SAC3D1 activates Wnt/β-catenin signalling in hepatocellular carcinoma

HAITAO WANG and XIUFANG SHI

Laboratory Center of Bozhou People's Hospital, The Bozhou Hospital Affiliated to Anhui Medical University, Bozhou, Anhui 236800, P.R. China

Received March 17, 2022; Accepted June 16, 2022

DOI: 10.3892/mmr.2022.12833

Abstract. β-catenin accumulates in hepatocellular carcinoma (HCC); therefore, understanding the mechanism of Wnt/β-catenin pathway activation is important for HCC therapy. SAC3 domain containing 1 (SAC3D1) is involved in numerous types of cancer, such as gastric cancer. To the best of our knowledge, however, the role of SAC3D1 in HCC has not yet been elucidated. Here, the expression of SAC3D in HCC was examined by quantitative PCR, western blotting and immunohistochemistry. The function of SAC3D1 in HCC were examined using Cell Counting Kit-8 and anchorage-independent growth assay. It was found that the levels of SAC3D1 mRNA and protein were upregulated in HCC. When SAC3D1 was overexpressed, the proliferation of HCC cells was promoted; when the expression of SAC3D1 was disrupted, HCC cell growth was inhibited. When the molecular mechanism was investigated using immunoprecipitation, it was found that SAC3D1 interacted with axin, inhibiting ubiquitination of β-catenin and elevating protein levels of β-catenin. In summary, the present study revealed the promoting function of SAC3D1 in the progression of HCC. SAC3D1 may be a promising target for HCC therapy.

Introduction

Hepatocellular carcinoma (HCC) is the third most common type of malignant tumour in China (1). Even following treatment with chemotherapy, radiotherapy and immunotherapy, its five-year survival rate remains <20% in China (2,3). Further research into the molecular mechanism underlying the occurrence of HCC is therefore key for identifying novel therapeutic targets.

Correspondence to: Professor Haitao Wang, Laboratory Center of Bozhou People's Hospital, The Bozhou Hospital Affiliated to Anhui Medical University, 616 Duzhong Road, Bozhou, Anhui 236800, P.R. China
E-mail: wanghaitao778868@126.com

Key words: SAC3 domain containing 1, β-catenin, axin, hepatocellular carcinoma

Multiple studies have shown the tumorigenic function of β-catenin in tumorigenesis and β-catenin is the core molecule of the Wnt/β-catenin pathway (4,5). Its stability is regulated by the intracellular β-catenin degradation complex. Composed of scaffold proteins (APC and axin) and kinases (glycogen synthase kinase 3β and casein kinase 1α), this degradation complex phosphorylates β-catenin (6). E3 ubiquitin ligase binds to phosphorylated β-catenin, causing its ubiquitination and subsequent degradation (7). When Wnt protein binds to LDL receptor-related protein 5 and 6 and Frizzled on the cell membrane, Frizzled recruits dishevelled segment polarity protein 1 (Dvl) via its C-terminus. Dvl recruits axin in the degradation complex via its DIX domain, thereby promoting dissociation of the complex (8). This results in increased protein levels of β-catenin; β-catenin enters the cell nucleus, where it activates expression of target genes, such as Axin2, c-Myc (8). In HCC, this cascade is abnormally activated due to an axin mutation and a constitutively activating mutation of β-catenin (9,10). The Wnt/β-catenin pathway is hijacked to promote proliferation and migration of HCC cells and remodel the tumour microenvironment, thereby reprogramming tumour metabolism to promote progression of HCC (11-15). Therefore, it is important to determine the regulatory mechanism of this pathway to improve HCC treatment.

SAC3D1 is a protein that binds to spindle assembly (16). SAC3D1 is extensively expressed, particularly in liver and kidney tissue. SAC3D1 functions in multiple biological processes (such as cell cycle regulation) (17). Inflammatory signals upregulate expression of SAC3D1 (18), although its function in tumours is poorly understood.

The present study evaluated the role of SAC3D1 in HCC and the molecular mechanisms underlying its biological function.

Materials and methods

Cell lines. HCC (MHCC97 and Huh7), hepatoblastoma (HepG2) and 293T cell lines were provided by The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. All cell lines were used following authentication by STR. Cells were incubated in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), penicillin and streptomycin) with 5% CO_2 at 37˚C. Lipofectamine® 8000 (Beyotime Institute of

Biotechnology) was used to transfect plasmids (1 μg/μl) into cells at room temperature for 20 min.

Overexpression and knockdown of SAC3D1. The coding sequence) of SAC3D1 was inserted into the vector of pLVX. For knockdown of SAC3D1, the oligo was annealed and inserted into the vector of pLKO.1. The oligo sequences for the knockdown of SAC3D1 were: short hairpin (sh)SAC3D1 1#, 5'-aaggag tacagccgacccg-3'; shSAC3D1 2#, 5'-aagccctggcccgcttcgc-3'.

Clinical tissue. A total of 30 HCC and adjacent non-cancerous tissue (2 cm away from the cancerous tissues) samples were obtained from Bozhou People's Hospital (Bozhou, China) from June 2019 to May 2021 (age, 31-82 years old, 13 females and 17 males) after written informed consent was obtained from patients. These patients did not receive the chemo- or radiotherapy before the surgery. The present study was approved by the Ethics Committee of Bozhou People's Hospital (E-2019-04).

Reverse transcription-quantitative (RT-q)PCR. TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract RNA from the tissue. PrimeScript™ RT kit (Takara Biotechnology Co., Ltd.) was used for RT according to the manufacturer's instructions. SYBR-Green kit (Toyobo Life Science) was used for qPCR. The thermocycle conditions were: 95˚C for 5 min, 94˚C for 30 sec, 56˚C for 15 sec, 72˚C for 20 sec; 35 cycles, and 4˚C forever. The mRNA levels of SAC3D1 were calculated according to the $2^{-\Delta\Delta Cq}$ method (19). The primer sequences were as follows: SAC3D1 forward, 5'-tctttctgctctataa-3' and reverse, 5'-cggaaggcagcatcta-3' and 18S forward, 5'-aggccctgtaattggaat gagtc-3' and reverse, 5'-gctcccaagatccaactacgag-3'.

Immunohistochemistry (IHC). Tissues were fixed with 4% formalin at 4˚C overnight, embedded with paraffin and cut into 5 μm sections. After being dewaxed with xylene and rehydrated in descending alcohol series, antigen retrieval was performed for 30 min in EDTA solution at 100˚C. Tissue was cooled to room temperature, treated with 3% hydrogen peroxide to quench the endogenous peroxidase activity and blocked with 10% BSA for 20 min at room temperature. After washing three times, tissue sections were incubated with SAC3D1 antibody (Abcam, ab122809, 1:200) for 8 h at 4˚C. Tissue was washed and incubated with horseradish peroxidase (HRP)-linked IgG (Cell Signaling Technology, #7074, 1:100) for 2 h at room temperature. Signals were developed with DAB and nuclei were counterstained with hematoxylin at room temperature for 5 min. The signals were examined under the light microscope (magnification, 20 fold) and analyzed with Vectra 2.0 (InForm).

Western blotting. RIPA (Cell Signaling technology, #9806) buffer was used to extract protein from cells and tissue. Then, centrifugation (4˚C, 12000g) for 2 h was performed, the supernatant was collected in a clean tube and concentration was determined via BCA kit. Proteins (20 μg/lane) were separated by electrophoresis using 10% SDS-PAGE and transferred to a PVDF membrane, which was blocked using 10% BSA for 30 min at room temperature. Then, the membrane was incubated with primary antibody for ≥8 h at 4˚C. The next day, membranes were washed twice, incubated with the HRP-linked secondary antibody overnight at 4˚C, and signals were visualized using chemiluminescence reagent (Millipore cat. no. WBKLS0050) and analyzed using Image Lab software 5.0 (Bio-Rad Laboratories, Inc.). The antibodies were as follows: Anti-SAC3D1 (1:1,000; cat. no. 25857-1-AP; ProteinTech Group, Inc.), anti-tubulin (1:4,000; cat. no. sc-5286; Santa Cruz Biotechnology, Inc.), anti-Flag (1:3,000; cat. no. F9291; Sigma-Aldrich; Merck KGaA), anti-ubiquitin (1:1,000; cat. no. #3936; Cell Signaling Technology, Inc.), anti-GAPDH (1:3,000; cat. no. 6004-1-Ig; ProteinTech Group, Inc.), anti-β-catenin (1:1,000; cat. no. 51067-2-AP; ProteinTech Group, Inc.), anti-histone (1:1,000; cat. no. 17168-1-AP; ProteinTech Group, Inc.), anti-axin (1:1,000; cat. no. 16541-1-AP; ProteinTech Group, Inc.) and anti-hemagglutinin (HA; 1:5,000; cat. no. 51064-2-AP; ProteinTech Group, Inc.).

MTT assay. Cells were incubated in fresh DMEM containing 10% MTT reagent (5 mg/ml in PBS) for 4 h. Dimethyl sulfoxide was used to dissolve the purple formazan and optical density at 540 nm was evaluated.

Anchorage-independent growth assay. A total of 500 μl gel [20% FBS, 40% 2X RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.)and 40% agar (~1.25%)] was added into a 24-well plate. When the gel solidified, the cell suspension ($1x10^4$ cells/ml) in RPMI-1640 was made. Then, gel [25% FBS (Gibco), 37.5% 2X RPMI-1640 (Gibco), 37.5% agar (1%) and 0.8% 2 mM L-glutamine] was made, the cell suspension was added to the gel and mixed and 500 μl mixture was added to each well. When the gel solidified, the plate was placed in the incubator at 37˚C. After 14 days, colonies (>50 cells) were photographed by a light microscope with 10-fold magnification and counted manually.

Ubiquitination assay. Cells were incubated with proteasome inhibitor MG132 (20 μM, Sigma-Aldrich; Merck KGaA) overnight at 37˚C. Immunoprecipitation lysis buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% NP-40 and protease and phosphatase inhibitors] was used to extract protein. A total of 1 μg β-catenin antibody (cat. no. #9562; Cell Signaling Technology, Inc.) and 500 μg total cell lysate was used to perform immunoprecipitation at 4˚C. After 8-10 h, Protein A/G beads (~40 μl; Bimake; cat. no. B23202) was added for 4 h incubation at 4˚C. After washing with TBST, 1X loading buffer was added to beads for 5 min at 100˚C. The immunoprecipitate was examined by western blot analysis and anti-ubiquitin antibody as aforementioned.

Immunoprecipitation assay. pLvx and pCMV-HA plasmids were obtained from Adgene. pLvx/Flag-SAC3D1 and PCMV/HA-Axin plasmids were co-transfected into cells using Lipofectamine 8000 (Beyotime Institute of Biotechnology) at room temperature for 20 min. After 48 h, immunoprecipitation lysis buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% NP-40 and protease and phosphatase inhibitors] was used to extract protein from cells. After centrifugation (4˚C, 12,000 g) for 20 min, the supernatant was collected 20 μl. Beads conjugated with anti-Flag antibody (0.25 μg) (cat. no. A2220; Sigma-Aldrich; Merck KGaA) were incubated with the 750 μl supernatant (containing 500 μg protein) for 2 h. Then, wash buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% NP-40] was used to wash the beads. The immunoprecipitate was

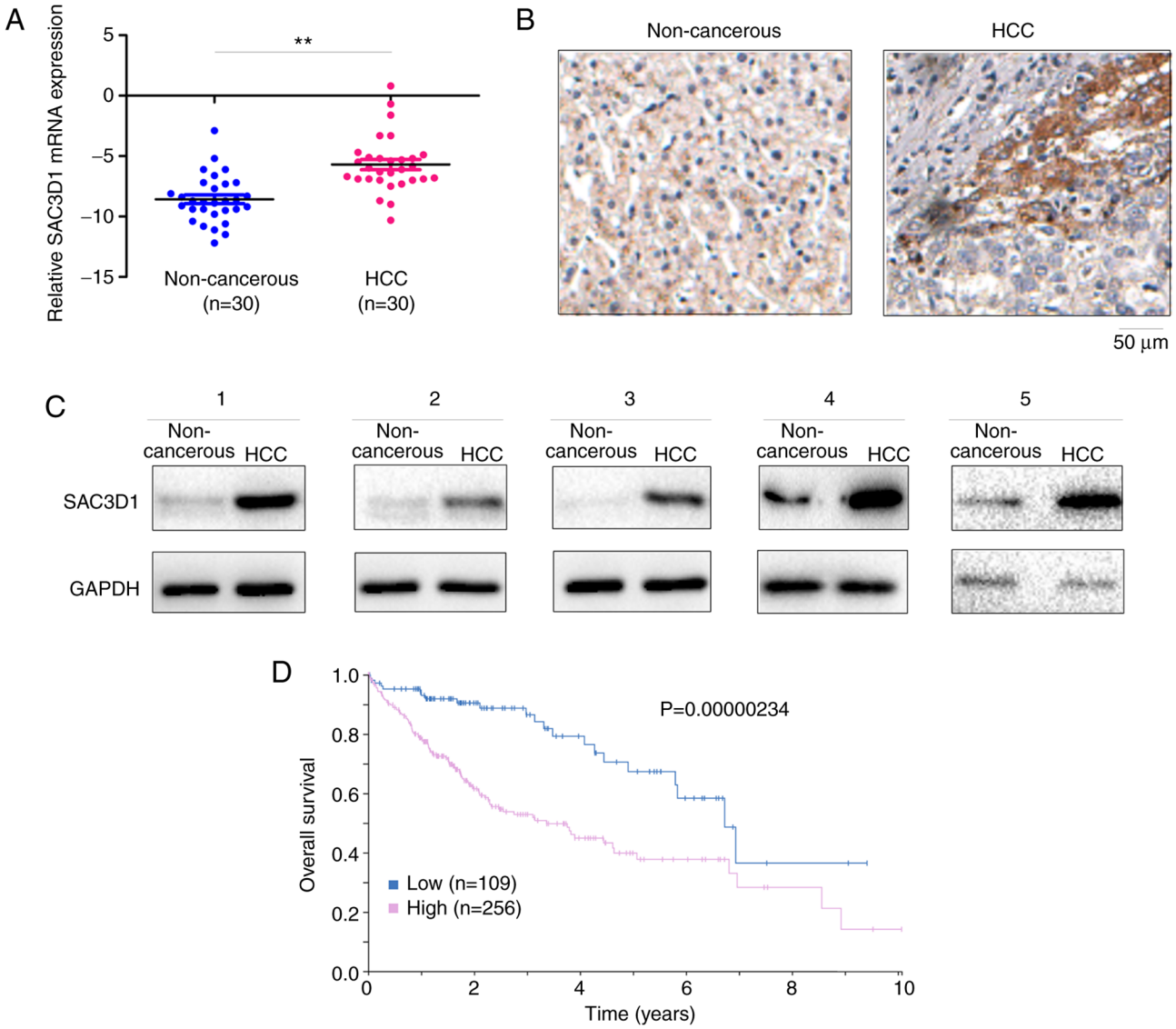

Figure 1. SAC3D1 is overexpressed in HCC. (A) Levels of SAC3D1 mRNA in 30 HCC and adjacent non-cancerous tissue samples were evaluated by quantitative PCR. Levels of SAC3D1 protein in tumour and adjacent non-cancerous tissue samples were evaluated by (B) immunohistochemistry and (C) western blotting. (D) Human Protein Atlas database was mined to analyse expression of SAC3D1 and survival. **P<0.01. SAC3D1, SAC3 domain containing 1; HCC, hepatocellular carcinoma.

eluted by 1X loading buffer and boiled at 100˚C for 5 min, then examined by western blot analysis as aforementioned.

Human Protein Atlas (HPA) database. To analyze the correlation between the expression of SAC3D1 and survival, HPA database was used (proteinatlas.org/search/SAC3D1). The Cut-off value is FPKM 5.6.

Statistical analysis. Data are presented as the mean ± standard deviation of two or three experimental repeats. Parametric one-way analysis of variance followed by Tukey-Kramer's post hoc test was used to test differences between groups using SPSS 15.0 (SPSS, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

SAC3D1 expression is upregulated in HCC. To determine expression of SAC3D1 in HCC, mRNA transcripts of SAC3D1 in 30 HCC tissue and paired adjacent samples were measured. The mean mRNA level of SAC3D1 in HCC tissue was significantly elevated (Fig. 1A). The results were verified by IHC staining (Fig. 1B). SAC3D1 protein level in 5 tumour and paired non-cancerous tissue samples was analysed. In HCC tissue, the level of SAC3D1 protein was relatively high (Fig. 1C). From the Human Protein Atlas database, it was determined that higher SAC3D1 expression was associated with poorer outcome (Fig. 1D). These findings indicated that SAC3D1 expression may be associated with HCC.

SAC3D1 promotes growth of HCC cells both in liquid media and on soft agar. To investigate the role of SAC3D1 in HCC cells, exogenous SAC3D1 was first stably expressed in Huh7 and MHCC97H cells (Fig. 2A). The effect of SAC3D1 expression on cell proliferation were investigated. MTT assay showed that SAC3D1 accelerated cell proliferation (Fig. 2B). A growth assay on soft agar was performed to determine

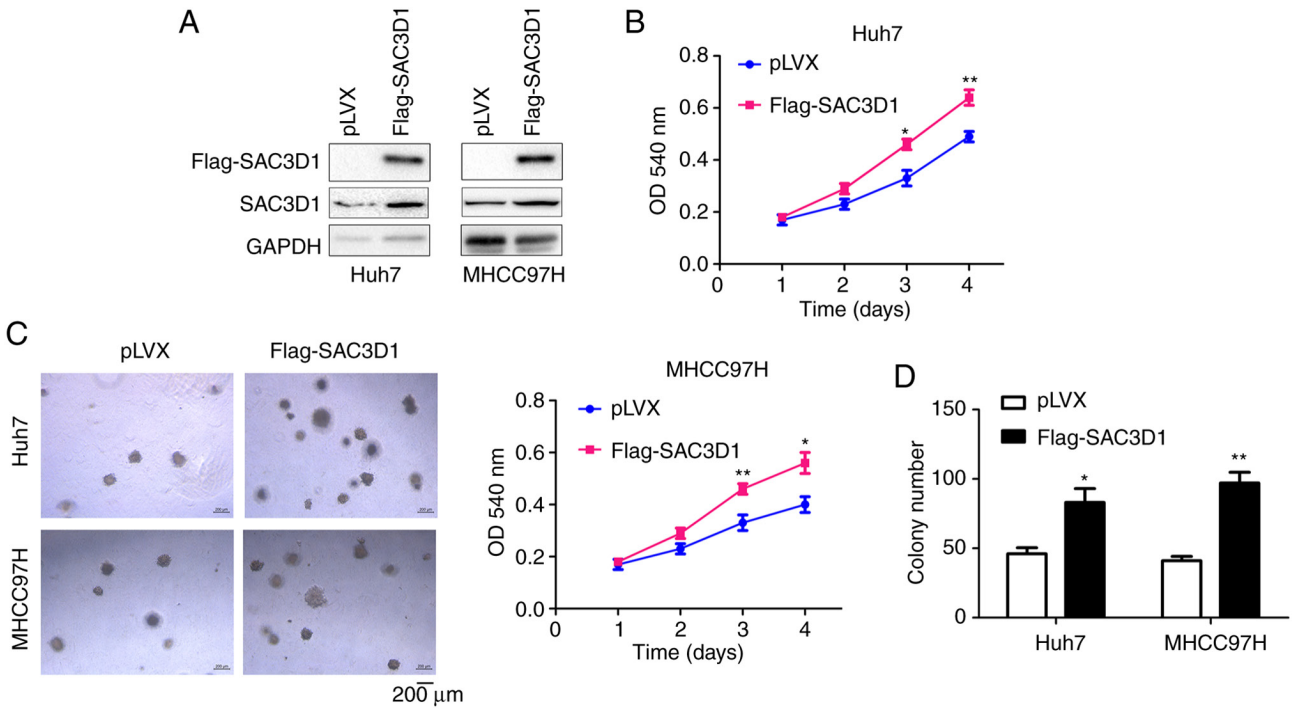

Figure 2. SAC3D1 promotes aggressive behaviour of HCC cells. (A) Overexpression of Flag-SAC3D1 in Huh7 and MHCC97H cells was evaluated by western blotting. (B) Proliferation of HCC cells was examined by MTT assay. (C) Anchorage-independent colony formation of HCC cells was evaluated on soft agar and (D) analysed. *P<0.05 Huh7/pLVX vs. Huh7/Flag-SAC3D1; **P<0.01 MHCC97H/pLVX vs. MHCC97H/Flag-SAC3D1. SAC3D1, SAC3 domain containing 1; HCC, hepatocellular carcinoma; OD, optical density.

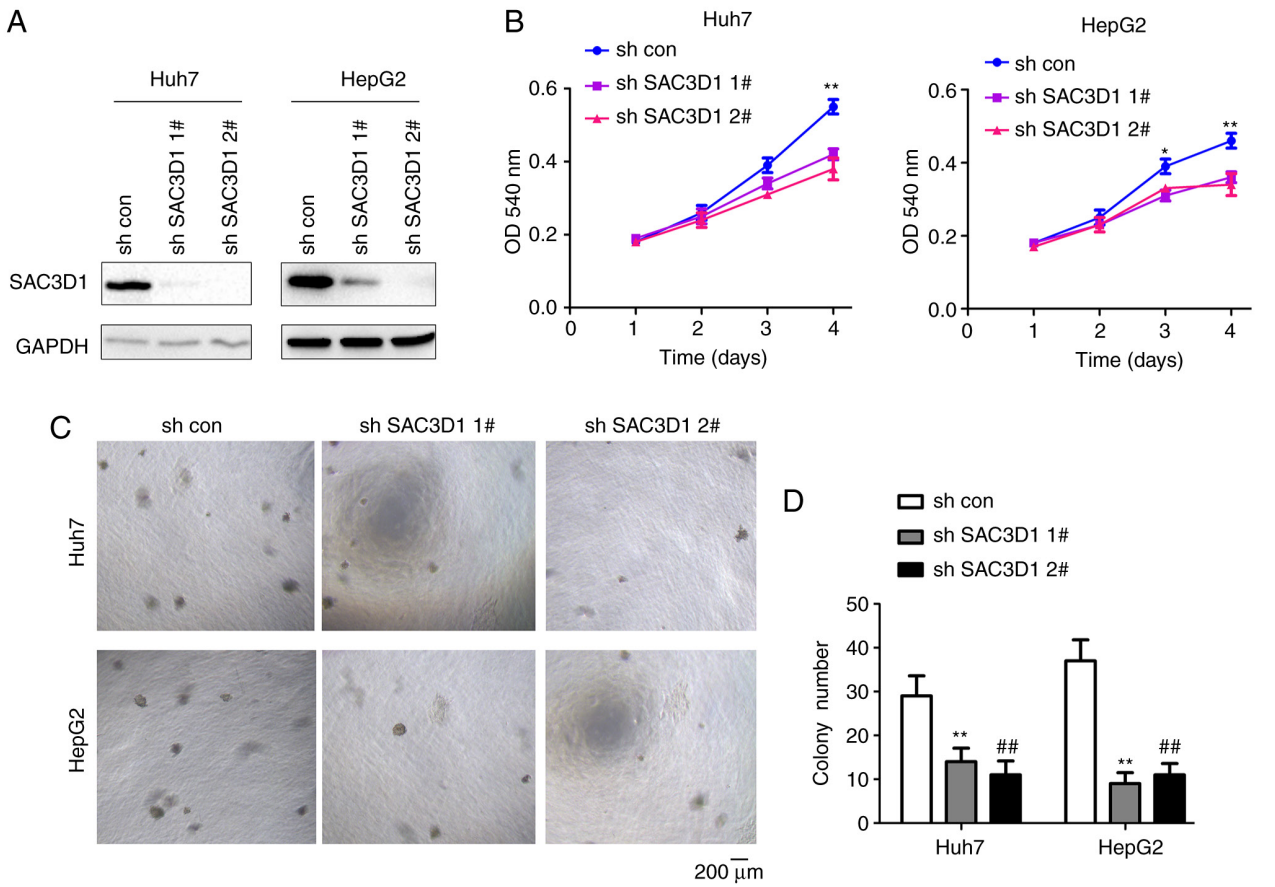

Figure 3. Knockdown of SAC3D1 impairs aggressive cell behaviour. (A) Efficiency of knocking down SAC3D1 in Huh7 and HepG2 cells was evaluated by western blotting. (B) Proliferation of HCC cells was analysed by MTT assay. (C) Anchorage-independent colony formation of HCC cells was (D) evaluated on soft agar. *P<0.05, **P<0.01 vs. sh SAC3D1 1#; ##P<0.01 vs. sh SAC3D1 2#. SAC3D1, SAC3 domain containing 1; HCC, hepatocellular carcinoma; OD, optical density; sh, short hairpin; con, control.

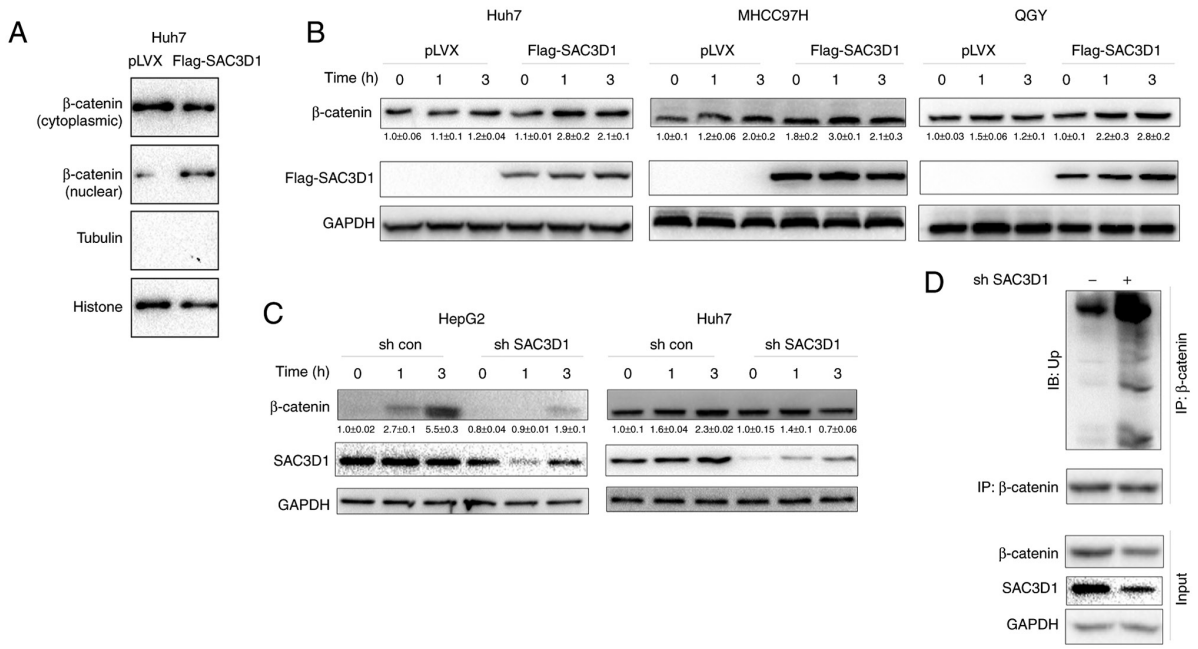

Figure 4. SAC3D1 induces β-catenin accumulation. (A) Nuclear protein was extracted from Huh7 cells and levels of β-catenin protein was examined by western blotting. (B) SAC3D1 enhanced protein levels of β-catenin. (C) Knockdown of SAC3D1 decreased levels of β-catenin. (D) Knockdown of SAC3D1 enhanced ubiquitination of β-catenin. MG132 (10 μM) was used to treat cells for 8 h and ubiquitination assay was performed. SAC3D1, SAC3 domain containing 1; HCC, hepatocellular carcinoma; sh, short hairpin; con, control; IB, immunoblot; IP, immunoprecipitate; Ub, ubiquitin.

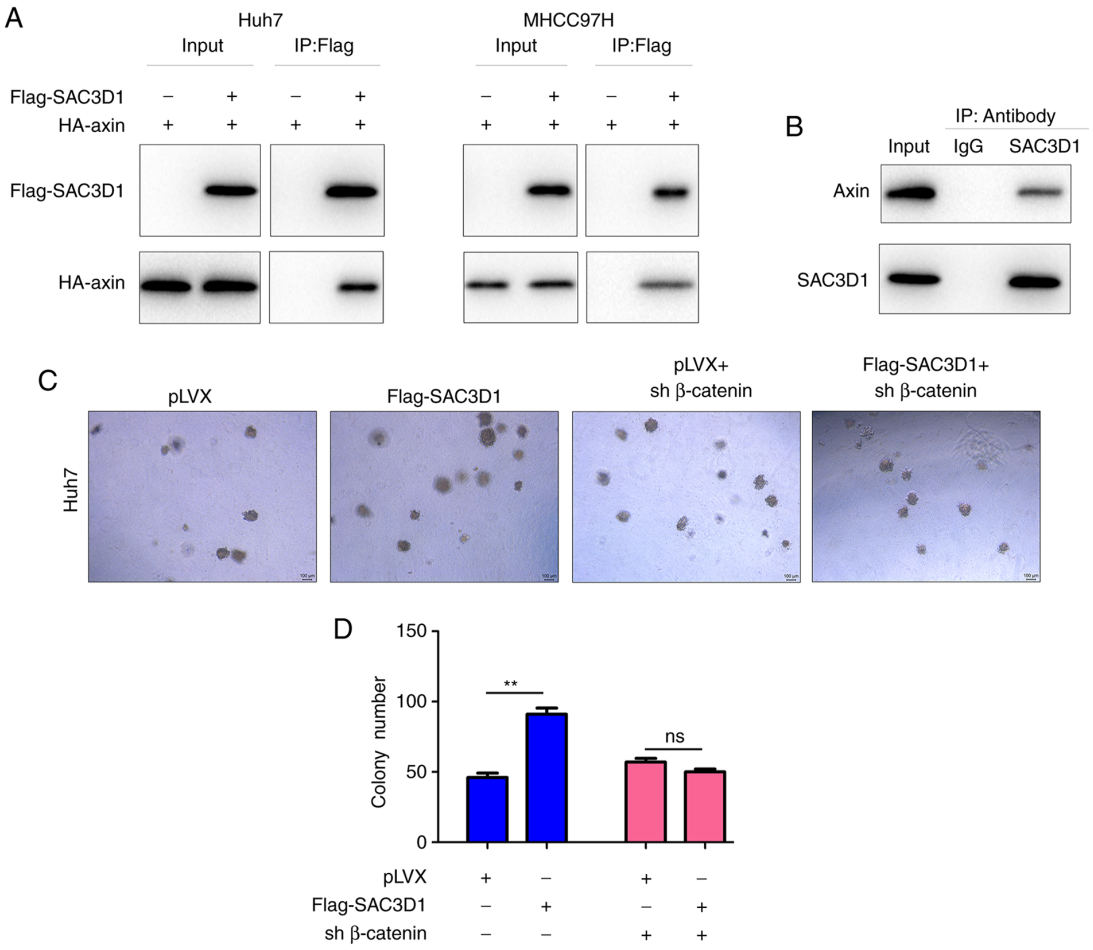

Figure 5. SAC3D1 interacts with axin. (A) Co-IP was performed to evaluate binding between Flag-SAC3D1 and HA-axin. Huh7 and MHCC97H cells were transfected with vectors. (B) Binding between endogenous SAC3D1 and axin in Huh7 cells was evaluated by co-IP. (C) Anchorage-independent assay and (D) analysis was performed to examine whether Wnt/β-catenin signalling mediates the oncogenic role of SAC3D1. **P<0.01. SAC3D1, SAC3 domain containing 1; co-IP, co-immunoprecipitation; sh, short hairpin; ns, not significant; HA, hemagglutinin.

the effect of SAC3D1 expression on anchorage-independent growth (Fig. 2C and D). The results showed that overexpression of SAC3D1 promoted cell growth.

SAC3D1 was then knocked down in two HCC cell lines using shRNA (Fig. 3A). Downregulation of SAC3D1 inhibited the proliferation HepG2 and Huh7 cells (Fig. 3B), as well as their growth on agar (Fig. 3C and D).

SAC3D1 promotes β-catenin accumulation. To test whether SAC3D1 regulates the Wnt/β-catenin signalling pathway, the effect of SAC3D1 on levels of nuclear β-catenin in control Huh7 cells and Huh7 cells overexpressing SAC3D1 was evaluated. SAC3D1 promoted nuclear localization of β-catenin (Fig. 4A), suggesting that SAC3D1 elevated levels of nuclear β-catenin protein. Consistently, Wnt3a promoted accumulation of β-catenin when SAC3D1 was overexpressed (Fig. 4B) and knockdown of SAC3D1 inhibited elevation of nuclear protein levels induced by Wnt3a (Fig. 4C). Moreover, knockdown of SAC3D1 elevated levels of ubiquitinated β-catenin (Fig. 4D).

SAC3D1 forms a complex with axin. To study the molecular mechanism underlying stabilization of β-catenin by SAC3D1, the interaction between SAC3D1 and each protein in the β-catenin degradation complex was analysed. In Huh7 and MHCC97H cells, exogenously expressed SAC3D1 (Flag-SAC3D1) interacted with axin (HA-Axin) in the degradation complex (Fig. 5A). Consistent with this observation, endogenously expressed SAC3D1 and axin formed a complex in Huh7 cells (Fig. 5B). Moreover, knockdown of β-catenin abolished the promoting effect of SAC3D1 on anchorage-independent growth of Huh7 cells, suggesting that the oncogenic role of SAC3D1 was dependent on Wnt/β-catenin signalling (Fig. 5C and D).

Discussion

Previous study has shown that expression of SAC3D1 is upregulated in HCC (16). Previous analysis of The Cancer Genome Atlas database showed that high expression of SAC3D1 is associated with poor outcome (GEPIA.cancer-pku.cn /detail.php?gene=SAC3D1). However, the function of SAC3D1 in the progression of HCC, as well as the molecular mechanism involved, remain unknown. Here, levels of SAC3D1 mRNA and protein were increased in HCC tissue and the proliferation and colony formation of HCC cells were enhanced by SAC3D1. SAC3D1 interacted with axin, activating the Wnt/β-catenin cascade. These findings reveal the promotive role of SAC3D1 in the progression of HCC.

In HCC tissue, the Wnt/β-catenin cascade is overactive. The reasons for its abnormal activation include loss of function mutation of Axin, constitutively activating mutation of β-catenin (10,20-22), upregulation of Wnt ligand, and downregulation of secreted frizzled-related protein (23-25). These abnormal changes are directly associated with the function of β-catenin degradation complex. Therefore, the regulatory mechanism of the β-catenin degradation complex in HCC may be valuable for identifying novel therapeutic targets. Here, SAC3D1 interacted with axin in the degradation complex, indicating the mechanism by which SAC3D1 activates the Wnt/β-catenin cascade. Therefore, a promising treatment approach may be to develop a small molecule drug to block the interaction between SAC3D1 and axin in the degradation complex.

The effect of SAC3D1 on the progression of HCC was studied in an HCC cell model, revealing that SAC3D1 promoted cell growth. In future, clinical specimens of HCC should be collected to confirm the association between levels of SAC3D1 and HCC clinical features. An animal model should be used to study the role of SAC3D1 in the occurrence and progression of HCC. In summary, SAC3D1 was upregulated in HCC and promoted progression of HCC by activating Wnt/β-catenin signalling.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HW designed the study and wrote the manuscript. XS performed the experiments. Both authors have read and approved the final manuscript. HW and XS confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Bozhou People's Hospital (approval no. E-2019-04).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J and Finn RS: Hepatocellular carcinoma. Nat Rev Dis Primers 7: 6, 2021.
2. Altomonte J. Liver cancer: Sensitizing hepatocellular carcinoma to oncolytic virus therapy. Nat Rev Gastroenterol Hepatol 15: 8-10, 2018.
3. Bruix J, da Fonseca LG and Reig M: Insights into the success and failure of systemic therapy for hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 16: 617-630, 2019.
4. Schunk SJ, Floege J, Fliser D and Speer T. WNT-β-catenin signalling-a versatile player in kidney injury and repair. Nat Rev Nephrol 17: 172-184, 2021.
5. Moon RT, Kohn AD, De Ferrari GV and Kaykas A: WNT and β-catenin signalling: Diseases and therapies. Nat Rev Genet 5: 691-701, 2004.
6. Kypta RM and Waxman J: Wnt/β-catenin signalling in prostate cancer. Nat Rev Urol 9: 418-428, 2012.

7. Leavy O. Mucosal immunology: β-catenin calms the gut. Nat Rev Immunol 10: 682, 2010.
8. Mosimann C, Hausmann G and Basler K: Beta-catenin hits chromatin: Regulation of Wnt target gene activation. Nat Rev Mol Cell Biol 10: 276-286, 2009.
9. Clevers H. Axin and hepatocellular carcinomas. Nat Genet 24: 206-208, 2000.
10. Ishizaki Y, Ikeda S, Fujimori M, Shimizu Y, Kurihara T, Itamoto T, Kikuchi A, Okajima M and Asahara T: Immunohistochemical analysis and mutational analyses of beta-catenin, Axin family and APC genes in hepatocellular carcinomas. Int J Oncol 24: 1077-1083, 2004.
11. Tian W, Li J, Wang Z, Zhang T, Han Y, Liu Y, Chu W, Liu Y and Yang B: HYD-PEP06 suppresses hepatocellular carcinoma metastasis, epithelial-mesenchymal transition and cancer stem cell-like properties by inhibiting PI3K/AKT and WNT/β-catenin signaling activation. Acta Pharm Sin B 11: 1592-1606, 2021.
12. Huang Y, Sheng H, Xiao Y, Hu W, Zhang Z, Chen Y, Zhu Z, Wu D, Cao C and Sun J: Wnt/β-catenin inhibitor ICG-001 enhances the antitumor efficacy of radiotherapy by increasing radiation-induced DNA damage and improving tumor immune microenvironment in hepatocellular carcinoma. Radiother Oncol 162: 34-44, 2021.
13. Yu Y, Zhang L and Xu J: LncRNA BANCR promotes proliferation of hepatocellular carcinoma Huh-7 cells by activating Wnt/beta-catenin signaling pathway. Minerva Gastroenterol (Torino) Jun 11, 2021 (Epub ahead of print).
14. Yang T, Xu R, Huo J, Wang B, Du X, Dai B, Zhu M, Zhan Y, Zhang D and Zhang Y: WWOX activation by toosendanin suppresses hepatocellular carcinoma metastasis through JAK2/Stat3 and Wnt/β-catenin signaling. Cancer Lett 513: 50-62, 2021.
15. Adebayo Michael AO, Ko S, Tao J, Moghe A, Yang H, Xu M, Russell JO, Pradhan-Sundd T, Liu S, Singh S, *et al*: Inhibiting glutamine-dependent mTORC1 activation ameliorates liver cancers driven by β-catenin mutations. Cell Metab 29: 1135-1150. e6, 2019.
16. Han ME, Kim JY, Kim GH, Park SY, Kim YH and Oh SO. SAC3D1: A novel prognostic marker in hepatocellular carcinoma. Sci Rep 8: 15608, 2018.
17. Liu AG, Zhong JC, Chen G, He RQ, He YQ, Ma J, Yang LH, Wu XJ, Huang JT, Li JJ, *et al*: Upregulated expression of SAC3D1 is associated with progression in gastric cancer. Int J Oncol 57: 122-138, 2020.
18. Nakajima H, Tamura T, Ito M, Shibata F, Kuroda K, Fukuchi Y, Watanabe N, Kitamura T, Ikeda Y and Handa M: SHD1 is a novel cytokine-inducible, negative feedback regulator of STAT5-dependent transcription. Blood 113: 1027-1036, 2009.
19. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
20. Guan CN, Chen XM, Lou HQ, Liao XH, Chen BY and Zhang PW: Clinical significance of axin and β-catenin protein expression in primary hepatocellular carcinomas. Asian Pac J Cancer Prev 13: 677-681, 2012.
21. Park JY, Park WS, Nam SW, Kim SY, Lee SH, Yoo NJ, Lee JY and Park CK: Mutations of beta-catenin and AXIN I genes are a late event in human hepatocellular carcinogenesis. Liver Int 25: 70-76, 2005.
22. Kitao A, Matsui O, Yoneda N, Kozaka K, Kobayashi S, Sanada J, Koda W, Minami T, Inoue D, Yoshida K, *et al*: Hepatocellular carcinoma with β-catenin mutation: Imaging and pathologic characteristics. Radiology 275: 708-717, 2015.
23. Yu J, Xie Y, Li M, Zhou F, Zhong Z, Liu Y, Wang F and Qi J: Association between SFRP promoter hypermethylation and different types of cancer: A systematic review and meta-analysis. Oncol Lett 18: 3481-3492, 2019.
24. Xu C, Zeng XH, Wang L, Tao SQ, Wu QX, Zhu P, Deng GH and Wang YM: sFRP-4, a potential novel serum marker for chronic hepatitis B-related hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int 14: 164-170, 2015.
25. Bengochea A, de Souza MM, Lefrançois L, Le Roux E, Galy O, Chemin I, Kim M, Wands JR, Trepo C, Hainaut P, *et al*: Common dysregulation of Wnt/Frizzled receptor elements in human hepatocellular carcinoma. Br J Cancer 99: 143-150, 2008.