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Knockdown of HJURP inhibits non-small cell lung cancer cell proliferation, migration, and invasion by repressing Wnt/β-catenin signaling

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Abstract. – OBJECTIVE: Holliday junction-recognizing protein (HJURP) was found to be upregulated in several tumors, including non-small cell lung cancer (NSCLC), and the effect of HJURP on NSCLC remains unknown. The objective of the present study was to explore the clinical significance of HJURP and its function in regulating the progression of NSCLC.

PATIENTS AND METHODS: Reverse Transcriptions-Polymerase Chain Reaction (RT-PC) and Western blot were performed to detect the expression levels of HJURP in NSCLC tissues and cell lines. The association of HJURP expression level with various important clinicopathological parameters was evaluated. Then, the effects of HJURP expression on tumor cell behavior *in vitro* were analyzed by the Cell Counting Kit-8 (CCK-8), EdU assays, colony formation, flow cytometry, and transwell assays. The protein levels of related proteins of the Wnt/β-catenin pathway were determined using the Western blot assay.

RESULTS: Our study showed that HJURP was significantly upregulated in both NSCLC tissues and cell lines. The higher expression of HJURP was associated with advanced TNM stage, distant metastasis, and poor prognosis. Our data from *in vitro* assays confirmed that the knockdown of HJURP suppressed NSCLC cells proliferation, migration, invasion, epithelial-mesenchymal transition (EMT) progress, and induced cells apoptosis. Notably, through Western blot analysis, we found that HJURP suppression remarkably decreased β-catenin, cyclin D1 and c-myc expression level in NSCLC cell lines.

CONCLUSIONS: Our findings provide clues regarding the role of HJURP as a tumor promoter in NSCLC via the activation of the Wnt/β-catenin pathway, indicating HJURP may be a promising therapeutic target for NSCLC.

Key Words:
HJURP, Wnt/β-catenin pathway, NSCLC, Prognosis, Metastasis, Proliferation.

Introduction

Lung cancer is the most frequent malignancy and a major cause of cancer-related death in the world, accounting for 18% (1.4 million) of cancer deaths in 2015 in the light of global cancer statistics[1]. In China, cancer data show that approximately over half a million patients died from lung cancer each year[2]. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer[3]. This disease is often diagnosed at an advanced stage because of its speedy growth and early tendency to spread to other organs and tissues[4,5]. Despite the rapid advancements in chemotherapy, radiation therapy, and lung resection for NSCLC patients, the 5-year survival rate of NSCLC is still below 15% related to recurrence and distant metastasis of this disease[6,7]. Therefore, further studies are necessary to better understand the pathogenesis of NSCLC.

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The Holliday junction recognition protein (HJURP) is an exclusive companion for the centromere CENP-A deposition during the early G1 phase[8]. In the human being, HJURP has been demonstrated to be a critical regulator of DNA binding and phosphorylation that is involved in the regulation of chromosomal segregation and cell division[9,10]. Emerging evidence reveals that HJURP expression was significantly up-regulated after DNA damage inductions, collaborates with components of the DNA repair machinery, and functions in homologous recombination[11,12]. Recently, the biological effects of HJURP attracted growing attention due to its dysregulation in several tumors such as hepatocellular carcinoma, bladder cancer, and glioblastoma[13-15]. In addition, the oncogenic roles of HJURP were also reported. For instance, Chen et al[16] reported that HJURP expression was increased in hepatocellular carcinoma and its knockdown resulted in the. decreased cell growth *in vitro* and *in vivo* by destabilizing p21. On the other hand, the prognostic value of high HJURP expression was also confirmed in breast carcinoma and ovarian carcinoma[17,18]. In lung cancer, HJURP was also reported to be overexpressed in lung cancer[19]. However, its potential function and the mechanism involved in the progression of NSCLC remain largely unclear.

In this study, we found that HJURP was up-regulated in NSCLC, and its expression was associated with TNM stage, distant metastasis and poor prognosis in patients with NSCLC. Moreover, HJURP silencing decreased NSCLC cell proliferation and metastasis, and increased apoptosis via suppression of the Wnt/β-catenin signaling. The present data provided novel insights into the potential molecular effects of HJURP/Wnt/β-catenin signaling in NSCLC and will offer a novel therapeutic target for the treatment of NSCLC.

Patients and Methods

Human Samples

Paraffin-embedded tumor tissue samples and adjacent normal tissue specimens from 74 NSCLC patients that underwent surgery from April 2015 to August 2017 were collected in Sichuan Cancer Hospital & Institute. Tissues were collected immediately after surgical resection, frozen in liquid nitrogen, and preserved at –80°C. Prior to surgery, all the patients did not receive any antitumor therapy. This investigation was approved by the Ethics Committee of Sichuan Cancer Hospital & Institute and the informed consent was obtained from all the patients who participated in the study.

Cell lines And Cell Culture

All cell lines used in this work (BEAS-2B, H1975, A549, HCC827, and H1299) were purchased from Shanghai Ruiyu Biological Co. Ltd. (Xuhui, Shanghai, China). The BEAS-2B cells were human immortalized epithelial cells and other cell lines were NSCLC cells. Cells were all cultured using Sciencell Roswell Park Memorial Institute-1640 (RPMI-1640) medium (ZQXZ Biotechnology, Yangpu, Shanghai, China) containing 1% antibiotics and 10% fetal bovine serum (FBS) in an incubator with 5% CO_2 at 37°C.

Cell Transfection

The small interfering RNA (siRNA) against HJURP were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Songjiang, Shanghai, China). The cell transfection was conducted using a siRNA Fect transfection kit (KangLing, Minhang, Shanghai, China). Briefly, cells after transfection were trypsinized and re-plated into 12-well plates. After the cell confluence reached 60-70%, 5 μl of siRNAFect reagent was mixed with siRNAs (50 pmol) in Opti-MEM medium for 15-20 min. Then, the mixture was added into cells and the medium was changed with fresh complete medium after 5-7 h.

Reverse Transcriptions PCR Assays

Total RNA from relevant tissue samples or NSCLC cells were extracted using TRIzol reagent. After the concentration of the isolated RNA was determined by a QuickDrop spectrophotometer apparatus (Molecular Devices, Pudong, Shanghai, China), 2 μg of total RNA was then reversely transcribed into cDNA using a Ray script cDNA Synthesis kit (Generay, Xuhui, Shanghai, China). The qRT-PCR assays were subsequently carried out to detect the mRNA levels of HJURP, β-catenin, cyclin D1, and c-myc using a SYBR Real-Time PCR kit (GenePharma, Suzhou, Jiangsu, China). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control of the mRNA levels determination. Fold changes in mRNA levels were calculated using

Table I. Sequence of the primers used in this study.

Genes	Primer sequences(5′-3′)
HJURP: Forward	CCACGCTGACCTACGAGAC
HJURP: Reverse	CTCACCGCTTTTTGAATCGGC
GAPDH: Forward	GGAGCGAGATCCCTCCAAAAT
GAPDH: Reverse	GGCTGTTGTCATACTTCTCATGG

the $2^{-\Delta\Delta Ct}$ method. All the primers used in this study were displayed in Table I.

Western Blot Assays

Cells were lysed with radioimmunoprecipitation assay (RIPA) lysis buffer (Tide Radar, Haidian, Beijing, China). Afterwards, a bicinchoninic acid (BCA) protein assay kit (Rong-Bio, Minhang, Shanghai, China) was applied to measure the concentrations of the protein samples. Then, 20 μg (per well) of proteins were separated using 8-10% of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Next, proteins were electronically transferred to the polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Thereafter, primary antibodies targeting the following proteins: vimentin, N-cadherin, β-catenin, cyclin D1, and c-myc, were separately employed to incubate the membranes overnight at 4°C. On the second day, Tris-Buffered Saline and Tween (TBST) was used to wash the membranes three times. Membranes were further incubated with corresponding secondary antibodies. Finally, an enhanced chemiluminescence (ECL) Western blotting Substrate kit (Qunji, Qingpu, Shanghai, China) was utilized to measure these proteins on a GelView 6000 Pro detection system (New Biochem, Guangzhou, Guangdong, China). The anti-N-cadherin, anti-cyclin D1, and anti-GAPDH primary antibodies were purchased from CusaBio Co. Ltd. (Wuhan, Hubei, China). The anti-vimentin, anti-β-catenin, anti-c-myc primary antibodies were obtained from Aviva System Biology Co. Ltd. (Daxing, Beijing, China).

Cell Counting Kit-8 (CCK-8) Assays

A CCK-8 assay kit (XuanLing, Jinshan, Shanghai, China) was utilized to determine the proliferative rates of A549 and H1299 cells after transfection with HJURP siRNAs. In brief, the treated cells were collected and re-plated into 96-well plates (2000 cells/well). A microplate reader (NYW-MB96; NYAW, Fengtai, Beijing, China) was employed to examine the optical density (OD) absorbance at 490 nm (OD 490 nm) after the CCK-8 reagent (10 μl) was added into each well for 1-2 h. The values of OD 490 nm were determined on 2, 3, and 4 days after being plated in the plates.

Table II. Relationship between HJURP expression and clinicopathologic features in NSCLC.

Characteristic	Case number	HJURP expression		*p*-value
		High	Low	
Age (years)				0.345
<60	39	18	21	
≥60	35	20	15	
Gender				0.363
Male	51	28	23	
Female	23	10	13	
Histological type				0.337
Adenocarcinoma	41	19	22	
Squamous carcinoma	33	19	14	
TNM stage				0.018
I+II	45	18	27	
III+IV	29	20	9	0.033
Distant metastasis				
Yes	19	14	5	
No	55	24	31	
Lymph node status				0.194
Yes	19	13	6	
No	55	25	30	

EdU Assays

EdU assays using the Cell-Light™ EdU Imaging Kit (RiboBio, Guangzhou, Guangdong, China) were also performed to assess the proliferation of A549 and H1299 cells after transfection with indicated siRNAs. Briefly, treated cells were plated into 48-well plates (3000 cells/well) and cultured for 48 h. Subsequently, the cells were treated with EdU reagent (10 μmol/L) for 2 h at 37°C, followed by a treatment with paraformaldehyde (4%), Triton X-100 (0.5%) and Apollo reaction cocktail (100 μl). After being stained with 4',6-diamidino-2-phenylindole (DAPI) for 20 min, cells were observed and photographed using an IX70 fluorescence microscope (Olympus, Shinjuku, Tokyo, Japan).

Cell Colony Formation Assays

A549 and H1299 cells after transfection with the indicated siRNAs were maintained in 6-wells plates (at a density of 1000 cells/well) at 37°C with 5% CO_2 for 2-3 weeks. Then, paraformaldehyde (4%) was utilized to fix the cell colonies and crystal violet dye (0.1%; Sigma-Aldrich, Pudong, Shanghai, China) was applied to stain the colonies. Colonies with more than 50 cells were then counted and imaged using a microscope (Olympus, Shinjuku, Tokyo, Japan).

Flow Cytometry Analysis

An apoptosis assay kit (BoGoo Biotech., Jiading, Shanghai, China) was applied to determine the cell apoptosis using flow cytometry. HJURP siRNAs or negative control siRNAs-transfected A549 and H1299 cells were trypsinized into single-cell suspensions, and Annexin V-FITC, as well as propidium iodide (PI) were added to the cells. After incubation in the dark for 15 min, cells were collected, washed using ice-cold phosphate-buffered saline (PBS), and subjected to flow cytometry analysis using a FACSCalibur flow cytometer (BD Biosciences, Pudong, Shanghai, China).

Wound Healing Assays

The migration ability of HJURP siRNAs or negative control siRNAs-transfected A549 and H1299 cells were evaluated by wound healing assays. In short, the cells after treatment with indicated siRNAs were placed in 6-well plates and cultured for 24-48 h until the cell confluence reached 90-100%. Then, an artificial wound was generated by a 200 μl pipette tip and the wound closure at 0 h, as well as 48 h was observed and imaged using a microscope (Olympus, Shinjuku, Tokyo, Japan).

Transwell Invasion Assays

Cell invasion of HJURP siRNAs or negative control siRNAs-transfected A549 and H1299 cells were determined by transwell invasion assays using Matrigel pre-coated Millipore transwell chamber inserts (pore size: 8 μm; Solarbio, Tongzhou, Beijing, China). Thereafter, the treated A549 and H1299 cells were trypsinized into single-cell suspension (without serum). Then, 200 μl the cell suspension (5×10^4 cell/well) were added into the upper chamber and 600 μl culture medium (with 15% FBS) were added into the bottom wells. 24 h later, paraformaldehyde (4%) was used to fix the cells which were invaded through membranes and crystal violet dye (0.1%; Sigma-Aldrich, Pudong, Shanghai, China) was employed to stain these cells. After rinsing with PBS twice, the invasive cells were counted and imaged using a microscope (Olympus, Shinjuku, Tokyo, Japan).

Statistical Analysis

Data analysis were carried out using the Statistical Package version 19.0 (SPSS, Chicago, IL, USA). For comparisons, the difference between the two groups was analyzed with Student's *t*-test. The multi-group comparison was performed using the One-way analysis of variance (ANOVA). The paired comparison was performed by the Student-Newman-Keuls (SNK) approach. The Chi-square test and Fisher's exact test were used to examine the association between HJURP expressions and various clinicopathological parameters. A value of $p < 0.05$ was considered statistically significant.

Results

Upregulation of HJURP Expression in NSCLC Tissues

To study the expression pattern of HJURP in the human NSCLC tissues, we first analyzed its expression in public gene profiling datasets GSE19188 from the Gene Expression Omnibus (GEO) database. As shown in Figure 1A, the expression pattern of dysregulated mRNAs was shown using a Heatmap. In addition, HJURP was one of the most up-regulated mRNAs in NSCLC (Figure 1B). Furthermore, we also analyzed chip data from TCGA datasets, finding similar results that HJURP was significantly up-regulated in NSCLC (Figure 1C). Then, to demonstrate the

results of bioinformatics analysis, we performed RT-PCR to detect the expression of HJURP in 74 NSCLC patients. As shown in Figure 1D, we found that the expression of HJURP was significantly up-regulated in NSCLC tissues compared to matched normal lung tissues ($p < 0.01$). Also, we found that the expression level of HJURP at both mRNA and protein level was increased in four NSCLC cell lines compared to BEAS-2B (Figure 1E and 1F). The A549 and H1299 cell lines have the highest expression of HJURP, thus, they were selected for the next study.

High HJURP Expression Levels Correlate With NSCLC Patients' Poor Prognosis

To explore the clinical significance of HJURP in NSCLC patients, we classified all NSCLC samples into two groups: low expression (n = 36) and high expression groups (n = 38). The relation between HJURP expression levels and clinicopathological features were shown in Table II. We found that high HJURP expression was associated with advanced TNM stage ($p = 0.018$) and distant metastasis ($p = 0.033$). However, there was no significant correlation of HJURP expression with other clinical features such as gender, age, histological type, and lymph node status ($p > 0.05$). We also analyzed the prognostic value of HJURP in NSCLC patients by analyzing clinical data from TCGA datasets, finding that high HJURP expression had a significant impact on overall survival and disease-free survival (Figure 1G). Thus, these results implied that upregulated HJURP expression may contribute to the progression and metastasis of NSCLC.

Silence of HJURP Suppressed The Cellular Growth of NSCLC Cells And Accelerated Cell Apoptosis

In the following study, we explored the effects of HJURP knockdown on the cellular growth and apoptosis of NSCLC cells. As the data from the

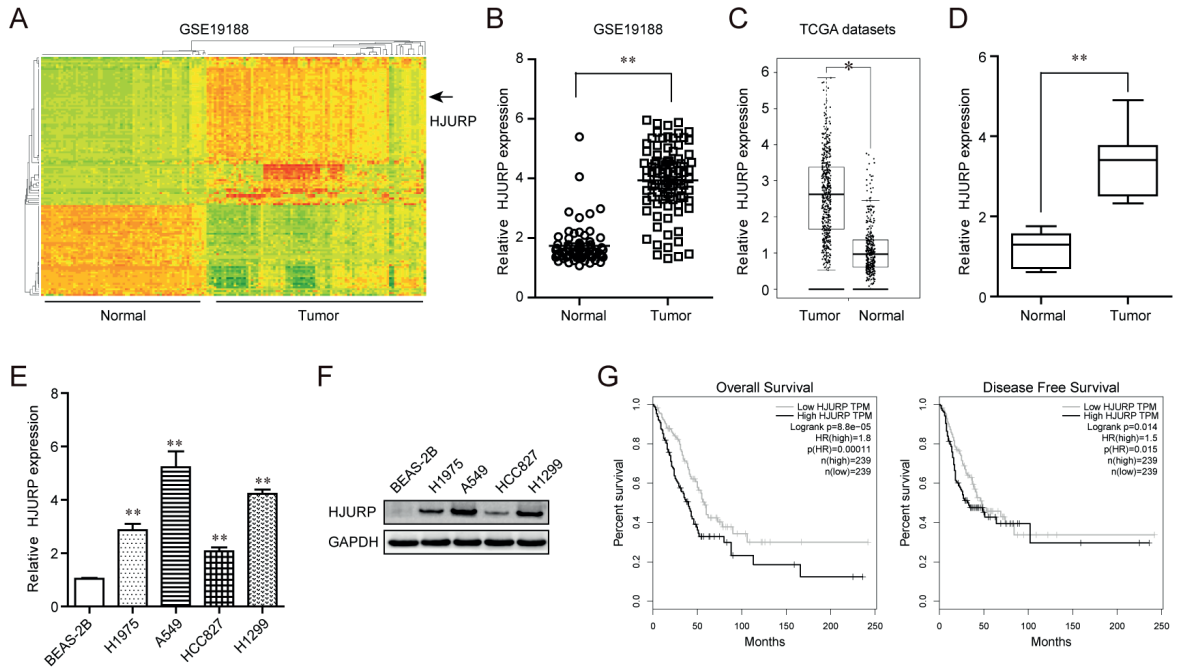

Figure 1. HJURP was up-regulated in lung cancer and its clinical significance. ***A,*** Heatmap showing mRNAs expressional levels in NSCLC tissues and their matched normal counterparts generated from RNA sequencing data from the GEO database (GSE19188). ***B,*** HJURP expression levels in lung tumors compared to normal lung tissues in publicly available gene expression data GSE19188. ***C,*** HJURP expression levels in lung tumors compared to normal lung tissues RNA sequencing data from the TCGA datasets. ***D,*** HJURP was significantly increased in primary human NSCLC tissues in comparison to adjacent-normal lung tissues. ***E, F,*** MRNA and protein expressions of HJURP were detected in NSCLC tissues and cells by RT-PCR and Western blot. ***G,*** Kaplan-Meier curves of the overall survival and disease-free survival of 478 NSCLC patients by analyzing clinical data from TCGA dataset. *$p < 0.05$, **$p < 0.01$.

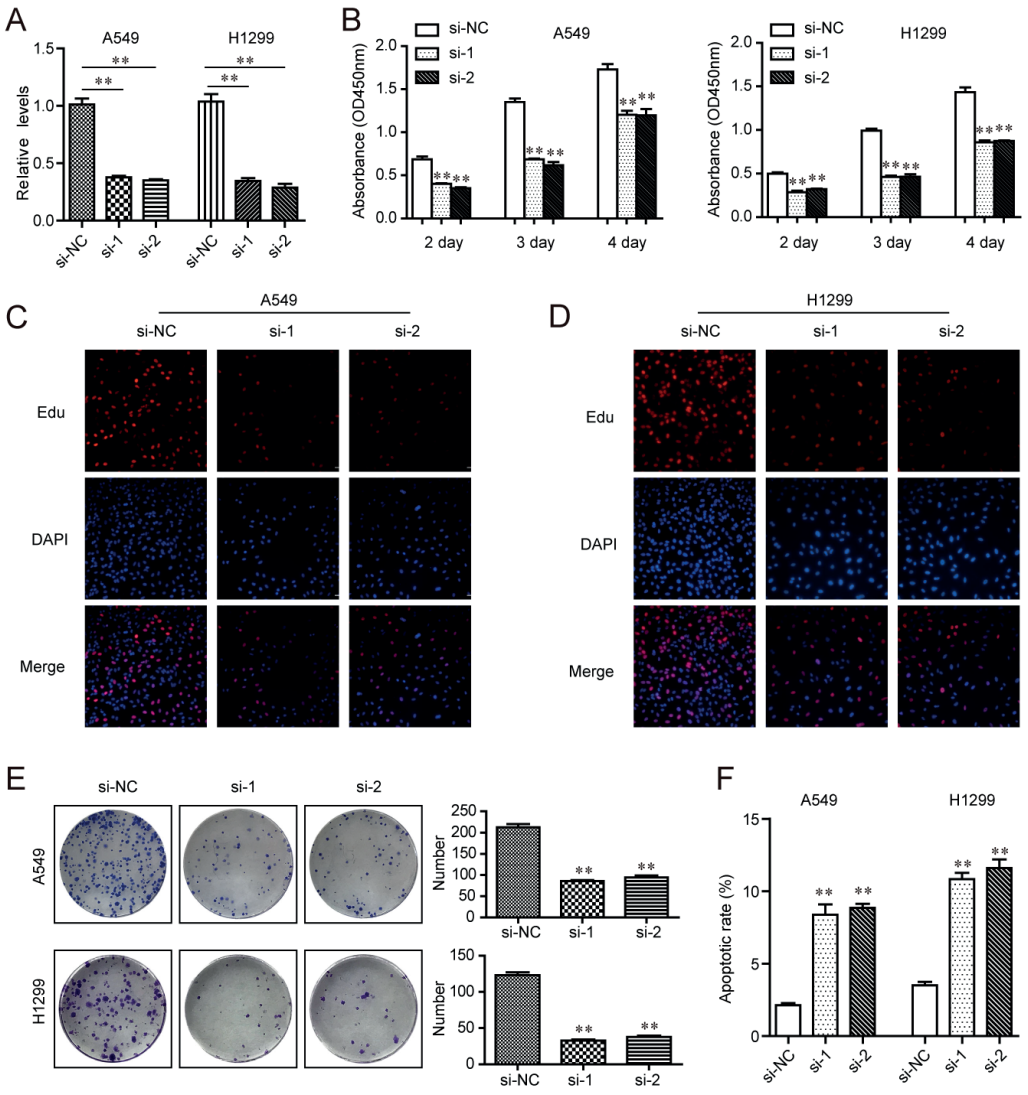

Figure 2. Knockdown of HJURP decreased cell proliferation and accelerated apoptosis of A549 and H1299 cells. ***A,*** Relative expression of HJURP in A549 and H1299 cells transfected with HJURP siRNAs (si-1 and si-2) and negative control siRNAs (si-NC). ***B,*** CCK-8 assays showed that down-regulation of HJURP inhibited the cell proliferation of A549 and H1299 cells. ***C, D,*** Representative results from EdU assays of A549 and H1299 cells transfected with si-1, si-2 and si-NC. Proliferative cells were labeled with red fluorescence. The nuclei were labeled with DAPI (blue fluorescence) (magnification: 100×). ***E,*** Cell colony assays showed that the colony formation ability was suppressed in HJURP siRNAs-transfected A549 and H1299 cells (magnification: 10×)**.** ***F,*** Knockdown of HJURP induced the apoptosis of A549 and H1299 cells. *$p < 0.05$, **$p < 0.01$.

qRT-PCR assays presented in Figure 2A, the expression levels of HJURP in the two NSCLC cell lines, A549 and H1299, significantly decreased after transfection with specific siRNAs targeting HJURP (si-1 and si-2) compared to cells transfected with negative control siRNAs (si-NC). Thereafter, the data of CCK-8 assays revealed that the cellular proliferation of HJURP silencing cells was significantly depressed compared to that of the control cells (Figure 2B). In addition, EdU assays were also performed to further evaluate the effects of HJURP depletion on the proliferation of NSCLC cells. According to the data, silence of HJURP by specific siRNAs was capable to inhibit the proliferation of A549 and H1299 cells (Figure 2C and D). Similarly, cell colony formation assays demonstrated that the clonogenic capability of A549 and H1299 cells was remarkably decreased after the deletion of HJURP (Figure 2E). Moreover, the cell apoptosis was also determined by the use of flow cytometry analysis and the results confirmed that knockdown of HJURP

markedly increased the apoptotic rates of A549 and H1299 cells. Collectively, our results indicated that HJURP might play an essential role in cellular proliferation of NSCLC cells.

HJURP Depletion Impaired the Invasion and Migration of NSCLC Cells

After confirming that HJURP was involved in the regulation of NSCLC cell proliferation, we next tried to investigate the influence of HJURP on the mobility of NSCLC cells. Transwell invasion assays illustrated that repressing the expression of HJURP notably reduced the invaded cell number of A549 and H1299 cells compared to the controls (Figure 3A and B). We then performed wound healing assays to test the impact of HJURP deficiency on cell migration. We found that HJURP depression also led to a markedly decreased migration of A549 and H1299 cells (Figure 3C). Therefore, these data revealed that HJURP affected the invasion and migration of NSCLC cells. As cellular invasion and migration were the key phenotypes of epithelial-mesenchymal transition (EMT), we then wondered if HJURP knockdown was able to change the expression of EMT-specific molecules in NSCLC cells. In Figure 3D it was shown that the protein levels of vimentin and N-cadherin were remarkably up-regulated with silencing HJURP expression in A549 and H1299 cells. Overall, these data suggested that HJURP played crucial roles in regulating the metastatic potentials of NSCLC cells via affecting EMT.

Knockdown of HJURP Suppressed the Activation of Wnt/β-Catenin Signaling in NSCLC

To further investigate the molecular mechanisms of the HJURP tumor-promoting role in NSCLC, we next aimed to employ qRT-PCR and

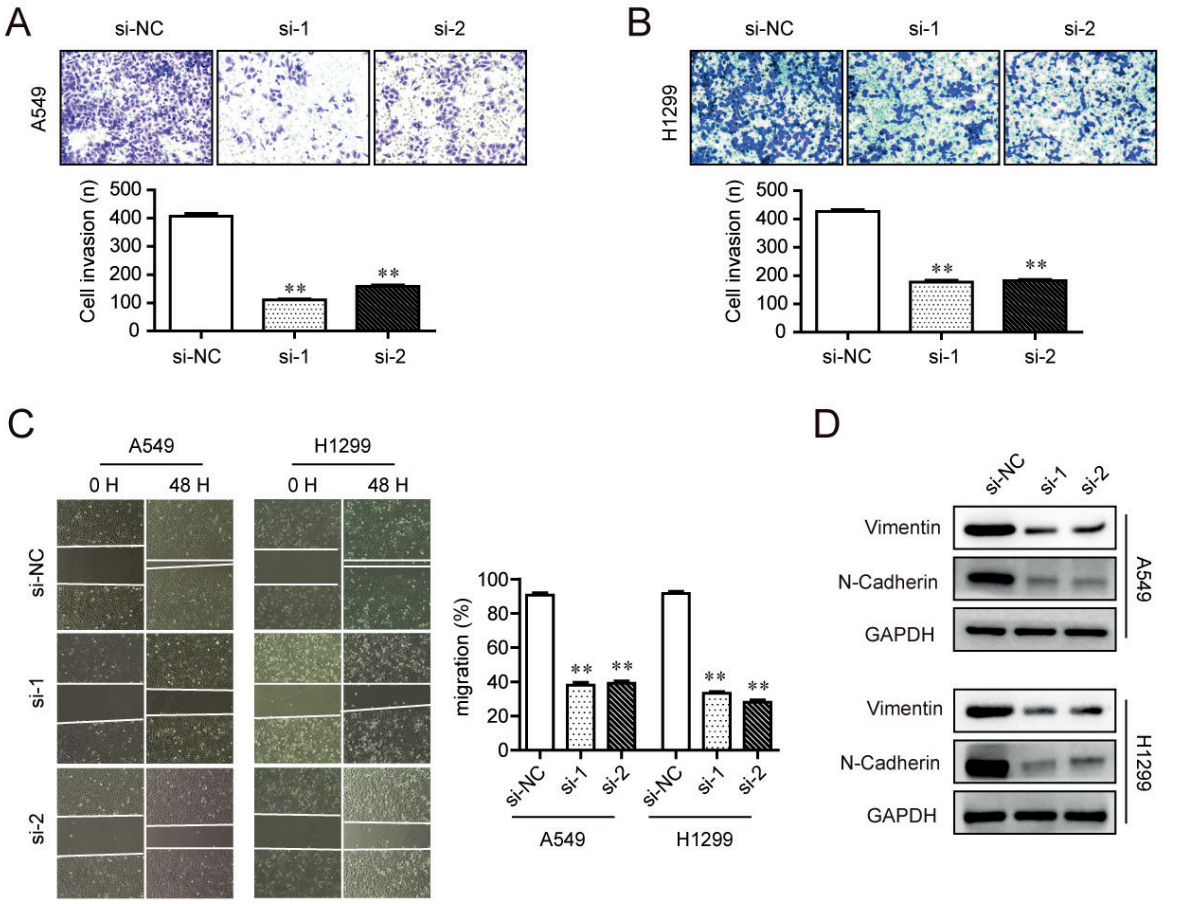

Figure 3. The effects of HJURP on cell invasion and migration. ***A, B,*** Transwell invasion assays determined cell invasion in A549 and H1299 cells transfected with si-1, si-2 and si-NC (magnification: 40×). ***C,*** Knockdown of HJURP reduced the migratory capacity of A549 and H1299 cells (magnification: 10×). ***D,*** Protein expression of vimentin and N-cadherin was decreased in A549 and H1299 cells. *$p < 0.05$, **$p < 0.01$.

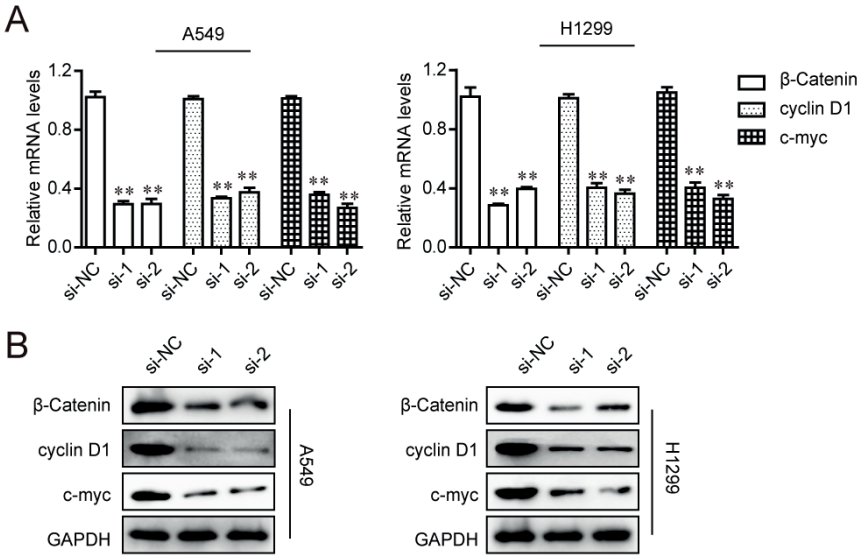

Figure 4. HJURP affected the activity of Wnt/β-catenin signaling in A549 and H1299 cells. **A,** QRT-PCR assays detected the mRNA expression of β-catenin, cyclin D1, and c-myc in A549 and H1299 cells after transfecting with si-1, si-2 and si-NC. **B,** Protein levels of β-catenin, cyclin D1, and c-myc in HJURP siRNAs or control siRNAs-transfected A549 and H1299 cells were determined by Western blot assays. *$p < 0.05$, **$p < 0.01$.

Western blot analysis to evaluate the expressing changes of relevant molecular signaling. Since Wnt/β-catenin signaling served as an essential player in modulating diverse phenotypes such as cellular growth, migration, and invasion of numerous cancer types, we focused on three key components of this signaling: β-catenin, cyclin D1, and c-myc. Using qRT-PCR assays, the mRNA expressing levels of β-catenin and its two downstream targets cyclin D1 and c-myc, were markedly decreased in A549 and H1299 cells, when the cells were transfected with HJURP siRNAs (Figure 4A). Consequently, by conducting Western blot assays, we also observed similar results with the data of qRT-PCR assays, i.e., that the repression of HJURP resulted in notably decreased expression of β-catenin, cyclin D1, and c-myc (Figure 4B). Therefore, our data indicated that the silence of HJURP inhibited the activation of Wnt/β-catenin signaling in NSCLC.

Discussion

NSCLC continuously contributes to cancer-related mortality and is a major public health burden in China[20]. Growing evidence has shown that altered patterns of several gene expressions correlate with various human diseases, especially various types of cancers[21-23]. The potential functions of tumor-related genes are very complex. The identification of several critical genes which could be used as novel biomarkers and therapeutic targets is urgent to improve the prognosis of NSCLC patients.

In this study, we further observed that HJURP expression was significantly up-regulated via analyzing microarray data and RT-PCR assays. Previously, increased expression of HJURP in lung cancer had been reported by Zhou et al[19]. However, the evidence was limited. Our results indicated that HJURP was highly expressed in NSCLC. Then, based on the clinical assays, we found that high HJURP expression was associated with TNM stage and distant metastasis, suggesting the tumor-promoting role of HJURP for clinical progress of NSCLC. Furthermore, we used an online bioinformatics statistics software which can calculate the data from TCGA datasets to explore the association between HJURP and clinical prognosis of NSCLC patients. Results showed that high expression of HJURP was associated with shorter overall survival and disease-free survival. However, due to the the limitation of clinical data, univariate and multivariate analysis were not performed to confirm whether the HJURP expression was an independent prog-

nostic factor for NSCLC patients. Further experiments were needed to solve this problem.

Although several studies on HJURP in the tumor have been reported, little is known about the biological function and mechanism of HJURP in tumors. Recently, Cao et al[14] reported that HJURP inhibition induces cell cycle dysregulation in bladder cancer cells via the. PPARγ-SIRT1 feedback loop. Hu et al[24] found that HJURP expression was significantly increased in hepatocellular carcinoma tissues and correlates with shorter overall survival of hepatocellular carcinoma patients. *In vitro* experiments indicated that HJURP acted as a tumor promoter in this disease due to its tumor-promoting roles in regulating hepatocellular carcinoma cell proliferation. Up to date, all previous studies revealed HJURP as an up-regulated gene in various tumors. However, the function of HJURP in NSCLC has not been investigated. In this study, to explore the specific effects of HJURP on NSCLC behaviors, we used si-HJURP to down-regulate the levels of HJURP in A549 and H1299 cells. Then, we performed a series of cells experiments and found that the knockdown of HJURP acted as a significant positive regulator in the modulation of proliferation, migration, and invasion of A549 and H1299 cells. Also, we found that down-regulation of HJURP promoted apoptosis. EMT is an essential mechanism involved in embryonic development and tissue repair and plays a key role in the early process of metastasis of cancer cells[25]. Identification of EMT status would help us to properly understand the mechanism of NSCLC metastasis. We found that N-Cadherin or Vimentin were significantly down-regulated in A549 and H1299 cells after transfected with si-HJURP, indicating that HJURP displayed functions in the metastasis of NSCLC cells via regulating the EMT progress.

The Wnt/β-catenin signaling pathway, a highly conserved molecular mechanism, plays a critical role in the regulation of the development of morphogenesis, gene transcription, differentiation, and proliferation[26,27]. This pathway has been involved in the modulation of various tumors, including cervical cancer, melanoma, glioblastoma, and NSCLC, via modulating its downstream targets, such as Cyclin D1, c-Myc, GSK-3, and so on[28-30]. A growing interest toward tumor-related genes in cancer has been sparked and several functional genes have been confirmed to display their oncogenic or anti-oncogenic roles by modulating various critical signaling pathways, including Wnt/β-catenin signaling pathway[31,32]. In this study, we used Western blot analysis to detect the protein expression of three key components of this signaling (β-catenin, cyclin D1, and c-myc) in A549 and H1299 cells after the HJURP knockdown. Results showed that the mRNA and protein level of these genes were decreased in HJURP knockdown group, which suggested that HJURP may act as a tumor promoter in NSCLC by promoting the Wnt/β-catenin pathway.

Conclusions

We showed that HJURP was upregulated in NSCLC tissues and cell lines, and provided the first evidence that HJURP promoted. NSCLC cell proliferation and metastasis by inactivating the Wnt/β-catenin pathway. The data described in this study suggested that HJURP is a potential candidate for further functional research in NSCLC and indicated the clinical value of HJURP as a novel therapeutic target for NSCLC patients.

Conflict of interest

The authors declare no conflicts of interest.

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