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SHCBP1 promotes cisplatin induced apoptosis resistance, migration and invasion through activating Wnt pathway

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ABSTRACT

Lung cancer is the leading cause for cancer death due to refractory nature to current treatment strategies, understanding the regulatory mechanism of therapy resistance of lung cancer is important for lung cancer therapy. Here, we aimed to study the role of SHCBP1 in lung cancer cisplatin resistance, we found SHCBP1 was upregulated in lung cancer tissues and cells, patients with high SHCBP1 had poor prognosis. SHC binding and spindle associated 1 (SHCBP1) overexpression promoted cisplatin induced apoptosis resistance, migration and invasion determined by apoptosis assay and transwell assay with or without Matrigel, while SHCBP1 knockdown inhibited cisplatin induced apoptosis resistance, migration and invasion. Wnt pathway promoted lung cancer progression, we found SHCBP1 activated Wnt pathway, characterized by promoting β-catenin nuclear translocation. Inhibition of Wnt pathway in SHCBP1 overexpression cells reversed the effect of SHCBP1 overexpression, confirming SHCBP1 promoted lung cancer progression through activating Wnt pathway. We also found SHCBP1 expression was positively corrected with Wnt pathway activity in lung cancer samples. In summary, we found SHCBP1 promoted cisplatin induced apoptosis resistance, migration and invasion through activating Wnt pathway, providing a potential target for lung cancer therapy.

1. Introduction

Lung cancer is the most common cancer worldwide, it has high metastasis ability and inherent drug resistance [1], many mutations are associated with lung cancer development, such as EGFR and KRAS mutation and EML4-ALK fusions [2,3], many drugs also been used for lung cancer therapy, such as Gefitinib [4] and Afatinib [5]. But there are few effective drugs for lung cancer treatment now. it's important to explore the regulatory mechanism of lung cancer progression and therapy resistance.

Many signaling pathways regulate lung cancer progression, many non-coding RNAs and genes regulate lung cancer progression through regulating Wnt pathway. For example, SOX9 promotes lung cancer cell metastasis and epithelial-mesenchymal transition through activating Wnt pathway [6]. ING5 inhibits lung cancer proliferation, migration, invasion and epithelial-mesenchymal transition through inhibiting Wnt pathway [7].

Shc SH2 domain-binding protein (SHCBP1) locates on 16q11.2 and is downstream of Shc adaptor proteins [8], many studies have showed it plays important roles in tumor progression recently. For example, SHCBP1 is upregulated in hepatocellular cancer (HCC) and promotes HCC cell proliferation, colony formation and soft agar colony formation [9]. SHCBP1 is also upregulated in breast cancer tissues and is positively corrected with advanced clinical stage and poor prognosis, it promotes breast cancer proliferation and inhibits apoptosis [10]. SHCBP1 is a downstream of SS18-SSX1 fusion gene and promotes synovial sarcoma cell proliferation, invasion, metastasis through regulating TGF-β1, MAPK/ERK and PI3K/AKT/mTOR signaling pathways [11,12]. From these studies, we found SHCBP1 might be an oncogene in above tumors, but its role in lung cancer progression isn't been studied, we conferred that SHCBP1 might regulated lung cancer progression. In this study, we studied the role of SHCBP1 in lung cancer progression and therapeutic resistance and found cisplatin induced apoptosis resistance, migration and invasion through activating Wnt pathway.

2. Materials and methods

2.1. Cell culture and tissues

Lung cancer cells NCI-H520, NCI-H460, 95D, NCI-H1915, A549,

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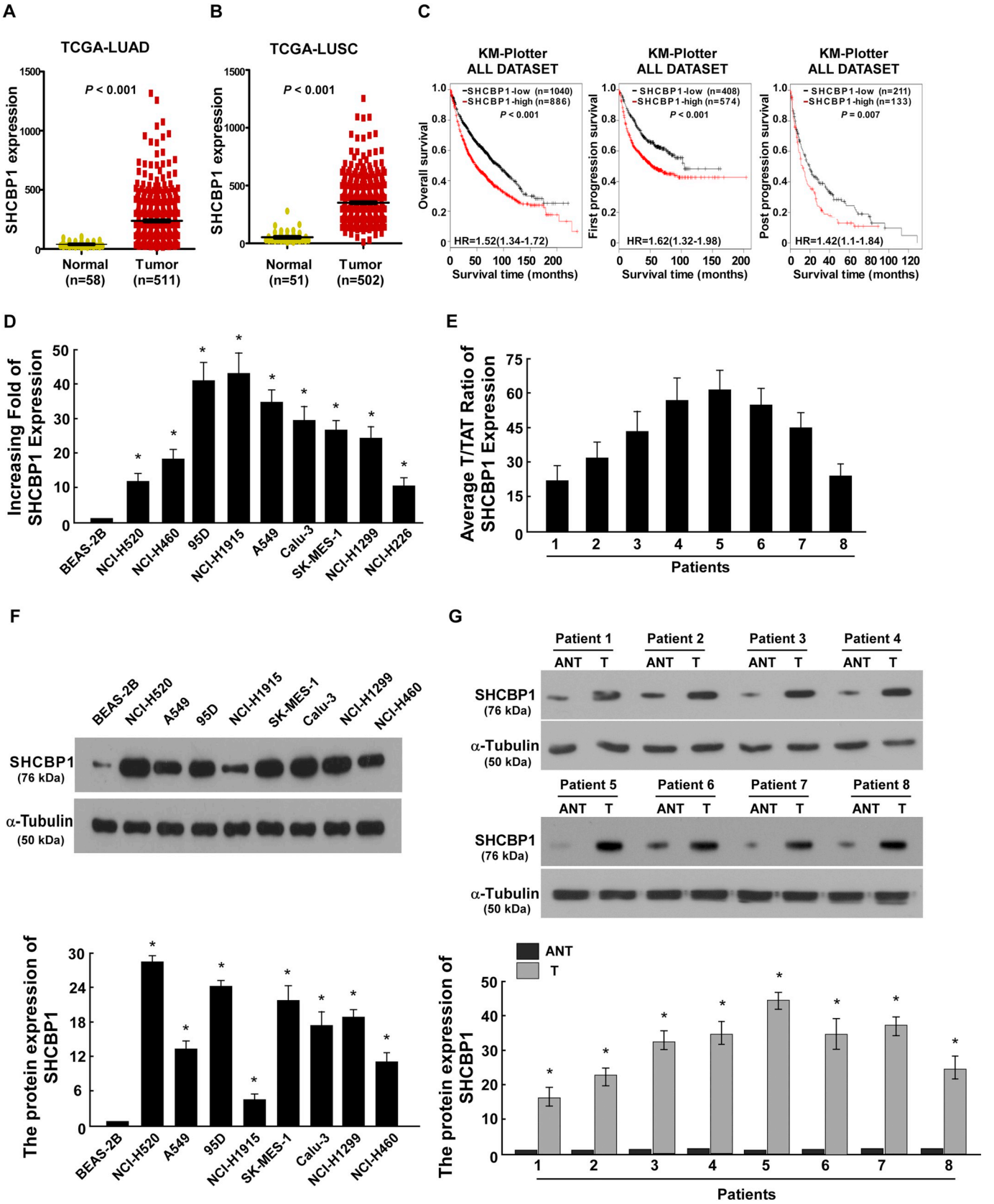

Fig. 1. SHCBP1 is upregulated is lung cancer cells and tissues. A. SHCBP1 was significantly upregulated in lung adenocarcinoma (TCGA-LUAD). Data was downloaded from TCGA dataset. B. SHCBP1 was significantly upregulated in lung squamous cell carcinoma (TCGA-LUSC). Data was downloaded from TCGA dataset. C. Kaplan-Meier analysis suggested that patients with high SHCBP1 expression had poor prognosis. D. Q-PCR analysis suggested that SHCBP1 was upregulated in lung cancer cells. E. Q-PCR analysis suggested that SHCBP1 was upregulated in lung cancer tissues (T) compared to adjacent normal tissues (ANT). F. Western blot analysis suggested that SHCBP1 was upregulated in lung cancer cells. G. Western blot analysis suggested that SHCBP1 was upregulated in lung cancer tissues (T) compared to adjacent normal tissues (ANT). α-Tubulin was used as the loading control. $^{*}P < 0.05$.

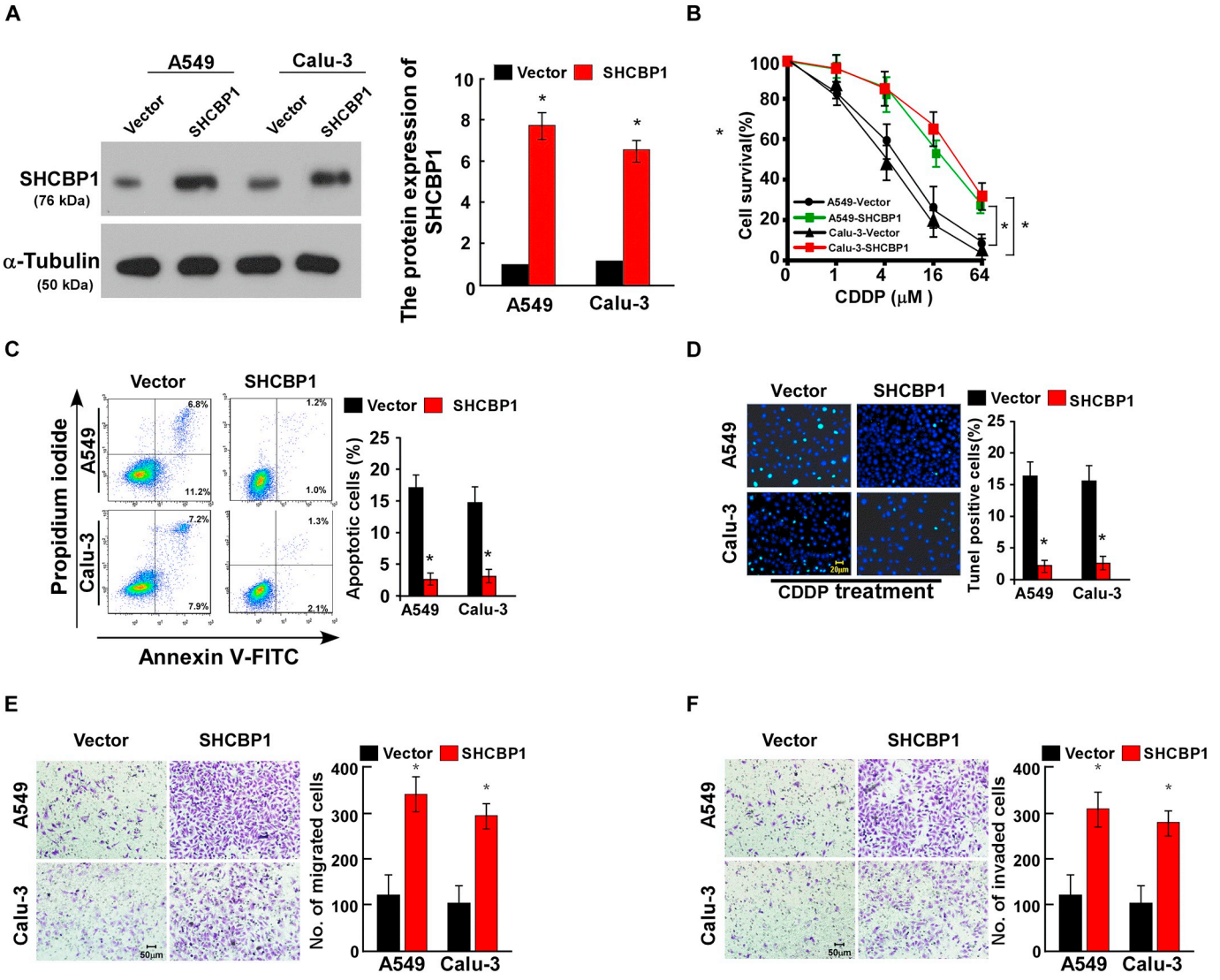

Fig. 2. SHCBP1 overexpression promoted CDDP induced apoptosis resistance, migration and invasion. A. Western blot analysis showed SHCBP1 was upregulated in cells after infection virus carried out SHCBP1 overexpression vector. B. Cell survival analysis showed SHCBP1 overexpression promoted CDDP induced apoptosis resistance. C. Annexin V/PI analysis showed SHCBP1 overexpression promoted CDDP induced apoptosis resistance. D. TUNEL analysis showed SHCBP1 overexpression promoted CDDP induced apoptosis resistance. E. Transwell analysis without Matrigel showed that SHCBP1 overexpression promoted cell migration. F. Transwell analysis with Matrigel showed that SHCBP1 overexpression promoted cell invasion. *$P < 0.05$.

Calu-3, SK-MES-1, NCI-H1299 and NCI-H226 and human bronchial epithelial cells BEAS-2B were obtained from ATCC or the cell banks of Shanghai Institute of Biological Sciences. They were cultured using DMEM medium (SH30022.01B, Hyclone) supplemented with 10% fetal bovine serum (sv30087.02, FBS, Hyclone). Lung cancer tissues and paired adjacent non-cancerous lung tissues were stored in liquid nitrogen until use. Prior patient consent and approval for the Institute Research Ethics Committee of Shunde Hospital of Southern Medical University were obtained for the use of these clinical materials for research purposes.

2.2. Plasmids, compounds, small interference RNA, infection and infection

The CDS of SHCBP1 was cloned onto retroviral plasmid pMSCV-puro, shRNAs for SHCBP1 were cloned to into lentiviral plasmid PLKO.1-puro, small interference RNA for β-catenin and its corresponding control oligonucleotides were purchased from RiboBio (Guangzhou, China). The retroviral plasmids with packing plasmid PLC were cotransfected into 293 T to generate retrovirus using Lipofectamine 2000 reagent (11668500, Thermo), the lentiviral plasmids with packing plasmid pM2.G and psPAX2 were cotransfected into 293 T to generate lentivirus using Lipofectamine 2000 reagent. Collected virus infected lung cancer cells, stable cell line was screened using puromycin (P8833, Sigma). TOPFlash and FOPFlash were obtained from YouBia Biotech (China). These plasmids were transfected into cells using Lipofectamine 2000, the firefly and Renilla luciferase activities were measured through a dual luciferase assay (E1910, Promega) 48 h after transfection. β-Catenin/TCF-mediated transcription inhibitor ICG-001 was purchased from Selleck, 3 μM was used.

2.3. Western blot and quantitative real-time RT-PCR (Q-PCR)

Cell lysates were prepared using RIPA buffer (20-188, Millipore) and separated on 12% SDS-PAGE gel. Nuclear proteins were isolated using Membrane and Cytosol Protein Extraction Kit (P0028, Beyotime Biotechnology). The primary antibodies were used the following: anti-SHCBP1 (ab122310), β-catenin (ab32572), p84 (ab487) and α-Tubulin (ab7291), they were purchased from Abcam. α-Tubulin was used as the loading control for total proteins, p84 was used as the loading control for nuclear proteins.

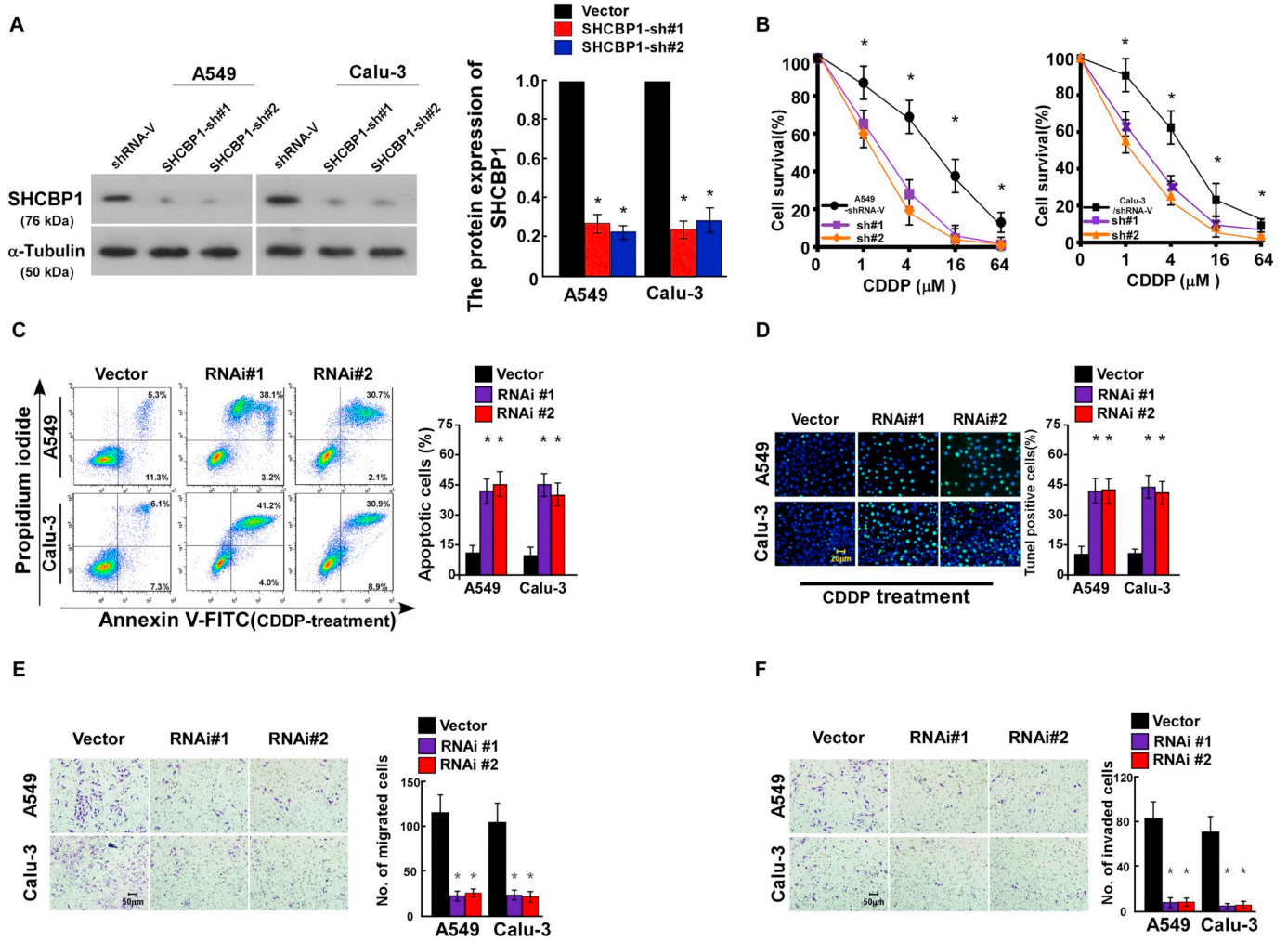

Fig. 3. SHCBP1 knockdown inhibited CDDP induced apoptosis resistance, migration and invasion. A. Western blot analysis showed SHCBP1 was downregulated in cells after infection virus carried out SHCBP1 shRNA vector. B. Cell survival analysis showed SHCBP1 knockdown inhibited CDDP induced apoptosis resistance. C. Annexin V/PI analysis showed SHCBP1 knockdown inhibited CDDP induced apoptosis resistance. D. TUNEL analysis showed SHCBP1 knockdown inhibited CDDP induced apoptosis resistance. E. Transwell analysis without Matrigel coated showed that SHCBP1 knockdown inhibited cell migration. F. Transwell analysis with Matrigel coated showed that SHCBP1 knockdown inhibited cell invasion. $^*P < 0.05$.

Total RNA was isolated using TRIzol regent (15596018, Thermo) and was reversely transcribed with HiScript Reverse Transcriptase (R101-1, Vazyme) according to the manufacturer's instructions. Q-PCR was carried out with AceQ qPCR SYBR Green Master Mix (Q511-02, Vazyme) according to the manufacturer's instructions on a CFX96 real-time PCR detection system (Bio-Rad). GAPDH was used for the normalization of the Q-PCR.

2.4. Apoptosis assay

For cell survival analysis, cells were seeded in 6-well plates and treated with CDDP (Selleck) of different concentration (1 μM, 4 μM, 16 μM and 64 μM). After one week, the cells were collected and counted. Annexin V-FITC Apoptosis Detection Kit and One Step TUNEL Apoptosis Assay Kit were purchased from Beyotime Biotechnology (China) and were used according to the manufacturer's instructions.

2.5. Migration and invasion analysis

Cells were harvested and resuspended in serum-free medium and were plated into chambers with Matrigel (invasion) or without Matrigel (migration) coated at a density of 2×10^4 every chamber. 5 μM CDDP was used. The chambers were inserted into the wells of a 24-well plate and incubated for 48 h in basal medium supplemented with 20% fetal bovine serum. The cells remaining on the upper surface of the membranes were removed, the cells adhering to the lower surface were fixed and stained using 0.05% crystal violet. OD_{570} was measured.

2.6. Statistical analysis

Global mRNA expression profiles of lung cancer were downloaded from The Cancer Genome Atlas (TCGA) dataset, gene set enrichment analysis (GSEA) was performed using GSEA software (http://www.broadinstitute.org/gsea/). The data were analyzed using SPSS 19.0 (SPSS), all error bars represent the mean ± s.e.m. derived from three independent experiments, the difference between two groups was analyzed using Student's *t*-test. The survival curves were calculated by the Kaplan-Meier method and the difference by the Log-rank test. *P* values < 0.05 were considered statistically significant.

3. Results

3.1. SHCBP1 is upregulated in lung cancer tissues and cells

To investigate the function of SHCBP1 in lung cancer progression, we characterized SHCBP1 expression in The Cancer Genome Atlas lung

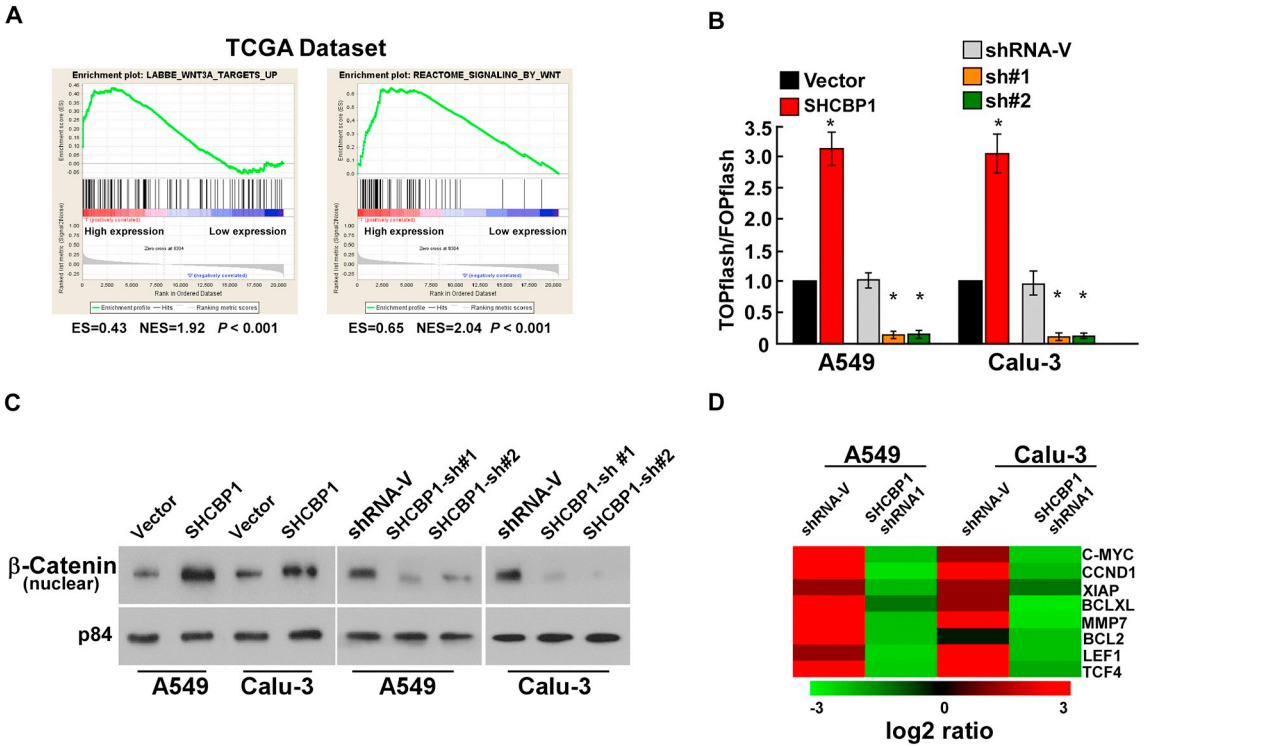

Fig. 4. SHCBP1 activated Wnt pathway. A. GSEA analysis showed SHCBP1 expression was positively associated with Wnt pathway. B. Luciferase reporter assay showed SHCBP1 overexpression significantly increased the luciferase activity, while SHCBP1 knockdown significantly inhibited the luciferase activity. C. Western blot analysis suggested that SHCBP1 overexpression increased the accumulation of β-catenin in nucleus, while SHCBP1 knockdown reduced the accumulation of β-catenin in nucleus, p84 was used as the loading control. D. Heatmap showed the effect of SHCBP1 level on the expression of C-MYC, CCND1, XIAP, BCLXL, MMP7, BCL2, LEF1 and TCF4. *$P < 0.05$.

adenocarcinoma (TCGA-LUAD) and The Cancer Genome Atlas lung squamous cell carcinoma (TCGA-LUSC) samples. Compared to normal lung tissues, SHCBP1 mRNA was significantly upregulated in lung cancer tissues (Fig. 1A and B). We used all microarrays for lung cancer in GEO Dataset to analyze the relationship between SHCBP1 expression and prognosis, Kaplan-Meier analysis indicated that patients with high SHCBP1 expression had a significantly shorter overall survival than patients with low SHCBP1 expression (Fig. 1C). We further determined SHCBP1 expression in lung cancer cell and lung cancer tissues, Q-PCR analysis found SHCBP1 was upregulated in lung cancer cells compared to human bronchial epithelial cells BEAS-2B, it also upregulated in lung cancer tissues compared to adjacent normal lung tissues (Fig. 1D and E). Western blot analysis confirmed that SHCBP1 expression was upregulated in lung cancer cells and lung cancer tissues (Fig. 1F and G). These results suggested that SHCBP1 was increased in lung cancer cells and tissues, it might promote lung cancer progression.

3.2. SHCBP1 overexpression inhibits chemotherapy induced apoptosis and induces cell invasion and migration

To investigate SHCBP1's function in lung cancer progression, we overexpressed SHCBP1 in lung cancer cells A549 and Calu-3, western blot analysis showed that SHCBP1 was upregulated in lung cancer cells using SHCBP1 overexpressing vector (Fig. 2A). Cisplatin (CDDP) is a well-known chemotherapy drug for various tumors, including lung cancer [13]. Cell survival analysis showed that cell survival rate was high in cells overexpressing SHCBP1 after treating CDDP compared to vector control, the effect of CDDP on cell survival was increased along with increased concentration. (Fig. 2B). Annexin V/PI analysis showed that SHCBP1 overexpression significantly inhibited apoptosis after treating CDDP (Fig. 2C). TUNEL assay showed that the TUNEL positive cells significantly reduced in cells overexpressing SHCBP1 after CDDP treatment (Fig. 2D). Transwell analysis without Matrigel coated showed that SHCBP1 overexpression significantly increased the number of migrated cells (Fig. 2E). Transwell analysis with Matrigel coated showed that SHCBP1 overexpression significantly increased the number of invaded cells (Fig. 2F). These findings suggested that SHCBP1 overexpression inhibited CDDP induced apoptosis and promoted migration and invasion.

3.3. SHCBP1 knockdown induced chemotherapy induced apoptosis and inhibited cell invasion and migration

To confirm above function of SHCBP1 in lung cancer progression, we knockdown SHCBP1 in the same cells, western blot analysis showed that SHCBP1 was knocked down in lung cancer cells using SHCBP1 shRNAs (Fig. 3A). Cell survival analysis showed that cell survival rate was low in cells knocking down SHCBP1 after treating CDDP compared to Scramble control (Fig. 3B). Annexin V/PI analysis showed that SHCBP1 knockdown significantly induced apoptosis after treating CDDP (Fig. 3C). TUNEL assay showed that the TUNEL positive cells significantly increased in cells knocking down SHCBP1 after CDDP treatment (Fig. 3D). Transwell analysis without Matrigel coated showed that SHCBP1 knockdown significantly reduced the number of migrated cells (Fig. 3E). Transwell analysis with Matrigel coated showed that SHCBP1 knockdown significantly reduced the number of invaded cells (Fig. 3F). Above results suggested SHCBP1 knockdown promoted CDDP induced apoptosis and inhibited migration and invasion.

3.4. SHCBP1 activates Wnt pathway

To investigate the regulatory mechanism of SHCBP1 inhibiting CDDP induced apoptosis and promoting migration and invasion, we used TCGA dataset and GSEA to screen signaling pathway associated

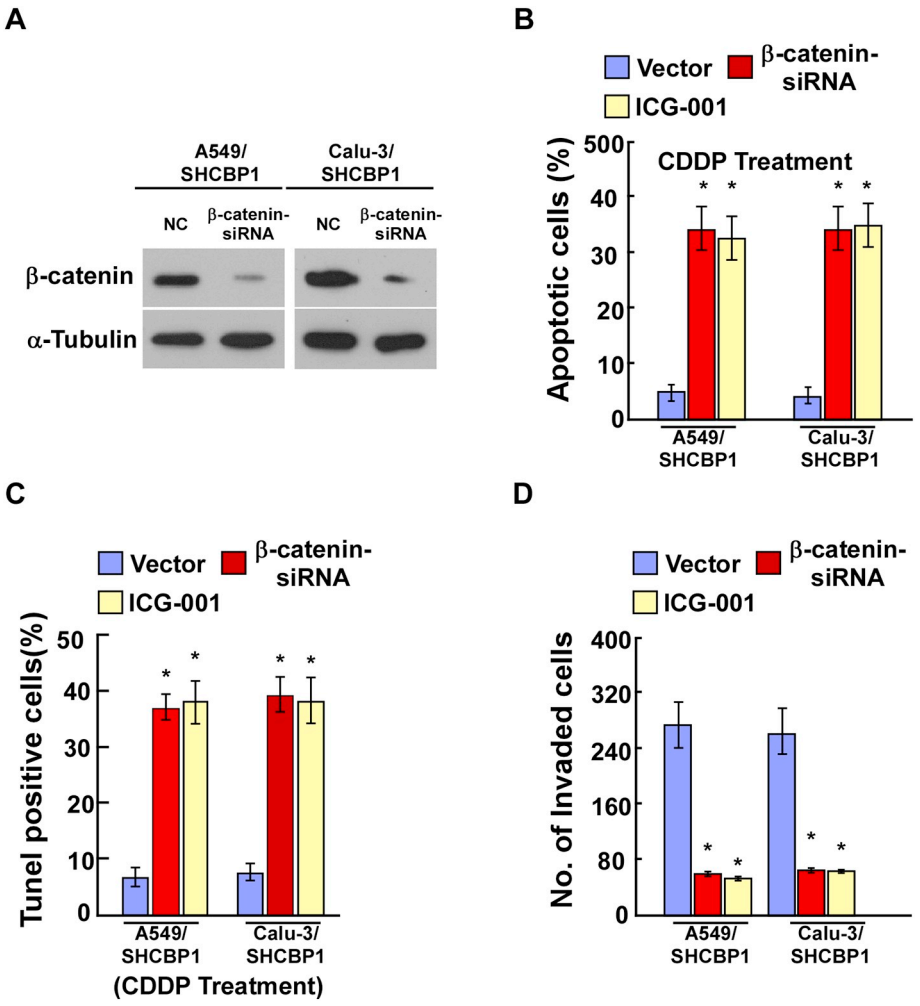

Fig. 5. Inhibition of Wnt pathway in SHCBP1 overexpression cells inhibited CDDP induced apoptosis resistance, migration and invasion. A. Western blot analysis showed β-catenin siRNA inhibited β-catenin expression in SHCBP1 overexpression cells. B. Annexin/PI analysis showed inhibition of Wnt pathway in SHCBP1 cells reduced CDDP induced apoptosis resistance. C. TUNEL assay showed that inhibition of Wnt pathway in SHCBP1 cells reduced CDDP induced apoptosis resistance. D. Transwell analysis with Matrigel coated showed that inhibition of Wnt pathway in SHCBP1 cells inhibited cell invasion. $^{*}P < 0.05$.

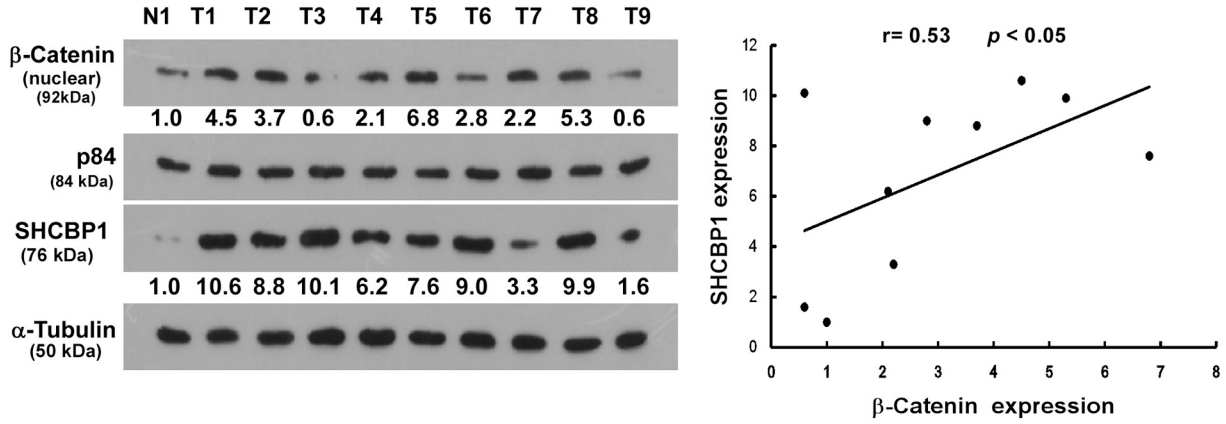

Fig. 6. SHCBP1 expression is positively corrected with Wnt pathway activity in lung cancer samples. The expression of SHCBP1 and β-catenin was determined using western blot, α-tubulin and p84 were used as the loading control for total proteins and nuclear proteins, respectively.

with SHCBP1 and found SHCBP1 was significantly positively corrected with Wnt pathway (Fig. 4A). TOP/FOPflash luciferase analysis is used to determine the transcription activity of Wnt pathway widely [14,15], luciferase activity analysis showed that SHCBP1 overexpression significantly increased Wnt pathway activation, SHCBP1 knockdown significantly inhibited Wnt pathway activity (Fig. 4B). β-Catenin translocating into nuclear is the marker of Wnt pathway activation [16]. Western blot analysis showed that SHCBP1 overexpression increased the accumulation of β-catenin in nucleus, SHCBP1 knockdown inhibited the accumulation of β-catenin in nucleus, suggesting SHCBP1 activated Wnt pathway (Fig. 4C). We also analyzed the expression of genes associated with apoptosis, migration and invasion, these genes

were also the targets of Wnt pathway. SHCBP1 knockdown inhibited C-MYC, CCND1, XIAP, BCLXL, MMP7, BCL2, LEF1 and TCF4 expression, confirming SHCBP1 knockdown induced apoptosis, inhibited cell migration and invasion (Fig. 4D). These results suggested SHCBP1 could activate Wnt pathway.

3.5. SHCBP1 inhibits CDDP induced apoptosis, promoted cell migration and invasion through activating Wnt pathway

To further explore the regulatory mechanism of SHCBP1 in lung cancer progression, we knocked down β-catenin using small inference RNA (siRNA) in SHCBP1 overexpressing cells, western blot showed that β-catenin siRNA inhibited β-catenin expression (Fig. 5A). ICG-001 is an antagonist for Wnt pathway [17,18]. Cell survival analysis showed apoptosis was significantly increased after ICG-001 or β-catenin siRNA treatment in SHCBP1 overexpression cells (Fig. 5B). TUNEL analysis also showed that TUNEL positive cells were significantly increased after ICG-001 or β-catenin siRNA treatment in SHCBP1 overexpression cells (Fig. 5C). Transwell analysis with Matrigel coated showed invaded cells were significantly reduced after ICG-001 or β-catenin siRNA treatment in SHCBP1 overexpression cells (Fig. 5D). Above results suggested inhibition of Wnt pathway, the effect of SHCBP1 on lung cancer progression was reversed. SHCBP1 inhibits CDDP induced apoptosis, promoted cell migration and invasion through activating Wnt pathway.

We also analyzed SHCBP1 and β-catenin expression in lung cancer tissues and found SHCBP1 expression was positively with the accumulation of β-catenin in the nucleus (Fig. 6), suggesting SHCBP1 activated Wnt pathway in clinical samples.

4. Discussion

In present study, we found SHCBP1 was upregulated in lung cancer cells and tissues, it inhibited CDDP induced apoptosis and promoted migration and invasion. Mechanism analysis suggested SHCBP1 activated Wnt pathway, it increased the accumulation of β-catenin in nucleus, inhibition of Wnt pathway in SHCBP1 overexpression cells promoted CDDP induced apoptosis and inhibited migration and invasion, confirming SHBP1 promoted lung cancer progression through activating Wnt pathway.

Shc participates in signal transduction pathways by interacting with cytoplasmic signaling molecule, it is a critical substrate for cytoplasmic tyrosine kinases, such as EGFR, PDGFR, ERBB2 and Ras, it could activate Ras [8]. Ras family including three genes, Hras, Mras and Kars plays critical role in the lung cell proliferation, growth, apoptosis and tumorigenesis, previous reports suggests SHCBP1 interacts with Shc [19–21]. We could interfere that SHCBP1 might regulate lung cancer progression. Our results confirmed that SHCBP1 induced chemotherapy resistance and promoted migration and invasion.

Wnt pathway is an important signaling pathway for various tumors [22,23], we found SHCBP1 increased the luciferase activity, suggesting SHCBP1 increased the transcription activity of TCF and LEF, SHCBP1 activated Wnt pathway. We also found SHCBP1 promoted the accumulation of β-catenin in nucleus, while SHCBP1 knockdown inhibited the accumulation of β-catenin in nucleus, confirming SHCBP1 activated Wnt pathway. We inhibited Wnt pathway treated with β-catenin siRNA or Wnt pathway inhibitor in SHCBP1 overexpression cells, and apoptosis induced by CDDP was increased, migration and invasion were reduced. These suggested that SHCBP1 promoted lung cancer progression through activated Wnt pathway.

In summary, we found SHCBP1 promoted CDDP induced apoptosis resistance, migration and invasion through activating Wnt pathway.

Declaration of competing interest

No potential conflicts of interest were disclosed.

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