



Sex Comb on Midleg Like-2 Accelerates Hepatocellular Carcinoma Cell Proliferation and Metastasis by Activating Wnt/ β -Catenin/EMT Signaling

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Purpose: The purpose of this study was to investigate the influences of sex comb on midleg like-2 (SCML2) on hepatocellular carcinoma (HCC) and potentially related mechanisms.

Materials and Methods: SCML2 expression in tumor tissues and cells was analyzed using the TCGA database and/or qRT-PCR. The proliferation of HCC cells was detected by CCK-8, colony formation, and EdU assays. The migration and invasion of HCC cells were detected by transwell and wound healing assays. Apoptosis of HCC cells was determined by flow cytometry. Additionally, qRT-PCR and Western blot were used to detect the expression of SCML2 and Wnt/ β -catenin/epithelial-mesenchymal transition (EMT) signaling. A xenograft model in mice was established to verify the in vitro findings.

Results: We found that SCML2 was highly expressed in HCC tissues and cells and that high expression of SCML2 was correlated with poor prognosis in HCC patients. SCML2 overexpression promoted proliferation, invasion, and migration and repressed apoptosis of HCC cells. The reverse results were obtained in SCML2-silenced cells. Further, we found that SCML2 activated the Wnt/ β -catenin/EMT pathway. SCML2 silencing reduced the protein levels of Wnt3a, β -catenin, N-cadherin, Vimentin, and Snail and enhanced E-cadherin protein expression both in vivo and in vitro.

Conclusion: SCML2 silencing inhibits the proliferation, migration, and invasion of HCC cells by regulating the Wnt/ β -catenin/EMT pathway.

Key Words: Sex comb on midleg like-2, hepatocellular carcinoma, proliferation, apoptosis, signaling pathway

INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignancy of the liver, with the third highest mortality rate among cancers worldwide.^{1,2}

Most HCC patients die from high rates of metastasis and post-operative recurrence.³ In current clinical practice, the primary treatments for HCC include liver transplantation, liver resection, transcatheter arterial chemoembolization, radiofrequen-

Received: February 26, 2021 **Revised:** August 26, 2021 **Accepted:** September 3, 2021

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•The authors have no potential conflicts of interest to disclose.

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cy ablation, and sorafenib.⁴ If HCC patients can be diagnosed at an early stage, hepatectomy or liver transplantation may cure HCC.⁵ However, surgery can be only performed in 15% of patients with HCC due to untimely detection of most HCCs and the potential for liver dysfunction in some patients.⁶ Also, when treating larger lesions (>3 cm), the rates of effective radiofrequency ablation monotherapy drop sharply.⁷ Additionally, the side effects of some drugs, such as sorafenib, are relatively large, and their therapeutic effects can decrease with long-term use, along with development of drug resistance.⁸ Ultimately, as the carcinogenesis of HCC is regarded as a multi-factor, multi-stage complex process,⁹ finding new diagnostic markers is of great significance for early diagnosis and effective treatment of HCC.

As one protein encoded by the *Drosophila Sex comb on midleg (Scm)* gene, sex comb on midleg like-2 (SCML2) is located close to SCML1, a gene cluster that may have originated before primate divergence.¹⁰ The Human SCML2 gene encodes two protein subtypes, SCML2A and SCML2B.¹¹ Sex comb on midleg (SCM) is required for the collection and suppressive effect of polycomb repressive complex 1 (PRC1) and PRC2, including a domain of undiscovered function, an SPM domain, two zinc fingers, and two malignant brain tumor (MBT) repeats.^{12,13} SCM plays an inhibitive impact on target genes via activation of MBT and SPM domains.¹⁴ It is worth noting that aberrant SCM function may be related with certain cancers and tissue growth.¹⁵ In the Xp22 region, SCML2 is regarded as a human gene that encodes 700 amino acids.¹⁶ SCML2 plays important roles in the regulation of ubiquitination and is a polycomb-group protein that codes transcriptional inhibitors requisite for mammalian normal progression.^{11,17} In a study by Yang, et al.,¹¹ SCML2 was identified as a new specific marker for gastroenteropancreatic cancers. Bioinformatics analysis showed that SCML2 might be recognized as an upstream regulatory molecule of amelogenin Y-linked protein in HCC.¹⁸ However, whether SCML2 exerts important roles on HCC has not been reported.

In our study, the results of bioinformatics analysis showed that SCML2 is highly expressed in HCC tissues and that high levels of SCML2 expression are associated with poor prognosis in HCC patients. Additionally, we found that SCML2 was up-regulated in HCC tumor tissues and cell lines. Moreover, we discovered that SCML2 high-expression facilitates proliferation and migration, while SCML2 ablation restrains proliferation and migration of HCC cells. The promoted effect of SCML2 on HCC might be achieved by regulating Wnt/ β -catenin/epithelial-mesenchymal transition (EMT) signaling. In summary, our study offers a basis for discovering new and more effective markers for HCC treatment.

MATERIALS AND METHODS

Bioinformatics analysis

Data on SCML2 expression in 371 HCC tissues (primary tumor)

and 50 adjacent non-tumorous tissue samples (normal) were acquired from the TCGA database (<https://cancergenome.nih.gov/>). The survival data were estimated using the Kaplan-Meier method and analyzed using the log-rank test.

Tissue specimens

In total, 53 HCC tissues and adjacent normal tissues were collected from HCC patients. These tissues were snap-frozen and stored at -80°C. The clinical characteristics of the 53 patients with HCC are described in Table 1. None of the 53 patients received radiation or chemotherapy before surgery. Informed consent was obtained from all participants in our study. The study protocol was approved by the Human Ethics Committee of Qingdao No.6 People's Hospital (201834).

Table 1. Correlation between SCML2 Expression and Clinicopathological Parameters in Patients with Hepatocellular Carcinoma

Clinical characteristics	n	SCML2		p value
		Low	High	
Sex				0.2749
Male	25	10	15	
Female	28	16	12	
Serum AFP (ng/mL)				0.1007
<400	29	11	18	
≥400	24	15	9	
HBsAg				0.1728
Negative	25	15	10	
Positive	28	11	17	
HBV				0.2749
Negative	24	14	10	
Positive	29	12	17	
HCV				0.4142
Negative	25	14	11	
Positive	28	12	16	
NASH				0.1655
No	31	18	13	
Yes	22	8	14	
Alcohol				0.1016
No	26	16	10	
Yes	27	10	17	
Tumor size				0.0060*
<5 cm	26	18	8	
≥5 cm	27	8	19	
TNM stage				0.0058*
I+II	24	17	7	
III+IV	29	9	20	
Lymph node metastasis				0.0024*
No	27	19	8	
Yes	26	7	19	

SCML2, sex comb on midleg like-2; AFP, alpha fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis.

* $p < 0.05$.

Culture of HCC cell lines

Human HCC cell lines Hep3B, Huh-7, and SMMC-7721 were obtained from the Chinese Academy of Medical Sciences Shanghai Cell Bank (Shanghai, China) and the normal hepatocyte cell line THLE-3 was purchased from American Type Culture Collection (Manassas, VA, USA). These cells were routinely cultured in DMEM medium including 10% FBS and 1% penicillin-streptomycin at 37°C and 5% CO₂.

Cell transfection

The si-control, siRNA (si)-SCML2#1, si-SCML2#2, pcDNA3.1 (pc)-SCML2, and pcDNA3.1 negative control (vector) (GenePharma, Shanghai, China) were used to downregulate or upregulate SCML2 levels in HCC cells. First, cells were seeded in 6-well plates and grown to 80% confluence. Afterwards, si-control and siRNAs were transfected into SMMC-7721 cells; pc-SCML2 and vector were transfected into Hep3B cells using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). After incubation for 48 h, the transfection efficiency was confirmed by qRT-PCR.

qRT-PCR

Total RNA in tumor tissues and cells was extracted using TRIzol reagent (Invitrogen), and cDNA was synthesized by a SuperScript III reverse transcriptase (TransGen Biotech, Beijing, China). qPCR was performed using the ABI Prism 7500 system (Applied Biosystems, Carlsbad, CA, USA) with SYBR Green qPCR Master Mix (Toyobo, Osaka, Japan). GAPDH was regarded as an internal control. The relative expression levels of SCML2 were determined on the basis of the formula of $2^{-\Delta\Delta Ct}$. Primers for qPCR are presented in Table 2.

Western blotting analysis

Total protein in tissues and cells was isolated by RIPA lysis with protease inhibitor (Thermo Fisher Scientific, Waltham, MA, USA). Protein specimens were isolated by 15% SDS-PAGE and transferred onto a PVDF membrane. Afterwards, the membranes were blocked with 5% skimmed milk for 1 h and then probed with primary antibodies (SCML2, Wnt3a, Vimentin, Snail, Thermo Fisher Scientific; β -catenin, E-cadherin, Sigma Aldrich, St. Louis, MO, USA; N-cadherin, Abcam, Cambridge, MA, USA) at 4°C overnight. The membranes were then incubated with secondary antibodies for 1 h at room temperature. After being washed with phosphate buffer saline (PBS) for 3×5 min, pro-

teins were visualized using enhanced chemiluminescence (GE Healthcare, Beijing, China).

Cell proliferation

CCK-8 assay was utilized to assess cell proliferation of SMMC-7721 and Hep3B cells. Cells were seeded at 1×10^3 cells/well in 96-well plates and cultured for 24 h, 48 h, and 72 h. Then, 10 μ L of CCK8 reagent was added to each well, and the plates were hatched for an additional 1.5 hours. Lastly, the OD₄₅₀ was analyzed utilizing a microplate reader (Elx800, BioTek, Winooski, VT, USA).

Colony formation assay

A total of 400 cells was seeded in 12-well plates at 37°C for 14 days. The formed cell colonies were immobilized with 4% paraformaldehyde at room temperature for 15 min. Afterwards, the colonies were stained with 0.5% crystal violet at room temperature for 1 h, followed by taking photos and counting under a microscope.

EdU assay

HCC cells (1.5×10^5) were seeded into a 24-well plate for 24 h and then incubated with 50 μ M 5-ethynyl-2'-deoxyuridine (EdU, RiboBio, Guangzhou, Guangdong, China) at 37°C for 2 h. The cells were then fixed with 4% paraformaldehyde for 30 min at room temperature. Afterwards, cells were incubated with glycine (2 mg/mL) for 5 min and 0.5% Trion X-100 for 10 min. The cells were then washed three times with PBS and incubated with 100 μ L DAPI solution at room temperature for 30 min. Finally, the images ($\times 200$) were captured by employing inverted microscopy (Nikon, Tokyo, Japan). The percentage of EdU-positive cells was tested from five random fields.

Transwell assay

In vitro invasion and migration of HCC cells were measured using transwell inserts pre-coated with or without Matrigel. Briefly, serum-free medium (200 μ L, including approximately 1×10^5 transfected cells) was inoculated into the upper chamber of each transwell. Complete medium (500 μ L, including 10% FBS) was put onto the lower chamber and incubated for 24 h at 37°C. The cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 10 min. Lastly, cell numbers were counted in five randomly selected fields using a light microscope at $\times 200$ magnification.

Wound healing assay

Cell migration capacity was determined by wound healing assay. HCC cells were plated in 6-well plates at a density of 5×10^5 cells/well and incubated at 37°C overnight. A cell-free wound gap was generated by scratching plates with a 10- μ L pipette tip. The rate of wound closure was monitored at different time points under a microscope.

Table 2. Primers Utilized in qRT-PCR

Name	Sequences
si-control	5'-CGAACUCACUGGUCUGACC-3'
si-SCML2#1	5'-GCCCTGACATTCTTGTGAA-3'
si-SCML2#2	5'-CCTGGCTTGCTGAACATCA-3'
SCML2 forward	5'-ACATTCTTGCCCTGGTGAA-3'
SCML2 reverse	5'-GCCACAGAGGTGACGATGAA-3'
GAPDH forward	5'-ACCTCAACTACATGGCTGAGAA-3'
GAPDH reverse	5'-CATGACGAACATGGGGGCAT-3'

Apoptosis rate detection

After 48 h transfection, HCC cells were collected and washed three times with 1×PBS. The cells were stained with Annexin VFITC and PI following the manufacturer’s protocol. Finally, the samples were tested on a flow cytometer. The results were analyzed using FlowJo software (BD, Franklin Lakes, NJ, USA).

In vivo tumor formation assay

The xenograft model was built with male BALB/c nude mice (6-weeks-old) purchased from Huafukang Bioscience Co, Inc (Beijing, China). The animal experiments were executed following guidelines from the Use Committee for Animal Care and approved by the institutional guidelines of our hospital.

SMMC-7721-si-SCML2 or SMMC-7721-si-control cells (5×10^6 cells) were injected subcutaneously into the right flank of nude mice. After injection for 5 days, tumor sizes were examined synchronously every 5 days and estimated using a simplified equation ($\text{length} \times \text{width}^2 \times 0.5$). Mice were sacrificed after five measurements, and the tumors were weighed.

Immunohistochemistry

The expression levels of SCML2 in tumor tissues from HCC patients and mice were detected by immunohistochemistry (IHC). Tumors were gathered and immobilized with 4% formaldehyde for 24 h at 4°C. Tissues were then embedded in paraffin and sectioned into 4- μm -thick sections. For IHC assay, paraffin sections

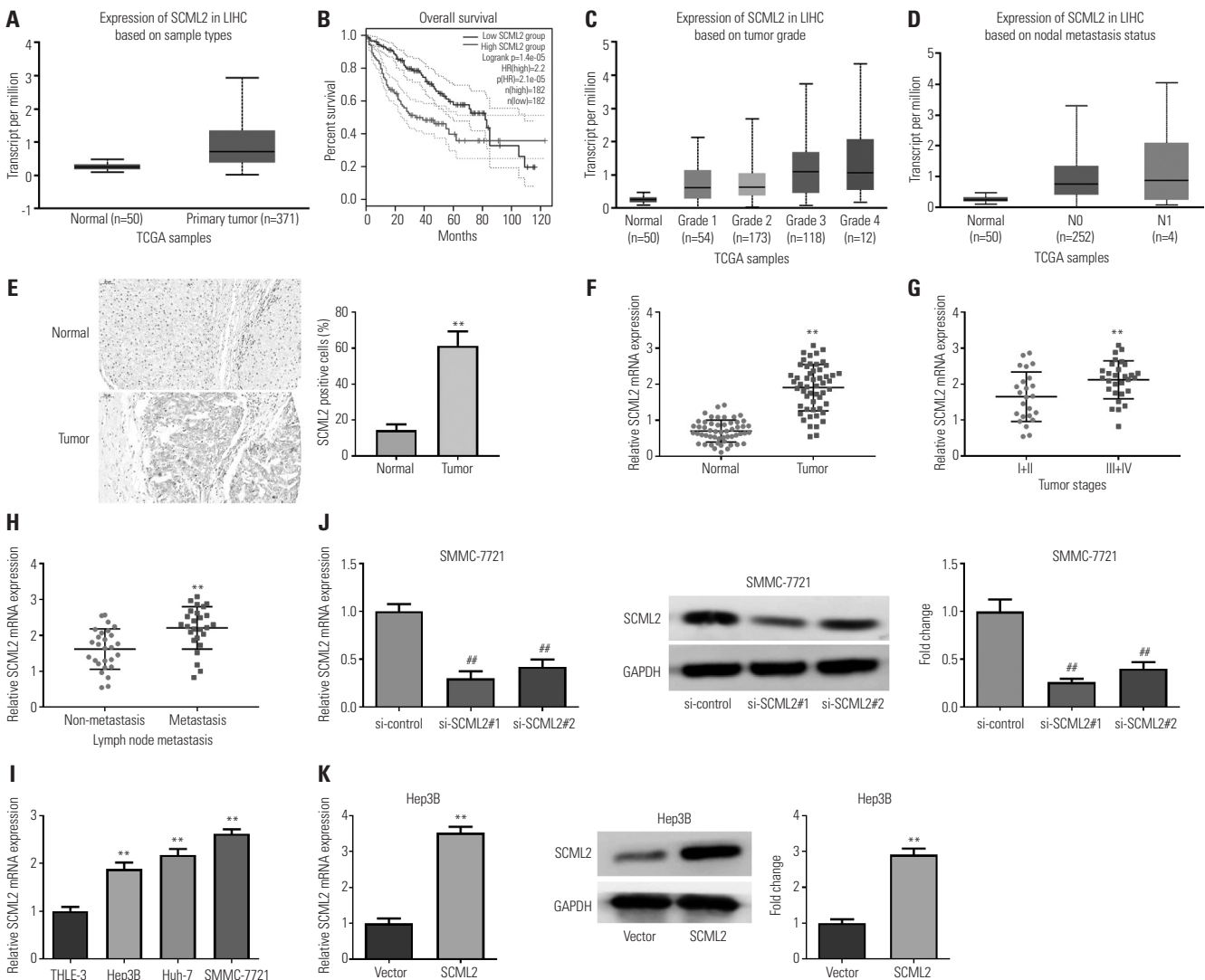


Fig. 1. SCML2 is highly expressed in HCC tissues and cells. (A) SCML2 mRNA levels in HCC tissues and normal samples based on the TCGA database. (B) The Kaplan-Meier method was applied to investigate overall survival in relation to SCML2 expression in HCC patients. (C) SCML2 was upregulated in four different individual cancer stages of HCC patients. (D) SCML2 was upregulated with metastasis in HCC patients. (E) Immunohistochemistry results between patients with HCC and paired adjacent normal tissues. $**p < 0.01$ vs. normal group. (F) SCML2 expression in normal tissues and HCC tumor tissues. $**p < 0.01$ vs. normal group. (G) SCML2 expression in HCC patients of different TNM stages (I+II, III+IV). $**p < 0.01$ vs. I+II group. (H) SCML2 expression in HCC patients without/with lymph node metastasis. $**p < 0.01$ vs. non-metastasis group. (I) SCML2 expression in THLE-3, Hep3B, Huh-7, and SMMC-7721 cells was measured by qRT-PCR. $**p < 0.01$. (J) SCML2 protein expression in SMMC-7721 cells. $**p < 0.01$ vs. si-control group. (K) SCML2 protein level in Hep3B cells. $**p < 0.01$ vs. vector group. SCML2, sex comb on midleg like-2; LIHC, liver hepatocellular carcinoma; HCC, hepatocellular carcinoma.

were dewaxed, re-hydrated, and exposed to antigen retrieval. Then, sections were sealed with 10% normal serum for 10 min and incubated with primary antibody against SCML2 (Sigma Aldrich) at 4°C overnight. The sections were then incubated with horseradish peroxidase-conjugated secondary antibody for 30 min at room temperature, and the immunocomplex was visualized using DAB solution (Thermo Fisher Scientific).

Gene set enrichment analysis

The gene set enrichment analysis (GSEA) algorithm was executed using the gsea software package (<http://www.gsea-msigdb.org/gsea/downloads.jsp>). The implemented parameters for GSEA were as follows: 1000 genome permutations, weighted enrichment statistics, genome sizes between 15 and 500, and signal-to-noise ratio. Genes were ranked according to their fold-change in different phenotypes. As for the gene set for analysis, the marker database was selected. A false discovery rate <0.05 was considered significant.

Data statistics

Experimental data were analyzed using SPSS 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 software (GraphPad Software, Inc., San Diego, CA, USA). The experimental data are expressed as a mean±SD. Student's t-test was applied to

analyze differences between groups. One-way analysis of variance with Bonferroni post-hoc test was used to analyze differences among multiple groups. *p* values <0.05 were regarded as statistically significant.

RESULTS

SCML2 is highly expressed in HCC tissues and cells

To determine whether SCML2 has an effect in HCC, we investigated SCML2 expression in HCC tissues (n=371) and adjacent normal specimens (n=50) based on the TCGA database. As shown in Fig. 1A, SCML2 expression was markedly overexpressed in HCC tissues relative to normal samples. Using a cut-off value (median value) for SCML2 expression, we separated 365 HCC specimens into two groups (high vs. low SCML2 expression). The overall survival rate in the high SCML2 expression group (n=182) was much lower than that in the low SCML2 expression group (n=182) (Fig. 1B). Moreover, we analyzed the expression of SCML2 in individual cancer stages and nodal metastasis status of HCC patients based on TCGA database. As shown in Fig. 1C and D, SCML2 was highly expressed in stage III+IV and N1. Then, we analyzed SCML2 expression in tumor tissues and normal tissues by IHC and qRT-PCR. The data

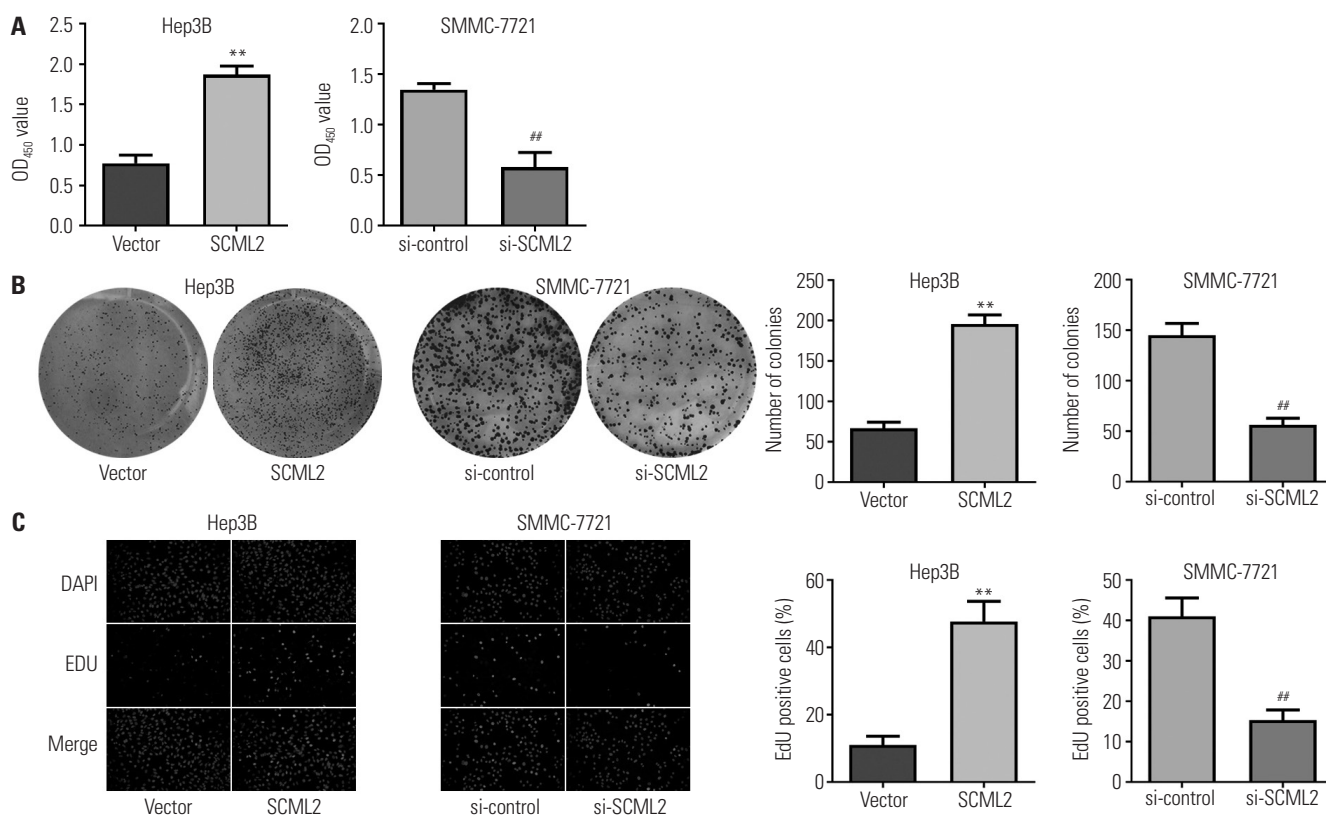


Fig. 2. SCML2 promotes the proliferation of HCC cells. (A) The influence of SCML2 silencing on cell proliferation in HCC cells was detected by CCK8 assay. (B) The images display the colony formation of pcDNA3.1-SCML2 transfected into Hep3B cells and si-SCML2 transfected into SMMC-7721 cells. (C) The impact of aberrant SCML2 expression on cell proliferation among HCC cells was detected by EdU assay. ***p*<0.01 vs. si-control group, #*p*<0.01 vs. vector group. SCML2, sex comb on midleg like-2; HCC, hepatocellular carcinoma.

showed that SCML2 expression was significantly higher in tumor tissues than in normal tissues (Fig. 1E and F). Additionally, high levels of SCML2 expression were associated with advanced TNM stage and nodal metastasis status. SCML2 expression in HCC patients with stage III and IV disease was higher than that in patients with stage I and II disease (Fig. 1G). SCML2 expression in HCC patients with nodal metastasis status was significantly higher than that in HCC patients without metastasis (Fig. 1H). Then, we detected the expression of SCML2 in HCC cell lines by qRT-PCR. The results showed that SCML2 was significantly increased in HCC cells (Hep3B, Huh-7, and SMCC-7721), compared with normal hepatocyte cells (THLE-3) (Fig. 1I). A high SCML2 expression level was observed in SMMC-7721 cells (Fig. 1I). Inversely, SCML2 mRNA expression was lower in Hep3B cells (Fig. 1I). Hence, si-SCML2 was transfected into SMMC-7721 cells, and pc-SCML2 was transfected into Hep3B cells in subsequent assays. As shown in Fig. 1J, si-SCML2#1 and si-SCML2#2 markedly reduced SCML2 expression in SMMC-7721 cells. Additionally, si-SCML2#1 was selected for subsequent experiments because of its lower expression. Furthermore, pc-SCML2 significantly raised SCML2 levels in Hep3B cells (Fig. 1K).

SCML2 promotes the proliferation of HCC cells

To investigate the biological effects of SCML2 on HCC cell proliferation, we performed CCK-8, colony formation, and EdU as-

says. The results of CCK-8 assay significant decreases in OD450 values upon SCML2 silencing and increases therein with SCML2 overexpression (Fig. 2A). The colony formation abilities of Hep3B cells were fortified by SCML2 overexpression, compared to the vector group, whereas those of SMMC-7721 cells were repressed by SCML2 silencing, compared to the si-control group (Fig. 2B). The results of EdU assays showed that the proliferative ability of HCC cells was increased with SCML2 overexpression and decreased with SCML2 silencing (Fig. 2C).

SCML2 promotes invasion and migration and inhibits apoptosis of HCC cells

We further explored the impact of SCML2 on HCC cell migration, invasion, and apoptosis by performing transwell, wound healing, and flow cytometry assays. As presented in Fig. 3A, SCML2 overexpression increased Hep3B cell invasion and migration, while SCML2 silence suppressed SMMC-7721 cell invasion and migration. Wound healing assays exhibited marked reductions in wound closure with SCML2 silencing, whereas it was significantly increased with SCML2 overexpression (Fig. 3B). Furthermore, SCML2 overexpression reduced the apoptosis of Hep3B cells, whereas SCML2 silencing increased apoptosis of SMMC-7721 cells (Fig. 3C).

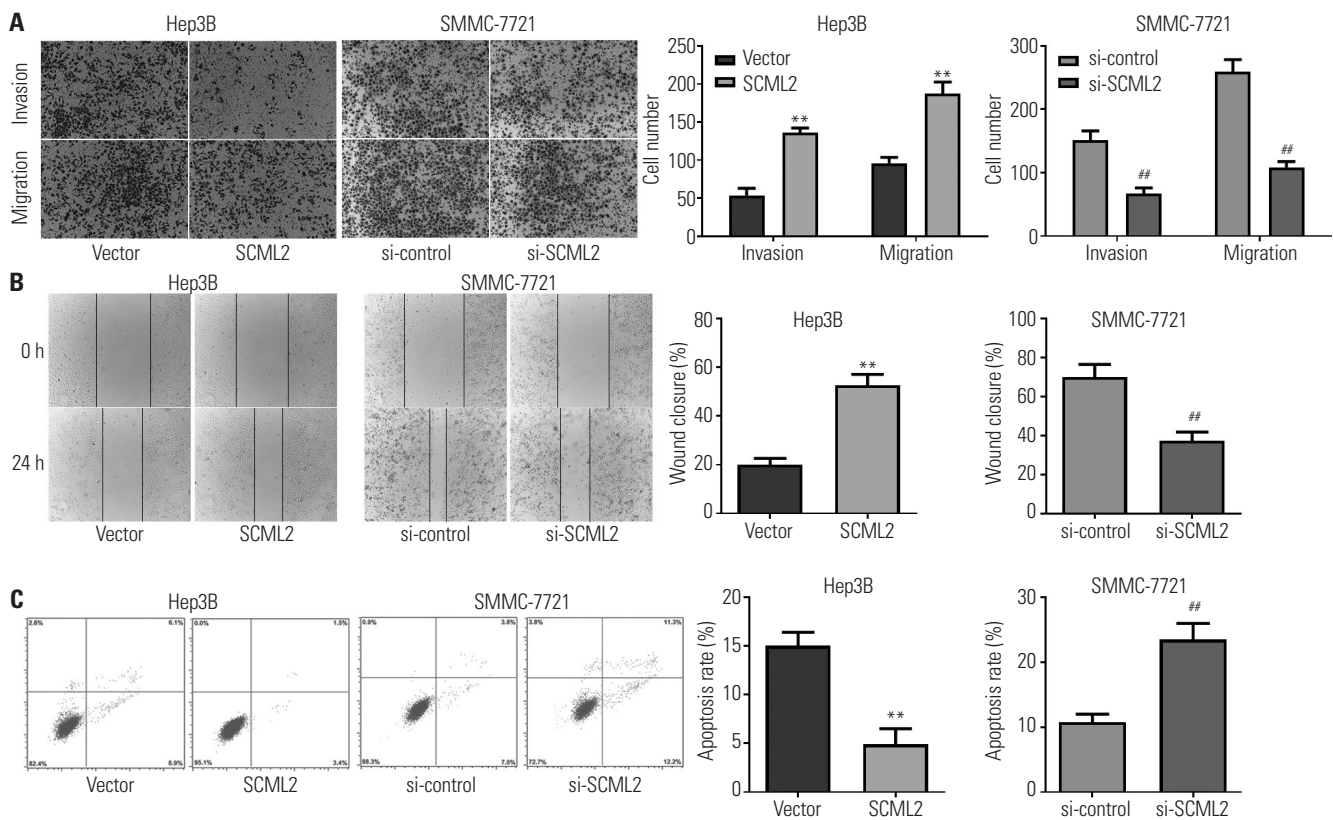


Fig. 3. SCML2 promotes invasion and migration and inhibits apoptosis of HCC cells. (A) The ability of migration and invasion of HCC cells was determined by transwell assay. (B) The migration ability of HCC cells was tested by wound healing assay (bar=100 μm). (C) The apoptosis of HCC cells was determined by flow cytometry. ***p*<0.01 vs. vector group, ##*p*<0.01 vs. si-control group. SCML2, sex comb on midleg like-2; HCC, hepatocellular carcinoma.

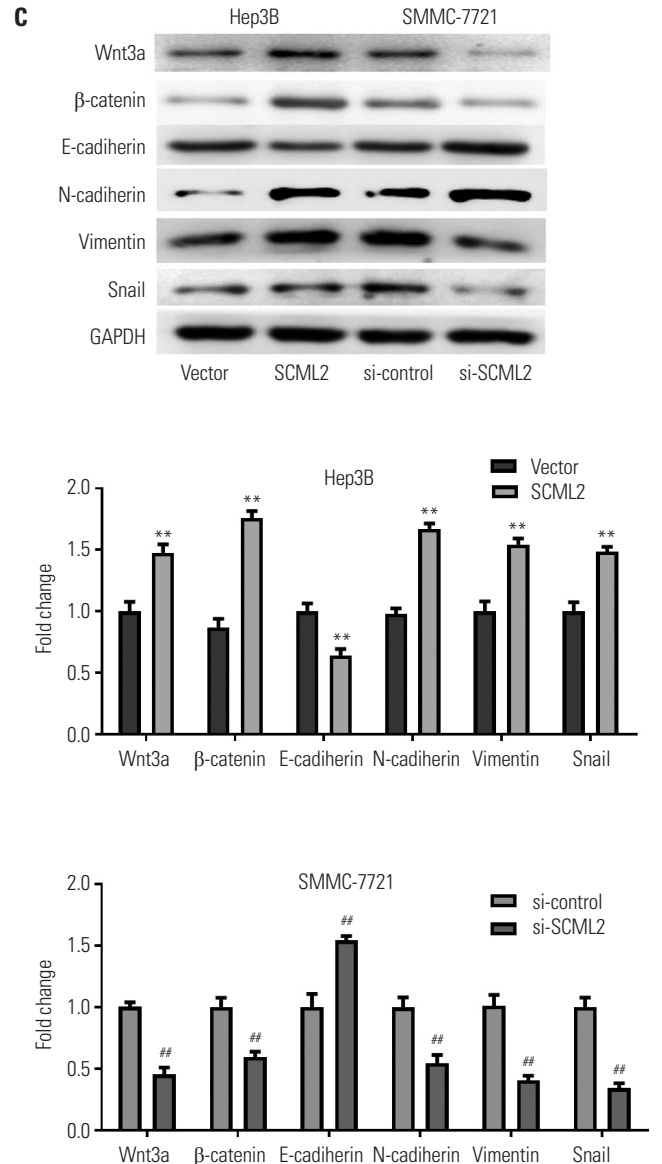
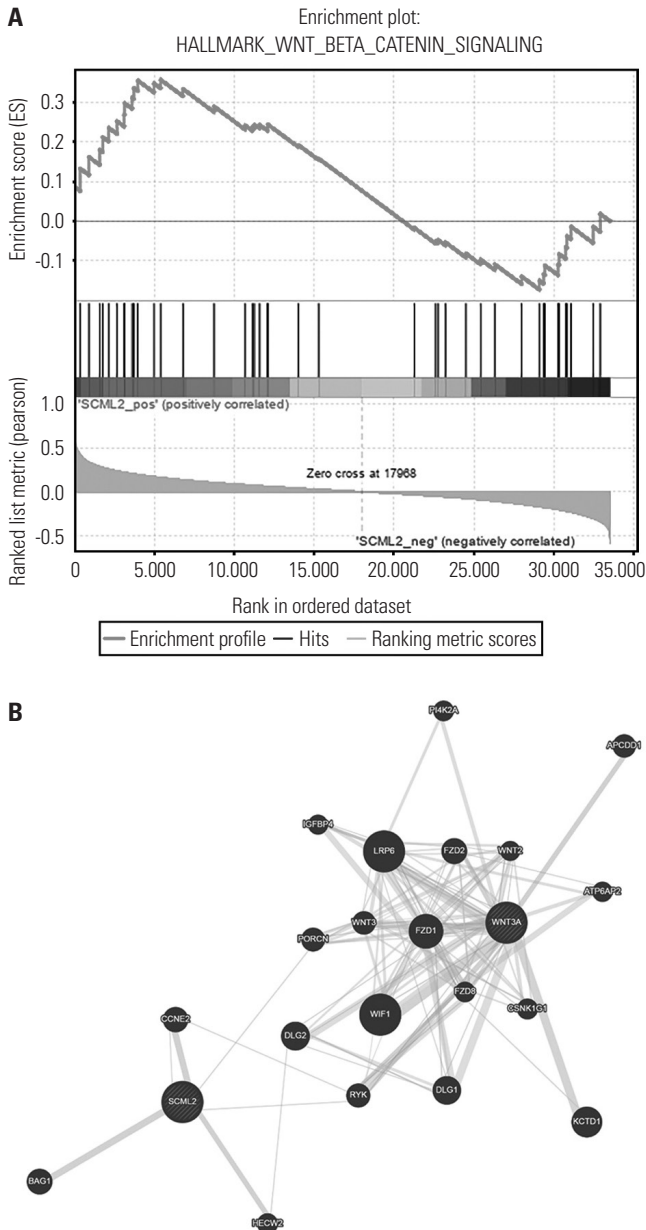


Fig. 4. SCML2 activates the Wnt/ β -catenin/EMT signaling pathway in HCC cells. The protein levels of Wnt3a, β -catenin, E-cadherin, N-cadherin, Vimentin, and Snail in HCC cells were detected by Western blot. ** $p < 0.01$ vs. vector group, # $p < 0.01$ vs. si-control group. SCML2, sex comb on midleg like-2; HCC, hepatocellular carcinoma; EMT, epithelial–mesenchymal transition.

SCML2 activates the Wnt/ β -catenin/EMT pathway in HCC cells

To obtain some understanding of the mechanism by which SCML2 affects cell growth and the motility of HCC cells, we performed GSEA to investigate SCML2 enriched pathways. As shown in Fig. 4A and B, SCML2 activated the Wnt/ β -catenin signaling pathway. Then, Western blotting was utilized to examine the expression of key markers (Wnt3a, β -catenin, E-cadherin, N-cadherin, Vimentin, and Snail) in Wnt/ β -catenin/EMT signaling. As displayed in Fig. 4C, SCML2 overexpression markedly enhanced the expression of Wnt3a, β -catenin, N-cadherin, Vimentin, and Snail proteins and reduced the protein levels of

E-cadherin in Hep3B cells. Meanwhile, silencing of SCML2 decreased protein expression of Wnt3a, β -catenin, N-cadherin, Vimentin, and Snail and increased E-cadherin protein levels in SMMC-7721 cells (Fig. 4C). In summary, the above data suggested that SCML2 regulates HCC cell growth and motility via Wnt/ β -catenin/EMT pathway signaling.

SCML2 silencing suppresses xenograft tumor growth in mice

The relationship between SCML2 and tumorigenesis was analyzed by establishing a subcutaneous xenograft tumor model in nude mice (n=4 per group). SMMC-7721-si-SCML2 or SMMC-

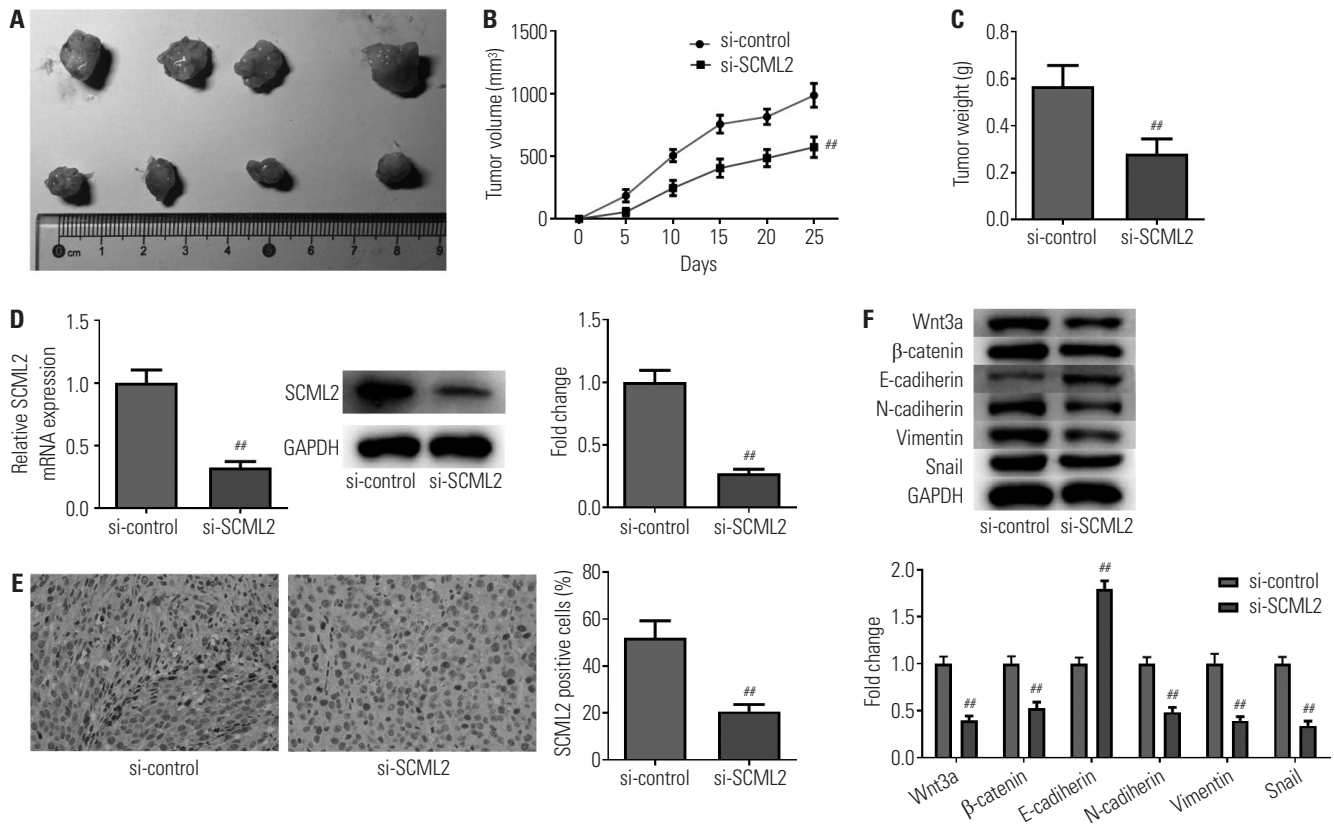


Fig. 5. SCML2 silencing represses HCC cell growth in vivo. (A) Tumors gathered from mice (n=4). (B) Tumor volume curves of mice injected with SMMC-7721-si- SCML2 or SMMC-7721-si-control. (C) Tumor weights were assessed. (D) SCML2 expression in tumors was detected by qRT-PCR and Western blot. (E) SCML2 expression in tumors was detected by immunohistochemistry. (F) Protein expression of Wnt3a, β-catenin, E-cadherin, N-cadherin, Vimentin, and Snail in HCC cells was detected by Western blot. ^{##}*p*<0.01 vs. si-control group. SCML2, sex comb on midleg like-2; HCC, hepatocellular carcinoma.

7721-si-control cells were subcutaneously injected into the right flank of nude mice. The xenograft tumor model with SMMC-7721-si-SCML2 cells revealed marked reductions in tumor volume and weight, compared with the SMMC-7721-si-control xenograft tumor model (Fig. 5A-C). Additionally, we detected SCML2 expression in mice tumors by qRT-PCR, Western blot, and IHC. The results in Fig. 5D and E showed that SCML2 expression in the si-SCML2 group was significantly decreased, compared with the si-control group. The data in Fig. 5F showed that SCML2 silencing inhibited the Wnt/β-catenin pathway. Collectively, these results indicated that SCML2 silencing could inhibit HCC cell proliferation in nude mice, in line with our in vitro results.

DISCUSSION

In this study, we explored the influences of SCML2 on HCC. We discovered that SCML2 is highly expressed in HCC tissues and cell lines. Moreover, high expression of SCML2 was related with poor prognosis in HCC patients. Additionally, we found that overexpression of SCML2 promoted cell growth, invasion, and migration and inhibited cell apoptosis in HCC by regulating the Wnt/β-catenin/EMT pathway. Overall, these results suggest

that SCML2 might be used as a clinical biomarker for HCC.

In this study, bioinformatics analysis indicated that SCML2 is overexpressed in HCC. SCML2A binds to non-coding RNAs to target and switch off certain genes in tumor cells,¹⁹ while SCML2B could bind to cyclin-dependent kinase 2 to modulate the cell cycle.²⁰ Thus, SCML2 could exert an effect on regulating cell-cycle machinery and influence cellular activity when it is ectopically expressed in transformed or tumor cells.²¹ Additionally, researchers have suggested that SCML2 is associated with some human cancers, including acute myeloid leukemia²² and malignant pediatric brain tumors.²³ Although Qi, et al.¹⁸ indicated that SCML2 might be related to HCC, the specific mechanism of action is still unclear, and further research is needed. In our study, we found that SCML2 was highly expressed in HCC tissues and HCC cell lines and that SCML2 overexpression accelerated cell proliferation and motility and suppressed cell apoptosis in HCC cells.

The Wnt/β-catenin pathway exerts a vital effect in modulation of the development of cell differentiation, migration, proliferation, and tumorigenesis and has been regarded as a target of EMT in carcinogenesis.²⁴ The study of Vilchez, et al.²⁵ demonstrated that Wnt/β-catenin signaling was aberrantly activated in HCC patients. As a target and prognostic factor for HCC, DDX39 promoted HCC cell growth, migration, and inva-

sion by activating the Wnt/ β -catenin pathway.²⁶ The high invasiveness of HCC cells in the proliferation process is the main reason for death in HCC patients.^{27,28} In the EMT process, epithelial cells lose cell-to-cell adhesion and obtain properties of migration.^{29,30} Corresponding to EMT characteristics, tumor cells overcome intercellular adhesion to gain invasiveness and motility, which is the first step in tumor progression.^{31,32} It is well known that E-cadherin is an epithelial marker, while N-cadherin, Vimentin, and Snail are mesenchymal markers of EMT.³³ EMT acts a vital role in tumor metastasis of HCC.^{34,35} Nevertheless, the effect of SCML2 on Wnt/ β -catenin or EMT has not been reported. Our study suggests that abnormal expression of SCML2 affects the expression of Wnt/ β -catenin/EMT pathway proteins. Overall, our results indicate that SCML2 influences HCC cell growth, motility, and apoptosis by modulating the Wnt/ β -catenin/EMT pathway.

In summary, we found that SCML2, as an oncogene, promotes HCC progression by activating the Wnt/ β -catenin/EMT pathway, which could be a possible new target for the treatment of HCC. One of the limitations of the study is the lack of in vivo experiments with metastatic lesions in mice. Additionally, whether SCML2 could affect the progression of HCC by regulating other pathways warrants further research.

AVAILABILITY OF DATA AND MATERIAL

The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

Conceptualization: Lei Du and Lina Wang. **Data curation:** Lina Wang and Jianming Lai. **Formal analysis:** Lina Wang and Xiaoli Zhi. **Funding acquisition:** Jianping Duan and Lei Du. **Investigation:** Wei Wu and Jianming Lai. **Methodology:** Lina Wang. **Project administration:** Lei Du and Lina Wang. **Resources:** Xiaoli Zhi and Jianming Lai. **Software:** Jianming Lai. **Supervision:** Hong Yang and Lei Du. **Validation:** Jianming Lai and Hong Yang. **Visualization:** Jianming Lai and Hong Yang. **Writing—original draft:** Lina Wang and Shaohua Fan. **Writing—review & editing:** Shaohua Fan and Xiaoli Zhi. **Approval of final manuscript:** all authors.

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