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Original Paper

Long Noncoding RNA HOST2 Promotes Epithelial-Mesenchymal Transition, Proliferation, Invasion and Migration of Hepatocellular Carcinoma Cells by Activating the JAK2-STAT3 Signaling Pathway

Yang Wu[a] Tan Yuan[b] Wei-Wei Wang[c,d] Peng-Lei Ge[a] Li-Qiang Gao[a] Gong Zhang[a] Zhe Tang[a] Xiao-Wei Dang[a] Yong-Fu Zh[a] Jian-Ying Zhang[e] Guo-Zhong Jiang[c,d]

[a]Department of Hepatobiliary and Pancreatic Surge[ry], the Fi[rst] Affiliated Hospital of Zhengzhou University, Zhengzhou, [b]Department of Respiratory, [Fi]fth Affiliated Hospital of Zhengzhou University, Zhengzhou, [c]Department of Path[ology], the [First] Affiliated Hospital of Zhengzhou University, Zhengzhou, [d]Department of Pathology, Sc[hool] of Basic Medicine, Zhengzhou University, Zhengzhou, [e]Institute of Medical and Pharmaceutical S[ciences], Zhengzhou University, Zhengzhou, China

Key Words
Hepatocellular carcinoma • Long noncoding RNA HOST2 • JAK2-STAT3 signaling pathway • Epithelial-mesenchymal transition • Proliferation • Migration • Invasion

Abstract
Background/Aims: This study aims to examine the effect of long noncoding RNA HOST2 (lncRNA HOST2) on epithelial-mesenchymal transition (EMT), proliferation, invasion and migration of hepatocellular carcinoma (HCC) cells *via* activation of the JAK2-STAT3 signaling pathway. ***Methods:*** HCC and para-cancerous tissues were collected from 136 HCC patients. Immunohistochemistry was used to detect the expression of JAK2 and STAT3. HCC SMMC7721 cells were grouped into blank, negative control (NC), HOST2 mimic and HOST2 inhibitor groups. The mRNA and protein expression levels of HOST2, JAK2, STAT3, E-cadherin, vimentin, Snail, Slug, Twist and Zeb1 in tissues and cells were determined by reverse transcription - quantitative polymerase chain reaction (RT-qPCR) and Western blotting, respectively. An MTT assay, scratch test and Transwell assay were applied to measure cell proliferation, migration and invasion, respectively. ***Results:*** The levels of JAK2, STAT3 and vimentin were higher in HCC tissues, while the expression of E-cadherin was lower in HCC tissues compared with

Y. Wu and T. Yuan contributed equally to this work.

Dr. Yang Wu
and Dr. Guo-Zhong Jiang
Department of Hepatobiliary and Pancreatic Surgery; Department of Pathology, the First Affiliated Hospital of Zhengzhou University, No. 1, Jianshe East Road, Zhengzhou 450052 (China)
Tel. +86-0371-67967122, E-Mail sunnywu@zzu.edu.cn; guozhongjiang@zzu.edu.cn

para-cancerous tissues. The silencing of HOST2 significantly decreased cell proliferation, migration and invasion, reduced the levels of HOST2, JAK2, STAT3 and vimentin, and elevated the expression of E-cadherin. HOST2 silencing also decreased the levels of Snail, Slug and Twist but increased the level of Zeb1 protein, while the opposite findings were observed in the HOST2 mimic group. ***Conclusion:*** These results reveal a possible mechanism in HCC in which LncRNA HOST2 may increase EMT and enhance proliferation, invasion and metastasis of HCC cells *via* activation of the JAK2-STAT3 signaling pathway.

Introduction

Hepatocellular carcinoma (HCC) is the 6th most common cancer worldwide and the 3rd most common cause of cancer-related deaths [1]. It is estimated that the incidence of HCC and HCC-related deaths have increased over the last several decades in many parts of the world [2]. The strongest risk factors for HCC are chronic hepatitis B virus (HBV) and/or hepatitis C virus (HCV) infections, which account for the vast majority of primary HCCs [3]. In areas with endemic HBV infection (where HBsAg prevalence is 8% or more), such as in sub-Saharan Africa and Eastern Asia, the disease burden is high with incidence rates of over 20 per 100, 000 individuals [4]. Potentially curative treatments for HCC include surgery (resection or transplant), radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI); globally, approximately 30-40% of HCC patients with very early-stage (Stage 0) or early-stage (Stage A) disease are eligible for these treatments [5]. Nevertheless, due to the current poor prognosis of HCC, novel diagnostic and prognostic biomarkers and therapeutic targets for HCC are urgently needed [6]. Therefore, determination of the molecular mechanisms that underlie HCC migration and invasion may aid in the identification of novel therapeutic targets and consequently lead to improved prognosis in the future [7].

Long noncoding RNA (LncRNA) is defined as an endogenous RNA that is longer than 200 nt in length [8]. Although many attributes of LncRNAs, such as patterns of expression, remain largely unknown, LncRNAs have been shown to play important roles in transcriptional, posttranscriptional, translational and epigenetic gene regulation [9, 10]. An increasing amount of evidence has revealed that different LncRNAs might be associated with HCC [11]. With regard to these latter effects, previous molecular-based studies found that certain LncRNAs promote tumor initiation, cancer cell growth, and metastasis during the development of HCC [12-14]. Human ovarian cancer-specific transcript 2 (HOST2) was once reported to be specifically expressed at a high level in human ovarian cancer [15]. A transcriptomic analysis demonstrated that an abnormal expression of LncRNAs, such as LncRNA HEIH in HCC tissues may promote tumor progression in patients with HBV-related HCC [16]. Moreover, HEIH expression is related to the recurrence and survival of HCC patients, which indicates that the expression of different LncRNAs might be associated with HCC [16, 17]. The janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling pathway plays an important role in the regulation of tumorigenesis and cell survival [18, 19]. In addition, recent studies have demonstrated the importance of the JAK/STAT signaling pathway in the development of HCC, which suggests the potential role of JAK/STAT inhibitors in the treatment of HCC [20, 21]. While the majority of research studies have focused on the discovery of protein-coding genes that are transcriptionally activated or repressed by JAK-STAT, a comprehensive understanding of regulatory networks must also include LncRNAs, whose expression is regulated by the JAK/STAT signaling pathway [22]. Thus, a better explanation of the disease-associated mechanisms of these small and single-stranded RNAs might provide new diagnostic and treatment modalities for diseases in the future. However, reports that are less relevant have also been published on LncRNA HOST2 expression in HCC, and less information is currently available with regards to the importance of the interactions between LncRNA HOST2 and the JAK2-STAT3 signaling pathway. Therefore, the present study aims to evaluate the potential role of LncRNA HOST2-mediated JAK2-STAT3 signaling on HCC cell migration and invasion.

Materials and Methods

Tissue samples

Between 2005 and 2011, 136 patients with pathologically diagnosed HCC who underwent surgery at the First Affiliated Hospital of Zhengzhou University were enrolled in this study. Among these subjects, 95 were male and 41 were female with a mean age of 52 years (range, 27 ~ 78 years). None of these subjects exhibited extrahepatic metastasis or received any therapeutic measures before surgery. In all, 136 paired HCC tissues and matched para-cancerous tissues (2 cm away from HCC tissues) were immediately obtained from fresh surgical specimens and were then immediately fixed in 10% formaldehyde solution and embedded in paraffin. After surgery, all subjects were followed-up for 12 to 56 months. Written informed consent was obtained from all subjects and/or their legal guardians. Ethical approval for this study was obtained from the Ethical Committee of the First Affiliated Hospital of Zhengzhou University.

Immunohistochemistry

The streptavidin-biotin-peroxidase (SP) method of immunohistochemistry was performed to detect the protein expression of JAK2 and STAT3 in HCC tissues and para-cancerous tissues. Paraffin-embedded samples were serially sectioned (4 μm thickness) and incubated at 60°C for 1 h, which was followed by deparaffinization in xylene and dehydration in decreasing concentrations of ethanol. After incubation in 3% H_2O_2 for 10 min and 3 washes in distilled water (3 min each time), the sections were subjected to antigen retrieval using a microwave oven, after which they were placed in an ice bath so that they could be cooled to room temperature. Then, the sections were washed in 0.01 M phosphate-buffered saline (PBS) (pH 7.4) and incubated with serum at 37°C for 40 min. The anti-human primary antibody against JAK2 (1:100, sc-278, Santa Cruz Biotechnology, CA, USA) and mouse anti-human primary antibody against STAT3 (1:150, sc-8019, Santa Cruz Biotechnology, CA, USA) were added to the sections, which were incubated at 37°C for 1 h, after which they were maintained at room temperature overnight. PBS was used in place of the primary antibody for the negative controls, which were washed 3 times in PBS for 3 min each time. After the biotin-labeled goat anti-mouse secondary antibody solution (1: 1000, AB1791, Abcam, Cambridge, MA, USA) was added, the sections were incubated at room temperature for 10 min. Subsequently, the tissues were incubated at room temperature for 10 min after the addition of HRP-streptavidin (E030100, EarthOx, San Francisco, CA, USA). Then, 3,3'-diaminobenzidine (DAB) (AR1000; Wuhan Boster Bioengineering Co., Ltd., Wuhan, Hubei, China) was added to develop a color-reaction, which was observed by microscopy (XSP-36, Shenzhen Boshida Optical Instrument Co., Ltd., Shenzhen, Guangdong, China).

All of the stained sections were assessed and evaluated by two independent pathologists who were blinded to this study [23]. JAK2 and STAT3 protein expression was located in the cytoplasm and indicated by brown granules in tissue color. The staining intensity scores were as follows: 0 points for no staining, 1 point for light yellow staining, 2 points for brown yellow staining, and 3 points for brown staining. The judgment criteria for protein expression score as follows: if the percentage of the stained cell count of the total number of counted cells was < 5%, the score was 0; if the percentage was 5% ~ 25%, the score was 1 point; if the percentage was 26% ~ 50%, the score was 2 points; if the percentage was more than 50%, the score was 3 points. The final score was the product of the above two scores: 0 points was the maximum value, less than 2 points indicated negative expression, and more than 2 points indicated positive expression [24].

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

After homogenization, the extraction of total RNA from HCC tissues was performed using an TRIZOL reagent (Invitrogen, Carlsbed, CA, USA). According to the manufacturer's instructions for the Quantitect Reverse Transcription Kit (Qiagen, Qiagen SpA., Milan, Italy) the extracted RNA was subjected to reverse

Table 1. Primer sequences for RT-qPCR. Note: RT-qPCR, reverse transcription-quantitative polymerase chain reaction; HOST2, human ovarian cancer-specific transcript 2; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; F, forward; R: reverse

Genes	Sequences (5'-3')
HOST2	F: FCTCAAATCAATCACGACCCT R: AATGTAGCAGGACGAGCC
JAK2	F: GTCAGCTACGATCGATCGAT R: CGTAGCTAGCCGGCATGCT
STAT3	F: CGTAGCGCTAGCTGATGCAT R: CTAGCGCTAGCTAGCTAGT
E-cadherin	F: CGGTGGTCAAAGAGCCCTTACT R: TGAGGGTTGGTGCAACGTCGTTA
vimentin	F: GAGAACTTTGCCGTTGAAGC R: GCTTCCTGTAGGTGGCAATC
GAPDH	F: ACAGTCCATGCCATCACTG R: AGTAGAGGCAGGGATGATG

transcription for cDNA preparation, and specific transcription primers were designed for HOST2, JAK2, STAT3, E-cadherin, vimentin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which were synthesized by TaKaRa Biotechnology (Dalian, China) (Table 1). Real-time fluorescent quantitative PCR was performed using the ABI 7500 Sequence Detection System (7500, ABI, Oyster Bay, NY, USA). The reaction conditions were pre-denaturation at 95°C for 10 min, and 40 cycles of denaturation at 95°C for 1 s, annealing at 60°C for 20 s, and extension at 72°C for 34 s. The reaction system contained SYBR Premix Ex TaqTM II 10 μL, PCR Forward Primer (10 μM) 0.8 μL, PCR Reverse Primer (10 μM) 0.8 μL, ROX Reference Dye 0.4 μL, cDNA template (2.0 μL), and sterile distilled water (6.0 μL). GAPDH served as an internal control the expression levels of HOST2, STAT3, E-cadherin, and vimentin. The $2^{-\Delta\Delta Ct}$ method presents the ratio of gene expression levels between the experimental group and the control group. ΔΔCt for each experimental sample was calculated as $\Delta\Delta Ct = \Delta Ct_{experimental} - \Delta Ct_{control}$. ΔCt for each sample was calculated as $Ct_{target\ gene} - Ct_{internal\ control}$. The Ct represents the amplification cycles when RT-qPCR reached the threshold. At this point, the growth was in the logarithmic phase. Each experiment was repeated 3 times. Forty-eight hours after transfection, the cells were collected to detect the mRNA expression according to the experimental method described above [25].

Western blotting

Western blot was performed to detect the protein expression of JAK2, STAT3. First, 1 mL tissue lysis buffer was added to the HCC tissues in a glass grinder [components: 50 mmol/L Tris, 150 mmol/L NaCl, 5 mmol/L ethylene diamine tetraacetic acid (EDTA), 0.1% sodium dodecyl sulfate (SDS), 1% NP-40, 5 μg/mL Aprotinin and 2 mmol/L phenylmethylsulfonyl fluoride (PMSF)]. The grinder was then placed in an ice bath to grind the homogenate; the protein lysate was finally incubated and vibrated at 4°C for 30 min and placed on a shaker for 10 min. The lysate was centrifuged for 20 min at 4°C and 12, 000 r/min to remove the grease layer. The resulting supernatant was used to test the protein concentration of each sample using a bicinchoninic acid (BCA) protein assay kit (20201ES76, Yi Sheng Biotechnology Co., Ltd., Shanghai, China). Finally, deionized water was added to adjust the sample size to 30 μg of protein per lane. After a 10% SDS separation gel and a stacking gel were prepared, the sample were added and mixed with loading buffer, which was followed by boiling at 100°C for 5 min. The boiling, incubation and centrifugation steps were conducted, and pipettes were used to add an amount of sample into each lane for electrophoretic separation. Next, the proteins on the gels were transferred to a nitrocellulose membrane. Then, the NCF was blocked in 5% skim milk powder at 4°C overnight. The membrane was incubated overnight with diluted rabbit polyclonal primary antibodies against JAK2 (ab38642, 1:500), STAT3 (ab93446, 1:1000), E-cadherin (ab77287, 1:500), vimentin (ab61780, 1:500), Bcl-2 (ab124512, 1:1000), Snail (ab53519, 1:1000), Slug (ab27568, 1:1000), Twist (ab50581, 1:1000) and GAPDH (ab9485, 1:2500) (all from Abcam Inc., Cambridge, MA, USA). The membrane was washed three times in PBS for 5 min each time. Immunoglobulin G (IgG) polyclonal antibody (bs-0361R-HRP, BOSS, Beijing, China) (1:200) labeled by horseradish peroxidase (HRP) was added to the membrane, which was taken and incubated for 1 h and washed 3 times in PBS for 5 min each time. Then, the HRP polyclonal antibody reacted with enhanced chemiluminescence (ECL) reagent (ECL808-25, Biomiga, USA) at room temperature for 1 min. This was followed by X-ray film development (36209ES01, Shanghai qcbio Science & Technologies Co., Ltd., Shanghai, China). With GAPDH as an internal control, the ratio between Bcl-2 and β-actin was taken to indicate the relative expression of the proteins. Forty-eight hours after transfection, the cells were collected and incubated in an ice bath for 30 min after the addition of 100 μL cell lysis buffer (YM-C1591, YuanMu Biological Technology Co. Ltd., China). Then, the samples were centrifuged at 4°C at 12, 000 r/min for 10 min to obtain supernatant. Finally, the procedure was conducted according to the aforementioned steps.

Cell sources

The human HCC cell line SMMC7721 (284, Cell Repository of the Chinese Academy of Sciences, China) was cultured in Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in an incubator containing 5% CO_2 and saturated humidity at 37°C. Cell passaging was performed once every 2 to 3 days. Cells in the logarithmic growth phase were collected for all experiments.

Cell transfection and grouping

SMMC7721 cells in a logarithmic growth phase were seeded in six-well plates and cultured until the cell density reached 50% confluence, at which point the cells were transfected using Lipofectamine 2000 (11668019, Thermo Fisher Scientific, USA). A total of 250 μL Opti-MEM (31985, Gibco, USA) was used to dilute 100 pmol HOST2 mimics, HOST2 inhibitors and negative control (added at a final concentration 50 nM); the solution was gently mixed and incubated at room temperature for 5 min. Then, 250 μL Opti-MEM was used to dilute 5 μL Lipofectamine 2000 reagent, which was gently mixed and incubated at room temperature for 5 min. After a second incubation at room temperature for 20 min, the mixture was added the cell culture wells. After culture in 5% CO_2 at 37°C for 6 to 8 h, the mixture was exchanged for complete medium (INV-00002, INNOVATE, Wuxi, Jiangsu, China), and the cells were cultured for another 24 h for further experiments. The cells were divided into the following four groups: (1) blank group (no transfection); (2) negative control group (NC group, negative control of HOST2 inhibitor group); (3) HOST2 mimic group (the cells transfected with HOST2 mimics); (4) HOST2 inhibitor group (cells transfected with HOST2 inhibitors).

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay

After transfection, when the cell density reached 80% confluence, the cells were washed 2 times in PBS; then, 0.25% trypsin (C0201, Beyotime Biotechnology Co., Shanghai) was used to digest the cells to generate a single cell suspension. After the cells were counted, cells in each group were collected, plated in 96-well plates at a density of 3 × 10^3 to 6 × 10^3 cells per well (200 μL media and cultured in an incubator. After 24 h, 48 h and 72 h, the cells were removed from the culture plates. When 10% MTT solution (5 g/L) (GD-Y1317, Guduo Biological Technology Co Ltd, Shanghai, China) was added to each culture plate, the cells were again cultured for 4 h, and then the grease layer was removed. Then, 100 μL dimethyl sulfoxide (DMSO) (D5879-100ML, Sigma, USA) was added, which was followed by shaking and gentle mixing for 10 min to fully dissolve the formazan crystals produced by living cells. Subsequently, a microplate reader (BS-1101, DeTie Laboratory Equipment Co Ltd, Nanjing, Jiangsu, China) was used to measure the optical density (OD) value of each well at 570 nm. The OD rates represented the speed of cell proliferation. The experiment was repeated three times, and a growth curve was generated for the mean OD value (y-axis) over time (x-axis).

Cell scratch test

Cells in the logarithmic growth phase were digested in trypsin. After centrifugation, the cell suspension was plated in 6-well plates and cultured at 5% CO_2 in an incubator at 37°C overnight. When the cells became 80% to 90% confluent, a 10 μL gun head was used to draw several scratches with the same force in the middle of each well. The plates were then washed twice in PBS. The cells were cultured for another 48 h, and then the migration rate of cell migration in the three groups was measured at random under the microscope.

Transwell assay

Trypsin and serum-free medium were used to digest the cells in a logarithmic growth phase and to prepare cells to an equal density, respectively. A total of 100 μL cell diluent from each group was seeded in the upper layer of a Transwell chamber, while 10% DMEM containing 10% FCS (600 μL) was added to the lower layer. Transwell chambers were removed, and cotton buds were used to wipe off the culture medium in the upper layer as well as the cells that failed to penetrate the upper layer. Then, 4% methanol was added to the chambers for fixation, which was followed by a Giemsa stain. Subsequently, a high-power lens was used to observe the number of cells that penetrated the membrane.

Statistical analysis

Data were analyzed using the SPSS 21.0 (IBM Corp. Armonk, NY, USA) software package. Measurement data are presented as the mean ± standard deviation (SD). Two groups were compared using a *t*-test, and multiple groups were compared using a one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

Results

JAK2 and STAT3 protein expression is higher in HCC tissues than in para-cancerous tissues

To detect whether JAK2 and STAT3 are differentially expressed in HCC tissues, immunohistochemistry was performed. The results are shown in Fig. 1. JAK2 and STAT3 proteins were primarily expressed in the cytoplasm of hepatocytes. JAK2 was expressed in 83 (61.35%) HCC tissues but was expressed in only 8 (5.80%) para-cancerous tissues. STAT3 was expressed in 98 (71.80%) HCC tissues and 17 (12.50%) para-cancerous tissues. Thus, HCC tissues exhibited significantly higher expression of JAK2 and STAT3 than para-cancerous tissues (all $P < 0.05$).

HOST2, JAK2, STAT3 and vimentin mRNAs are expressed at a high level and E-cadherin mRNA is expressed at a low level in HCC tissues

RT-qPCR was used to detect the mRNA expression levels of HOST2, JAK2, STAT3 and vimentin in HCC and para-cancerous tissues. As shown in Fig. 2, the RT-qPCR results demonstrated that HOST2, JAK2, STAT3 and vimentin mRNAs were expressed at a higher level in HCC tissues compared with para-cancerous tissues, while the expression of E-cadherin mRNA was lower in HCC tissues (all $P < 0.05$).

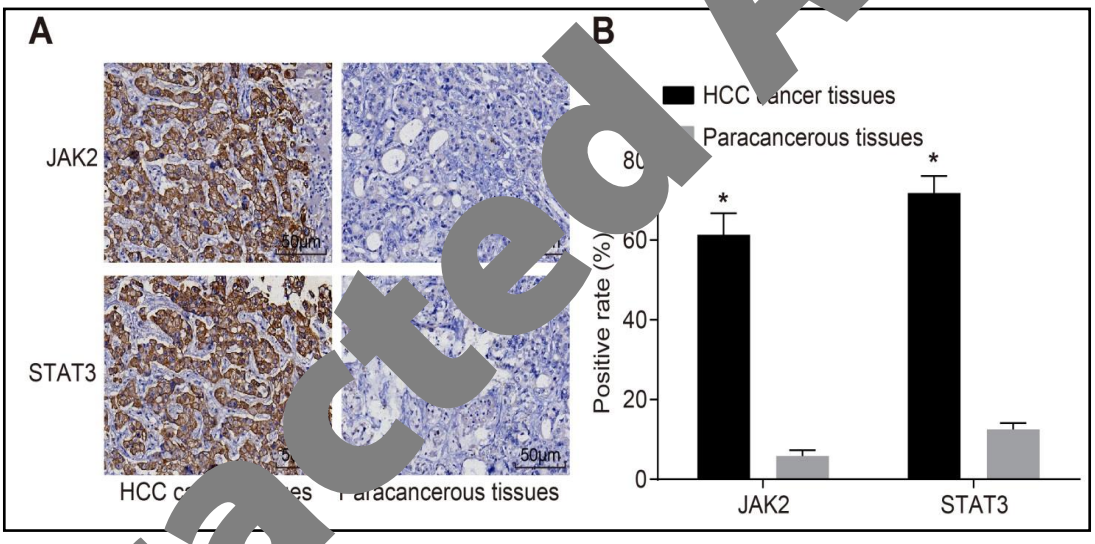

Fig. 1. Positive rate of JAK2 and STAT3 protein expression is higher in HCC tissues. Note: A: Protein expression of JAK2 and STAT3 in HCC and para-cancerous tissues; B: Positive rate of JAK2 and STAT3 protein expression in HCC and para-cancerous tissues; *, P<0.05, compared with para-cancerous tissues; Analysis of data in the map using a paired t test, n = 136; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; HCC, hepatocellular carcinoma.

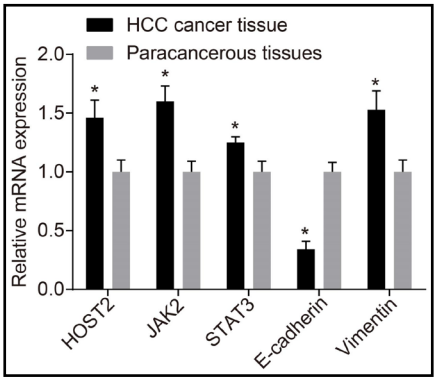

Fig. 2. HOST2, JAK2, STAT3 and vimentin are expressed at a high level, while E-cadherin is expressed at a low level in HCC tissues. Note: *, P<0.05, compared with para-cancerous tissues. Analysis of data in the map using a paired t test, n = 136; HCC, hepatocellular carcinoma; HOST2: human ovarian cancer-specific transcript 2; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

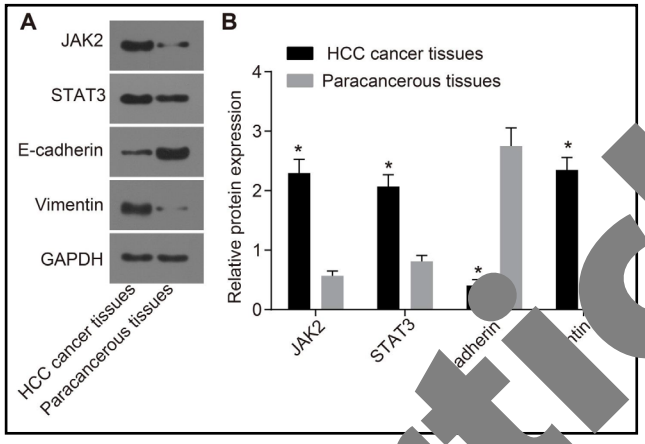

Fig. 3. JAK2, STAT3 and vimentin proteins are expressed at a high level, while E-cadherin is expressed at a low level in HCC tissue. Note: A: Gray value of JAK2, STAT3, E-cadherin and vimentin protein bands in HCC and para-cancerous tissues; B: Relative protein expression of JAK2, STAT3, E-cadherin and vimentin in HCC and para-cancerous tissues; *, P<0.05, compared with para-cancerous tissues. Analysis of data in the map using a paired t test, n = 136; HCC, hepatocellular carcinoma; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

JAK2, STAT3 and vimentin proteins are expressed at a high level and E-cadherin protein is expressed at a low level in HCC tissues

Western blotting was used to detect the protein levels of JAK2, STAT3, vimentin and E-cadherin in HCC and para-cancerous tissues. The Western blotting results are shown in Fig. 3, which suggested that compared with para-cancerous tissues, the expression of JAK2, STAT3, vimentin proteins was higher in HCC tissues, while the expression of E-cadherin protein was lower in HCC tissues (all $P < 0.05$).

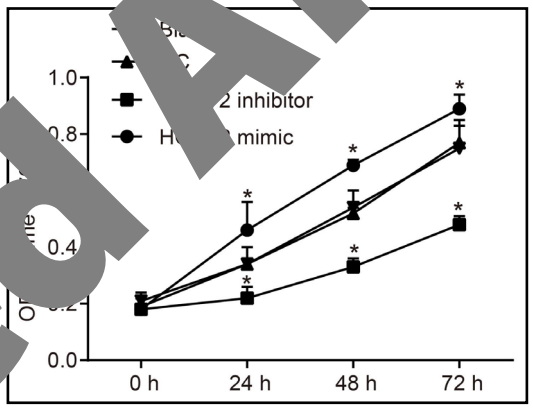

Fig. 4. HOST2 increases the proliferation of HCC cells. Note: *, P<0.05, compared with the blank control group. Analysis of data in the map using a paired t test, n = 3; OD, optical density; NC, negative control; HOST2: human ovarian cancer-specific transcript 2; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

HOST2 increases the proliferation of HCC cells

An MTT assay was performed to explore the effect of HOST2 expression on HCC cell viability. According to the results, no notable difference was observed in cell viability between the blank and NC groups ($P > 0.05$). Compared with the blank and NC groups, the cell growth rate and cell viability as well as the OD values at 48 h and 72 h in the HOST2 inhibitor group were significantly decreased, while cell viability and OD values at 48 h and 72 h in the HOST2 mimic group were significantly increased (all $P < 0.05$) (Fig. 4). These data indicated that LncRNA HOST2 may increase the viability of HCC cells.

HOST2 increases cell migration of HCC cells

To observe the migration ability of the cells, a cell scratch test was performed. As shown in Fig. 5, the scratch test revealed that, after a 48 h culture, no significant difference was observed in the migration distance between the blank group (411.34 ± 61.23) μm and the NC group (418.32 ± 56.21) μm ($P > 0.05$). Compared with the blank and NC groups, the migration ability of HCC cells was distinctly less in the HOST2 inhibitor group (248.26 ± 30.32) μm but was increased in the HOST2 mimic group (613.17 ± 71.43) μm ($P < 0.05$). These results suggested that LncRNA HOST2 may increase the migration ability of HCC cells.

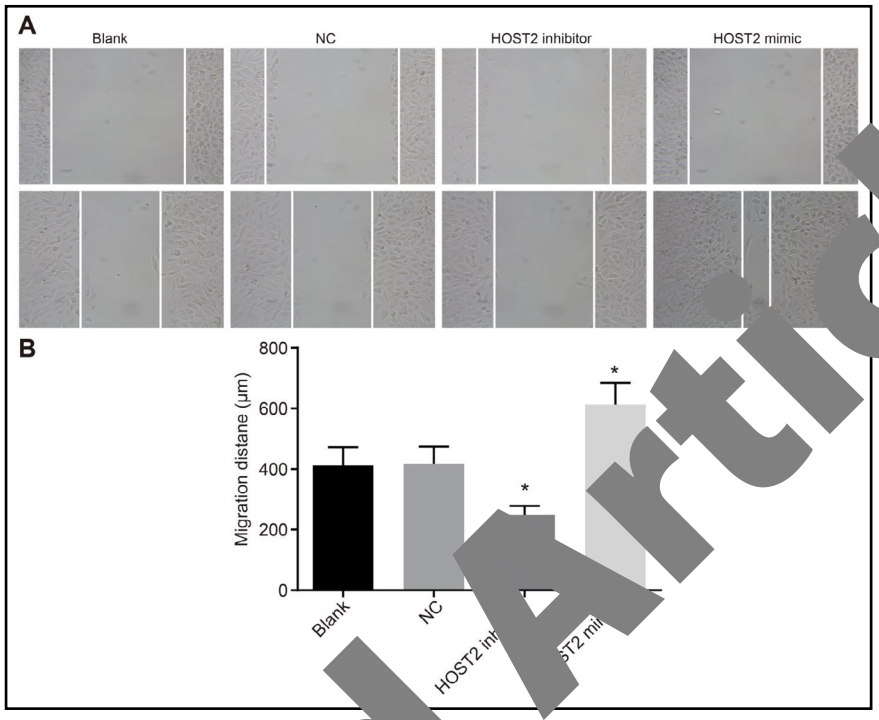

Fig. 5. HOST2 increases the migration ability of HCC cells. Note: A: Results of the cell scratch test in the four transfection groups; B: The migration ability of HCC cells in the four transfection groups; *, P<0.05, compared with the blank control group. Analysis of data in the map using a paired t test, n = 3; NC, negative control; HOST2: human ovarian cancer-specific transcript 2.

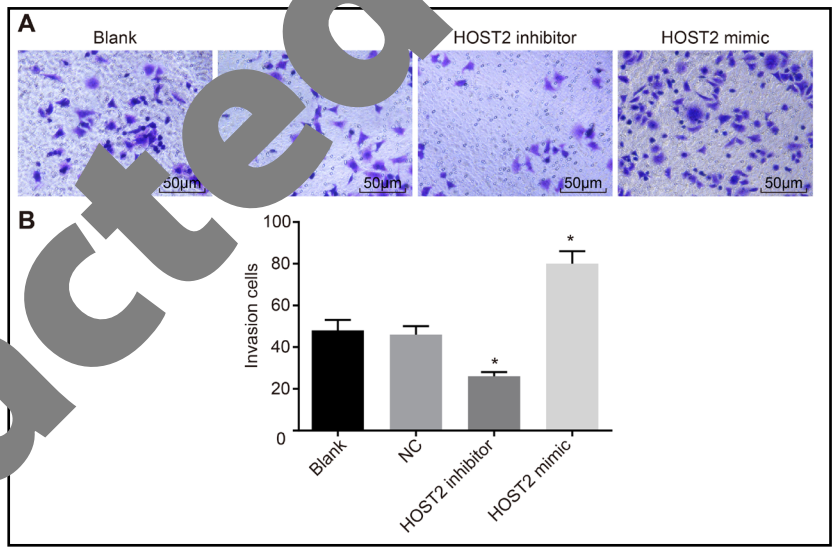

Fig. 6. HOST2 enhances the invasion ability of HCC cells. Note: A: Results of the Transwell assay in the four transfection groups; B: The invasion ability of HCC cells in the four transfection groups; *, P<0.05, compared with the blank control gro... Analysis of data ... map using a p...re... test, n = 3; ... neg... control; ... 2: hum... c... ... specific ...ipt 2.

... enhances the invasion ability of HCC cells

To further verify the effect of HOST2 on cell invasion, a Transwell assay was performed. Aft... a 48 h culture, the cell invasion ability did not differ significantly between the blank ...65 ± 4.13) and NC (47.67 ± 3.06) groups ($P > 0.05$). Compared with the blank and NC groups, the invasion ability of HCC cells was notably reduced in the HOST2 inhibitor group (25.76 ± 2.09) but was increased in the HOST2 mimic group (73.22 ± 5.86) ($P < 0.05$) (Fig. 6).

HOST2 leads to the increased expression of HOST2, JAK2, STAT, E-cadherin and vimentin mRNA in HCC cells

RT-qPCR was used to explore the effect of HOST2 on the mRNA expression levels of JAK2-STAT3 signaling pathway-related genes, and the results (Fig. 7) revealed no significant difference in the mRNA expression levels of HOST2, JAK2, STAT, E-cadherin and vimentin between the blank and NC groups (all $P > 0.05$). Compared with the blank and NC groups, in the HOST2 inhibitor group, the mRNA expression of HOST2, JAK2, STAT3 and vimentin was remarkably decreased (all $P < 0.05$), but the mRNA expression of E-cadherin was increased ($P < 0.05$). The HOST2 mimic group exhibited significantly increased mRNA expression of HOST2, JAK2, STAT3 and vimentin and an obvious decrease in the mRNA expression of E-cadherin (all $P < 0.05$).

HOST2 upregulates the expression of JAK2, STAT3 and vimentin proteins, but decreases the expression of E-cadherin protein

Western blotting was used to detect the effect of HOST2 on the expression of JAK2, STAT3, E-cadherin and vimentin proteins in HCC cells. The results revealed no significant difference in the expression of these proteins between the blank and NC groups (all $P > 0.05$). Compared with the blank and NC groups, in the HOST2 inhibitor group, the protein levels of JAK2, STAT3 and vimentin were significantly decreased, but the E-cadherin protein was increased (all $P < 0.05$). In the HOST2 mimic group, the protein [illegible] STAT3 and vimentin were increased, but the E-cadherin protein level was decreased (all $P < 0.05$), which demonstrated that HOST2 may increase EMT through activation of the JAK2/STAT3 signaling pathway.

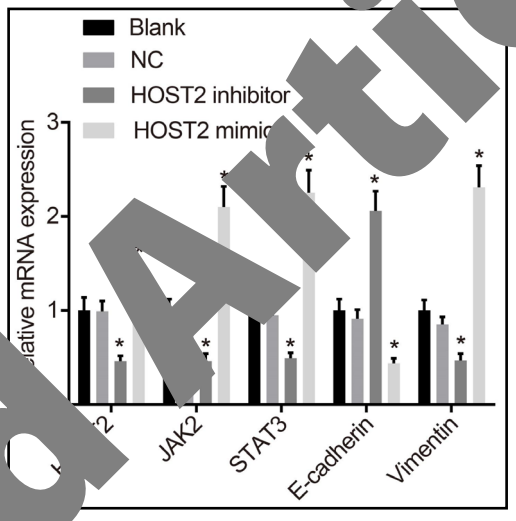

Fig. 7. HOST2 elevates the mRNA expression levels of HOST2, JAK2, STAT, E-cadherin and vimentin in HCC cells. Note: *, P<0.05, compared with the blank control group. Analysis of data in the map using a paired t test, n = 3; HOST2: human ovarian cancer-specific transcript 2; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

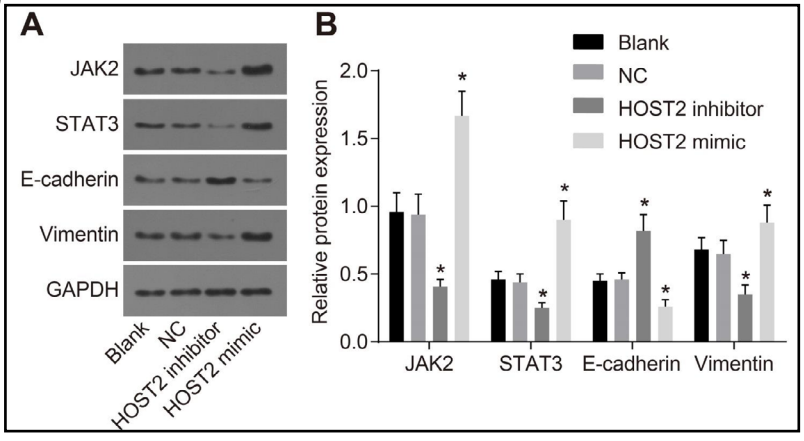

Fig. 8. HOST2 upregulates the protein expression of JAK2, STAT3, E-cadherin and vimentin. Note: A: JAK2, STAT3, E-cadherin and vimentin protein bands in the four transfection groups; B: Relative protein expression of JAK2, STAT3, E-cadherin and vimentin in the four transfection groups; *, P<0.05, compared with the blank control group. Analysis of data in the map using a paired t test, n = 3; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NC, negative control.

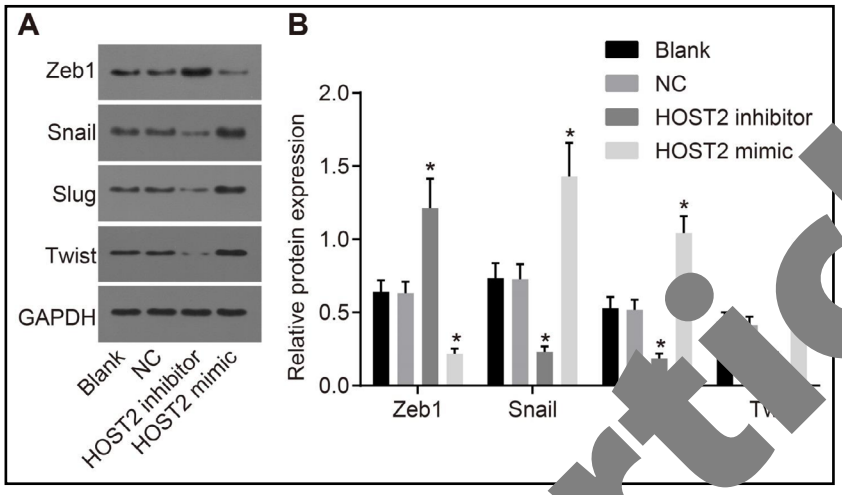

Fig. 9. HOST2 may regulate EMT by inducing Snail, Slug, Twist and Zeb1. Note: A: Zeb1, Snail, Slug and Twist protein bands in the four transfection groups; B: Relative protein expression levels of Zeb1, Snail, Slug and Twist in the four transfection groups; *, P<0.05, compared with the blank control group. Analysis of data in the map using a paired t test, n = 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; EM[illegible]ithelial-m[illegible]chymal transition; NC, negative control.

HOST2 may regulate EMT by inducing the expression of S[illegible]ug, Twist and Zeb1

To further explore the main factors that [illegible]gulate EM[illegible] HCC cells, we performed additional related experiments. Western blo[illegible] was used [illegible] detect the expression of EMT-related factors, including Zeb1 [26], Snail [illegible]lug [28], and Twist [29]. The results revealed no significant difference in the expre[illegible]e proteins between the blank and NC groups (all $P > 0.05$). Compared with the [illegible]k a[illegible] groups, in the HOST2 inhibitor group, the protein levels of Snail, Slug and Twi[illegible] significantly decreased, but the Zeb1 protein level was increased (all $P < 0$[illegible]. [illegible]he HOST2 mimic group, the protein levels of Snail, Slug and Twist were increased, [illegible] Ze[illegible]1 protein level was decreased (all $P < 0.05$), which demonstrated that HO[illegible]2 [illegible]ay [illegible]at[illegible]MT by inducing the expression of Snail, Slug, Twist and Zeb1 (Fig. 9).

Discussion

HCC is one [illegible]mos[illegible]mmon malignancies (the seventh most common cancer in males and the [illegible]t common in females), as it reaches an incidence of one million new cases [illegible]very [illegible]r [illegible]. In recent years, research on the biological functions of lncRNAs in vario[illegible]ncer[illegible]ding HCC [6, 31, 32], has exponentially grown. Here, we have shown that Lnc[illegible] HOST2 may increase EMT and accelerate proliferation, invasion and metastasis [illegible] cel[illegible]ough upregulation of the JAK2-STAT3 signaling pathway.

[illegible]itially, [illegible]r experiments on HCC cells demonstrated that the upregulation of HOST2 expr[illegible]ncreased cell proliferation, migration and invasion *via* the JAK2/STAT3 signaling [illegible]hway. This was in agreement with a previous study, which provided evidence that the [illegible]ncreased expression of LncRNAs in HCC tissues was primarily related to HBV infection [33]. D[illegible]e to the influence of many LncRNAs on several cell functions such as proliferation, apoptosis, [illegible]fferentiation and transformation, alterations in LncRNAs may lead to direct changes in the cellular response to both physiologic and pathologic processes [34]. A recent study also showed that persistently activated STAT3 enhances tumor cell proliferation, survival and invasion, while it suppresses anti-tumor immunity [35]. It has also been demonstrated that multiple LncRNAs are associated with HCC. For example, Wang *et al.* revealed that LncRNA-UCA1 upregulation and miR-216b inhibition could promote the progression of HCC [36], and Lv *et al.* showed that increased LncRNA H19 contributes to migration and invasiveness of HCC cells, which is accompanied by miR-675 downregulation [37]. STAT3 signaling promotes HCC progression through the suppression of apoptosis through the induction of

the expression of anti-apoptotic factors of the Bcl-2 family such as Mcl-1 and Bcl-xl [38]. Therefore, we assumed that HOST2 and the JAK2/STAT3 pathway may play an important role in HCC cell migration and invasion. To achieve a better understanding of the mechanisms of cell invasion and migration in terms of the JAK2/STAT3 signaling pathway, the role of this pathway as it relates to HOST2 was investigated.

Moreover, the results of our study also showed that the mRNA and protein expression levels of HOST2, JAK2 and STAT3 were higher in HCC tissues than in para-cancerous tissues and were also higher in the HOST2 mimic group. As an LncRNA, HOST2 expression is strongly associated with cancer development and progression [39]. The JAK2/STAT3 signaling pathway was shown to be a crucial pathway in the induction of autophagy in response to oxaliplatin [40]. Despite that tyrosine kinase signaling occurs through multiple pathways, signal transducer and activation of transcription 3 (STAT3) is a point of convergence for many nonreceptor and receptor tyrosine kinases and is constitutively activated and expressed at a high frequency in a wide range of cancer cells [41]. One of the hallmarks of inactivation of the JAK-STAT signaling pathway is the restriction in translocation of the nuclear and accumulated STAT3 in the cytoplasm [42]. Moreover, recent studies have revealed the importance of the JAK/STAT pathway in the development of HCC and have indicated that JAK/STAT inhibitors might be able to be used in the treatment of HCC [21, 43].

In addition, the results of RT-qPCR and Western blotting assays in HCC cells verified that the mRNA and protein expression levels of vimentin were increased in HCC tissues and in the HOST2 mimic group, while the mRNA and protein expression levels of E-cadherin were lower. This demonstrated that LncRNA HOST2 was involved in the upregulation of EMT in HCC cells. EMT involves multiple components, such as vimentin and E-cadherin. vimentin, a well-known metastasis marker, has been a therapeutic target because of its function in the reduction of cell migration [44]. vimentin expression is an late event in EMT and is preceded by a loss of epithelial features, which leads to the upregulation of mesenchymal genes [45]. E-cadherin, which promotes cell-cell contact and suppresses the malignant invasion and metastasis of epithelial cells, is associated with the invasiveness of HCC cells [46]. The loss of E-cadherin enables metastasis by disrupting intercellular contacts, an early step in metastatic dissemination [29]. Moreover, one of the hallmarks of EMT is the downregulation of E-cadherin (a cell adhesion molecule which is a transmembrane protein involved in the establishment of stable adherent junctions; other hallmarks of EMT include the upregulation of mesenchymal markers (vimentin, fibronectin and/or N-cadherin) [47]. Additionally, we also found that in the HOST2 mimic group, the protein levels of Snail, Slug and Twist were increased but the protein level of Zeb1 was decreased. Twist, Slug, and Snail, which control EMT during embryonic development, are highly expressed in multiple tumor types and are closely associated with metastasis and poor prognosis [48, 49]. Interestingly, Twist and ZEB1, which are known to repress E-cadherin, were upregulated following E-cadherin loss [29].

Conclusion

In conclusion, our study provided evidence that the increase in LncRNA-HOST2 demonstrated a great ability to promote HCC cell proliferation, migration and invasion. Nevertheless, in order to provide more precise estimates as to the confirmation of the effects of LncRNA-HOST2 in HCC patients *via* the JAK2-STAT3 signaling pathway, more studies with a larger sample size are essential. Additional studies may lead to the development of a new therapeutic genetic strategy for patients with HCC.

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Disclosure Statement

The authors declare no competing interests.

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