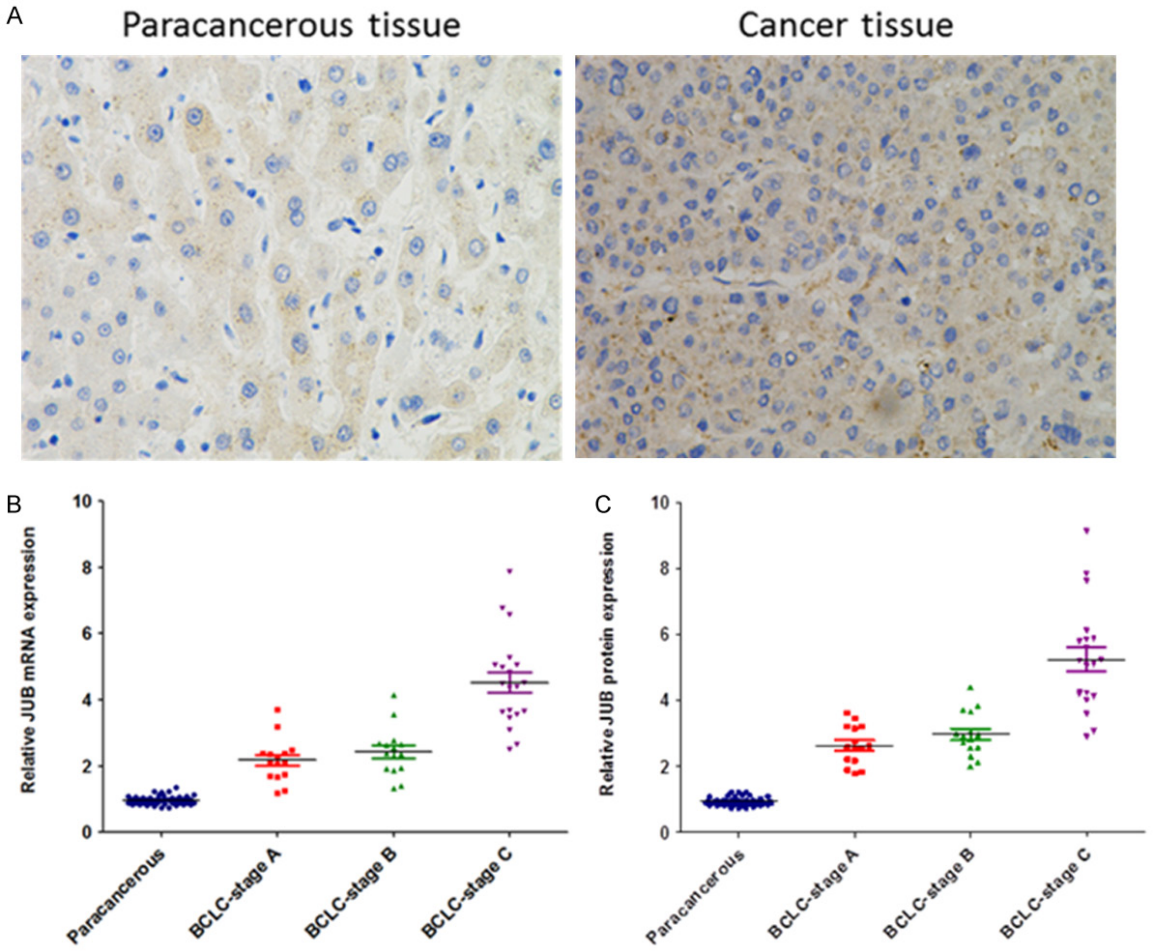

Figure 1. Overexpressed levels of JUB expression in HCC tissues. A. Immunohistochemistry staining of JUB in liver carcinoma and para-carcinoma tissues. B. Real time PCR analysis of JUB expression in HCC patients with BCLC stage and para-carcinoma tissues. C. Western blot detect the JUB protein expression level in HCC patients with BCLC stage and para-carcinoma tissues, β-actin served as the loading control. (*P<0.05, compare to para-carcinoma).

bands was analyzed by reflectance densitometry on a Gel-Pro Analyzer.

Statistical analysis

The χ^2 test was used to compare clinicopathological parameters in patients with JUB protein expression. The SPSS 16.0 software (Chicago, USA) was used to analyze the enumeration data. Each experiment was performed at least 3 times, on independent passages, usually in triplicates. Data were analyzed by Newman-Keuls test using Statistical software as indicated and are presented as mean ± SEM. $p<0.05$ was considered statistically significant. Results of time lapse microscopy experiments were analyzed with Wilcoxon test in R software.

Results

High expression of JUB in cancer cells is associated with HCC progression

To examine whether JUB involved in HCC progression, JUB mRNA and protein levels were tested in liver tissues from patients with different HCC stages. The disease stage was classified by the Barcelona Clinic Liver Cancer (BCLC) system. Among the 50 pathologically diagnosed HCC patients, 15 had BCLC-stage A, 15 BCLC stage B, and 20 BCLC-stage C. Expression of JUB was examined in all HCC tissues using an anti-JUB antibody. Immunostaining of JUB was detected in the majority of most HCC cancer cells (**Figure 1A**). As show in **Figure 1B** and

Table 1. Association between JUB expression and clinicopathological characteristics of 50 HCC patients

Variables	Cases (n=50)		JUB		*P* value*
	n	%	High (22)	Low (28)	
Age (years)					
≤60	32	64.00	14	18	0.889
>60	18	36.00	8	10	
Sex					
Male	43	86.00	20	23	0.156
Female	7	14.00	2	5	
Hepatitis B					
Absent	5	10.00	4	1	0.972
Present	45	90.00	34	11	
AFP					
Negative	18	36.00	11	7	0.653
Positive	32	64.00	24	8	
Tumor size					
≤3	9	18.00	3	6	0.232
>3	41	82.00	19	22	
Tumor number					
Single	39	78.00	19	20	0.232
>Multiple	11	22.00	3	8	
BCLC-stage					
Stage A	15	30.00	3	11	0.001
Stage B	15	30.00	4	10	0.002
Stage C	20	40.00	15	5	

Abbreviations and note: TNM, tumor-node-metastasis. *Pearson's χ^2 test.

1C both mRNA and protein expression of JUB was notably elevated in HCC patients with BCLC stage C compared to those with paracancerous tissues. Real-time PCR and Western blot analyses showed that JUB was upregulated on average ≤1.9-fold in patients with BCLC-stage C compared to those with BCLC-stage A or B (P=0.002). **Figure 1B** and **1C** showed that there were no significant difference of JUB expression between patients with BCLC-stages A and B (P>0.05).

We divided 50 patients into JUB high and JUB low groups based on JUB immune positivity. **Table 1** shows that 22 patients belonged to JUB high group and 28 JUB low group, respectively. Correlations between JUB expression and clinicopathological features were further analyzed. Consistent with the findings based on real-time PCR and Western blotting, there was a significant difference in JUB expression level between BCLC-stages C and A (P=0.001) or B (P=0.002), while there was no significant difference between BCLC-stages A and B. No other clinical characteristics, including age, gender, serum AFP level, hepatitis B virus infection, tumor number, tumor size, and Child-Pugh class were related to the expression of JUB.

High expression of JUB significantly enhances the ability of invasion and metastasis and EMT marker expression

Promotion effect of JUB on HepG2 cell migration was evaluated by scratch wound healing assay (**Figure 2A**). At timepoint 0 h, cells were scraped and photographed. Migration of cells into wounded areas was effectively increased in the JUB group after 24 h. This result suggests that JUB promoted migration of the HepG2 cells. JUB promoting invasion of HepG2 cell invasion was determined by transwell invasion assay. JUB significantly promoted invasion of the cells (**Figure 2B**). The expression level of EMT markers, N-cadherin, vimentin and E-cadherin were compared between the JUB transfected HepG2 cells and vector transfected HepG2 cells by Western blot assay. The results of this analysis confirmed that ectopic expression of JUB in HepG2 cells produced N-cadherin and vimentin in substantially higher amounts and with opposite E-cadherin changing in the cells (**Figure 2C**). The results also revealed that the vectors had no effect on the protein expression.

Silencing JUB significantly represses the ability of invasion and metastasis and EMT marker expression

To further confirm the role of JUB in promoting EMT in the opposite way, Huh-7 was selected for depletion of the endogenous expression of JUB. The effect of JUB on Huh-7 cell migration was evaluated by scratch wound healing assay (**Figure 3A**). The motility and invasive ability were impaired by silencing of JUB. Fewer cells were presented in the under-surface of the transwell chamber membrane (**Figure 3B**). The expression level of EMT markers, N-cadherin, vimentin and E-cadherin were detected by Western blotting. The results of this analysis confirmed that silencing of JUB in

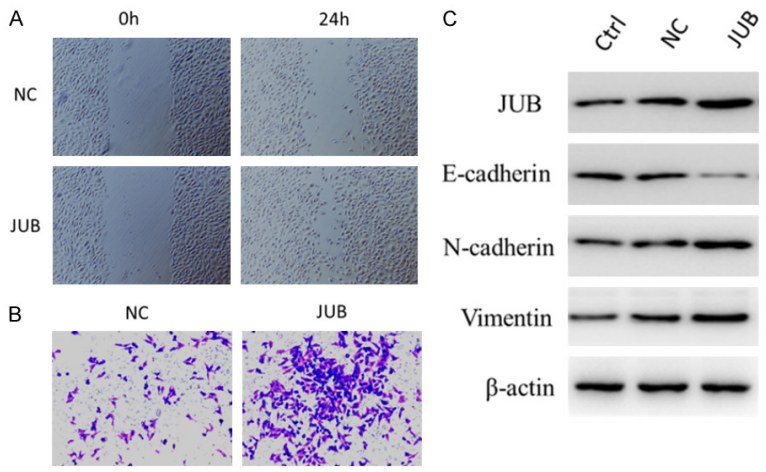

Figure 2. Overexpression of JUB enhances the motility and invasion of HepG2 cell and regulates EMT related gene expression. A. In vitro wound-healing assay. Confluent cell cultures were scratched with a pipette tip to produce a wound and analyzed by time-lapse video microscopy. Images of the wounded area immediately (0 hours) and 24 hours after the incision was made are shown. B. Invasion assay was performed with transwell-inserts coated with Matrigel. Images were taken with invert microscope (magnification, x100). C. Western blot analysis of E-cadherin, N-cadherin and Vimentin in HepG2 cell, in response to JUB over-expression. β-actin was used as loading control.

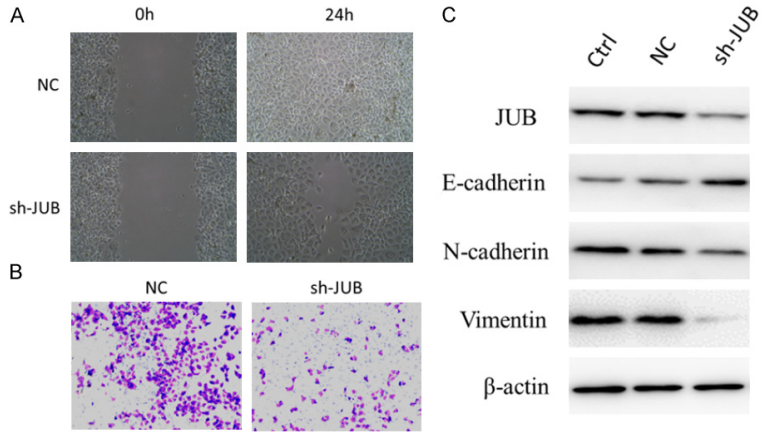

Figure 3. Knockdown of JUB inhibits the motility and invasion of Huh-7 cell and regulates EMT related gene expression. A. In vitro wound-healing assay. Confluent cell cultures were scratched with a pipette tip to produce a wound and analyzed by time-lapse video microscopy. Images of the wounded area immediately (0 hours) and 24 hours after the incision was made are shown. B. Invasion assay was performed with transwell-inserts coated with Matrigel. Images were taken with invert microscope (magnification, x100). C. Western blot analysis of E-cadherin, N-cadherin and Vimentin in Huh-7 cell, in response to JUB over-expression. β-actin was used as loading control.

Huh-7 cells produced N-cadherin and vimentin in substantially lower amounts and with opposite E-cadherin changing in the cells (**Figure 3C**).

The role of JUB on regulating β-catenin expression

To verify whether JUB promoted HCC cell EMT through the Wnt/β-catenin signaling pathway, β-catenin protein levels of the cell lysates were analyzed. The results showed that the protein level of β-catenin in the JUB cells was significantly increased compared with vector transected cells, JUB-silenced cells, and control cells (**Figure 4A**). DKK1, the β-catenin inhibitor, was used to co-incubate the JUB transfected HepG2 cells for 48 h. β-catenin expression was activated after JUB increased significantly after DKK1 co-incubation. However, expression JUB of was not affected. This suggested that JUB is an upstream protein inducing EMT through activating the β-catenin pathway (**Figure 4B**).

Discussion

JUB have been reported to regulate many events in cells. However, the clinical role of JUB in HCC progression and the molecular mechanisms haveremainedunclear.Inthisstudy, we show that JUB expression is correlated with clinical features of HCC, including invasive ability and BCLC st aging. Furthermore, we found that increase expression of JUB enhances the migration, invasion capacity, and EMT of HCC cells by activating the Wnt/β-catenin signaling pathway.

Metastasis is an important step of HCC progression. In this study, we focused on the clinical importance of JUB in HCC, and found that there was a positive association betw-

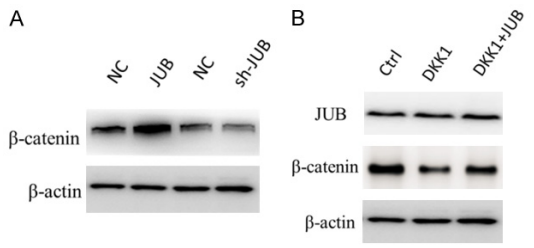

Figure 4. The role of JUB on regulating β-catenin expression. A. Western blot analysis of β-catenin in HCC cells when transfected with JUB over-expression or knockdown plasmids. β-actin was used as loading control. B. Western blot analysis of β-catenin and JUB expression in HCC cells when treatment with DKK1 or DKK1+JUB over-expression plasmid.

een JUB expression and HCC metastasis progression. As metastasis and invasion are major factors in the progression of HCC, we propose that JUB might be involved in HCC metastasis. Furthermore, we show that JUB expression was correlated with metastasis and invasion. First, we found that overexpression of JUB was associated with HCC metastasis in HCC tissues and cells. This result was consistent with a report that silencing of JUB resulted in inhibition of cell migration and invasion in Huh7 cells [25]. The migration and invasion capacities of HCC were increased in HepG2-JUB cell lines, suggesting that in JUB may function to promote HCC metastasis in patients. So far, few studies have explored how JUB regulate HCC metastasis.

It is known that EMT is very important in cancer metastasis and other human diseases [26-28]. A key step in the EMT is downregulation of E-cadherin [29]. In the process of cell adhesion, the E-cadherin-catenin complex plays an important role. Its dysfunction has been associated with an increased cell invasiveness and metastasis and reduction in cell differentiation. As a negative regulator of E-cadherin, snail is one of the key transcription factors promoting EMT [30]. Moreover, the mesenchymal marker N-cadherin has been particularly associated with metastatic dissemination in human cancers [31]. In this study, we found that JUB could modulate the EMT program of HCC cells. As an epithelial cell marker, vimentin was positively correlated with JUB expression levels. HepG2-JUB cells showed enhance EMT, indicated by enhanced motility and invasiveness, decreased expression of E-cadherin, and increased expression of vimentin and N-cadherin compared to vector. Silencing of JUB in Huh-7 cells dramatically impaired EMT, leading to impaired motility and invasiveness, increased expression of E-cadherin, and decreased expression of vimentin and N-cadherin compared with control cells.

Many reports have shown that EMT was regulated various signaling pathways [16]. We found that JUB regulates EMT and metastasis through the Wnt/β-catenin signaling pathway. Although Wnt/β catenin activation might not be required for the final effects, it is necessary for EMT. It was reported that tumor cells undergo EMT in response to Wnt/β-catenin activation. Inhibition of the pathway could induce a reversal in the expression of protein markers of EMT [32]. The results of our study show that JUB upregulation modulates Wnt/β-catenin activity in HepG2 cells so that HCC may obtain the ability of metastatic and invasion by this pathway activity. It is still unknown whether JUB is an indispensable molecule or just facilitates cell EMT. Its overexpression represents an important initiation step for HCC to gain a metastatic and/or invasion advantage. Intriguingly, when HepG2-JUB was co-cultured with Wnt/β-catenin inhibitor DKK1, the β-catenin protein expression was significantly inhibited but the JUB expression maintaining the normal. So, it can be presumed that JUB overexpression is necessary for induction of HCC EMT progression. JUB acts as the upstream molecule in this signaling pathway in favor of EMT. This result indicates that JUB may be an essential bio-factor in HCC metastasis and invasion, and that its depletion might be accompanied by inhibition of EMT and could be a new therapeutic target in metastatic carcinoma. These results also reveal that JUB mainly induces EMT via Wnt/β-catenin signaling pathway in HCC.

In conclusion, our findings suggest that JUB stimulates the invasiveness and metastasis of HCC cells. JUB may promote the EMT of HCC cells through Wnt/β-catenin signaling pathways. Thus, JUB might be a potential indicator of HCC EMT and metastasis, and our studies provide a theoretical basis for therapeutically targeting JUB for the treatment of HCC.

Acknowledgements

This work was supported by National Natural Science Foundation of China (81170417).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yitao Ding, Department of Hepatobiliary Surgery, Drum Tower Clinical Medical College of Nanjing Medical University, 321 Zhongshan Road, Nanjing 210008, China. Tel: 86 25-83304616; Fax: 86 25-83317016; E-mail: dingyitaodoc@hotmail.com

References

[1] Hernandez-Gea V, Toffanin S, Friedman SL and Llovet JM. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. Gastroenterology 2013; 144: 512-527.

[2] Zhang PF, Li KS, Shen YH, Gao PT, Dong ZR, Cai JB, Zhang C, Huang XY, Tian MX, Hu ZQ, Gao DM, Fan J, Ke AW and Shi GM. Galectin-1 induces hepatocellular carcinoma EMT and sorafenib resistance by activating FAK/PI3K/AKT signaling. Cell Death Dis 2016; 7: e2201.

[3] Llovet JM and Hernandez-Gea V. Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design. Clin Cancer Res 2014; 20: 2072-2079.

[4] Bosch FX, Ribes J and Borras J. Epidemiology of primary liver cancer. Semin Liver Dis 1999; 19: 271-285.

[5] Chen C, Zimmermann M, Tinhofer I, Kaufmann AM and Albers AE. Epithelial-to-mesenchymal transition and cancer stem(-like) cells in head and neck squamous cell carcinoma. Cancer Lett 2013; 338: 47-56.

[6] Krawczyk N, Meier-Stiegen F, Banys M, Neubauer H, Ruckhaeberle E and Fehm T. Expression of stem cell and epithelial-mesenchymal transition markers in circulating tumor cells of breast cancer patients. Biomed Res Int 2014; 2014: 415721.

[7] Loboda A, Nebozhyn MV, Watters JW, Buser CA, Shaw PM, Huang PS, Van't Veer L, Tollenaar RA, Jackson DB, Agrawal D, Dai H and Yeatman TJ. EMT is the dominant program in human colon cancer. BMC Med Genomics 2011; 4: 9.

[8] Kalluri R and Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009; 119: 1420-1428.

[9] Nieto MA. The ins and outs of the epithelial to mesenchymal transition in health and disease. Annu Rev Cell Dev Biol 2011; 27: 347-376.

[10] Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J and Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133: 704-715.

[11] Thiery JP, Acloque H, Huang RY and Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009; 139: 871-890.

[12] Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT, Isakoff SJ, Ciciliano JC, Wells MN, Shah AM, Concannon KF, Donaldson MC, Sequist LV, Brachtel E, Sgroi D, Baselga J, Ramaswamy S, Toner M, Haber DA and Maheswaran S. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science 2013; 339: 580-584.

[13] Chapman HA. Epithelial-mesenchymal interactions in pulmonary fibrosis. Annu Rev Physiol 2011; 73: 413-435.

[14] Kage H and Borok Z. EMT and interstitial lung disease: a mysterious relationship. Curr Opin Pulm Med 2012; 18: 517-523.

[15] He W, He S, Wang Z, Shen H, Fang W, Zhang Y, Qian W, Lin M, Yuan J, Wang J, Huang W, Wang L and Ke Z. Astrocyte elevated gene-1 (AEG-1) induces epithelial-mesenchymal transition in lung cancer through activating Wnt/beta-catenin signaling. BMC Cancer 2015; 15: 107.

[16] Iwai S, Yonekawa A, Harada C, Hamada M, Katagiri W, Nakazawa M and Yura Y. Involvement of the Wnt-beta-catenin pathway in invasion and migration of oral squamous carcinoma cells. Int J Oncol 2010; 37: 1095-1103.

[17] Tsuchiya R, Yamamoto G, Nagoshi Y, Aida T, Irie T and Tachikawa T. Expression of adenomatous polyposis coli (APC) in tumorigenesis of human oral squamous cell carcinoma. Oral Oncol 2004; 40: 932-940.

[18] Sabbah M, Emami S, Redeuilh G, Julien S, Prevost G, Zimber A, Ouelaa R, Bracke M, De Wever O and Gespach C. Molecular signature and therapeutic perspective of the epithelial-to-mesenchymal transitions in epithelial cancers. Drug Resist Updat 2008; 11: 123-151.

[19] Gilles C, Polette M, Mestdagt M, Nawrocki-Raby B, Ruggeri P, Birembaut P and Foidart JM. Transactivation of vimentin by beta-catenin in human breast cancer cells. Cancer Res 2003; 63: 2658-2664.

[20] Pan Y, Li X, Duan J, Yuan L, Fan S, Fan J, Xiaokaiti Y, Yang H, Wang Y and Li X. Enoxaparin sensitizes human non-small-cell lung carcinomas to gefitinib by inhibiting DOCK1 expression, vimentin phosphorylation, and Akt activation. Mol Pharmacol 2015; 87: 378-390.

[21] Goyal RK, Lin P, Kanungo J, Payne AS, Muslin AJ and Longmore GD. Ajuba, a novel LIM protein, interacts with Grb2, augments mitogen-activated protein kinase activity in fibroblasts, and promotes meiotic maturation of Xenopus oocytes in a Grb2- and Ras-dependent manner. Mol Cell Biol 1999; 19: 4379-4389.

[22] Sharp TV, Munoz F, Bourboulia D, Presneau N, Darai E, Wang HW, Cannon M, Butcher DN, Nicholson AG, Klein G, Imreh S and Boshoff C. LIM domains-containing protein 1 (LIMD1), a tumor suppressor encoded at chromosome 3p21.3, binds pRB and represses E2F-driven transcription. Proc Natl Acad Sci U S A 2004; 101: 16531-16536.

[23] Srichai MB, Konieczkowski M, Padiyar A, Konieczkowski DJ, Mukherjee A, Hayden PS, Kamat S, El-Meanawy MA, Khan S, Mundel P, Lee SB, Bruggeman LA, Schelling JR and Sedor JR. A WT1 co-regulator controls podocyte phenotype by shuttling between adhesion structures and nucleus. J Biol Chem 2004; 279: 14398-14408.

[24] Liang XH, Zhang GX, Zeng YB, Yang HF, Li WH, Liu QL, Tang YL, He WG, Huang YN, Zhang L, Yu LN and Zeng XC. LIM protein JUB promotes epithelial-mesenchymal transition in colorectal cancer. Cancer Sci 2014; 105: 660-666.

[25] Miao HL, Pan ZJ, Lei CJ, Wen JY, Li MY, Liu ZK, Qiu ZD, Lin MZ, Chen NP and Chen M. Knockdown of GPC3 inhibits the proliferation of Huh7 hepatocellular carcinoma cells through down-regulation of YAP. J Cell Biochem 2013; 114: 625-631.

[26] Acloque H, Adams MS, Fishwick K, Bronner-Fraser M and Nieto MA. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. J Clin Invest 2009; 119: 1438-1449.

[27] Ding W, You H, Dang H, LeBlanc F, Galicia V, Lu SC, Stiles B and Rountree CB. Epithelial-to-mesenchymal transition of murine liver tumor cells promotes invasion. Hepatology 2010; 52: 945-953.

[28] Ke AW, Shi GM, Zhou J, Huang XY, Shi YH, Ding ZB, Wang XY, Devbhandari RP and Fan J. CD151 amplifies signaling by integrin alpha-6beta1 to PI3K and induces the epithelial-mesenchymal transition in HCC cells. Gastroenterology 2011; 140: 1629-1641, e1615.

[29] Thiery JP and Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 2006; 7: 131-142.

[30] Zhu M, Yin F, Fan X, Jing W, Chen R, Liu L, Zhang L, Liu Y, Liang Y, Bu F, Tong X, Zheng H, Zhao J and Guo Y. Decreased TIP30 promotes Snail-mediated epithelial-mesenchymal transition and tumor-initiating properties in hepatocellular carcinoma. Oncogene 2015; 34: 1420-1431.

[31] Suh Y, Yoon CH, Kim RK, Lim EJ, Oh YS, Hwang SG, An S, Yoon G, Gye MC, Yi JM, Kim MJ and Lee SJ. Claudin-1 induces epithelial-mesenchymal transition through activation of the c-Abl-ERK signaling pathway in human liver cells. Oncogene 2013; 32: 4873-4882.

[32] Warrier S, Bhuvanalakshmi G, Arfuso F, Rajan G, Millward M and Dharmarajan A. Cancer stem-like cells from head and neck cancers are chemosensitized by the Wnt antagonist, sFRP4, by inducing apoptosis, decreasing stemness, drug resistance and epithelial to mesenchymal transition. Cancer Gene Ther 2014; 21: 381-388.