ORIGINAL ARTICLE

Observational Study

Smoc2 potentiates proliferation of hepatocellular carcinoma cells *via* promotion of cell cycle progression

Jing-Ran Su, Jing-Hua Kuai, Yan-Qing Li

Jing-Ran Su, Yan-Qing Li, Department of Gastroenterology, Laboratory of Translational Gastroenterology, Shandong University, Qilu Hospital, Jinan 250012, Shandong Province, China

Jing-Hua Kuai, Department of Gastroenterology, Shandong University, Qilu Hospital of Shandong University (Qingdao), Qingdao 266035, Shandong Province, China

Correspondence to: Yan-Qing Li, MD, PhD, Department of Gastroenterology, Laboratory of Translational Gastroenterology, Shandong University, Qilu Hospital, 107 Wenhuaxi Road, Jinan 250012, Shandong Province, China. liyanqing@sdu.edu.cn
Telephone: +86-531-82166090
Fax: +86-531-82166090

Abstract

AIM

To determine the influence of Smoc2 on hepatocellular carcinoma (HCC) cell proliferation and to find a possible new therapeutic target for preventing HCC progression.

METHODS

We detected expression of Smoc2 in HCC tissues and corresponding non-tumor liver (CNL) tissues using PCR, western blot, and immunohistochemistry methods. Subsequently, we down-regulated and up-regulated Smoc2 expression using siRNA and lentivirus transfection assay, respectively. Then, we identified the effect of Smoc2 on cell proliferation and cell cycle using CCK-8 and flow cytometry, respectively. The common cell growth signaling influenced by Smoc2 was detected by western blot assay.

RESULTS

The expression of Smoc2 was significantly higher in HCC tissues compared with CNL tissues. Overexpression of Smoc2 promoted HCC cell proliferation and cell cycle progression. Down-regulation of Smoc2 led to inhibition of cell proliferation and cell cycle progression. Smoc2 had positive effect on ERK and AKT signaling.

CONCLUSION
Smoc2 promotes the proliferation of HCC cells through accelerating cell cycle progression and might act as an anti-cancer therapeutic target in the future.

Key words: Smoc2; Hepatocellular carcinoma; Cell cycle; Proliferation

Core tip: In our study, we confirmed that Smoc2 was up-regulated in hepatocellular carcinoma (HCC) tissues and played an important role in regulating liver cancer cell proliferation. Besides, we verified that Smoc2 participated in promoting HCC cell proliferation mainly through regulation of cell cycle progression. We have not investigated the promotive role of Smoc2 in regulating cell proliferation, whether it is through cell cycle regulation only or involves regulation of cell apoptosis as well. Moreover, the exact mechanism of how Smoc2 regulates cell cycle remains unclear. The core contents of our study included Smoc2 promotion of HCC cell proliferation *via* accelerating cell cycle progression.

INTRODUCTION
Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, with high mortality rate and low early diagnostic rate[1]. HBV infection, alcohol abuse, aflatoxin exposure and HCV infection are identified as major causes of HCC. The current therapies available for HCC include surgery, interventional therapy, radio frequency therapy, radiotherapy, biological target therapy and so on[2]. All of these treatments have certain curative effects, but have inherent limitations and adverse effects, especially for HCC patients at the advanced stage[3]. Thus, it is urgent to find new treatment target for the sake of enhancing curative effect and reducing adverse effects, especially in advanced HCC patients.

Apart from the common etiologies of HCC listed above, certain oncogenes, cytokines, neurotransmitters, chemokines, extracellular secretory proteins and tumor microenvironment are thought to play important roles in origin and progression of HCC[4]. Therefore, oncogenes and tumor microenvironment, which facilitate HCC progression, can be chosen as therapeutic targets for HCC treatment[5].

The secreted protein acidic and rich in cysteine (SPARC; alternative names: osteonectin; ON or basement membrane-40; BM-40) family is recognized as extracellular matrix proteins[6]. A differential expression of SPARC in tumor tissue and its surrounding stroma compared to normal tissues has been reported for many different types of cancer[7]. And, SPARC was found to be up-regulated in several solid tumors and to facilitate tumor metastasis[8].

Secreted modular calcium-binding protein-2 (Smoc2) is a novel member of the SPARC family[9]. Previous study confirmed that Smoc2 could promote cell cycle progression of human umbilical vein endothelial cells by inducing the expression of transcripts required for cell cycle[10]. Other studies have shown that Smoc2 is necessary for DNA synthesis in the cell cycle and is likely to impact cell growth *in vitro* and *in vivo*[11]. However, to the best of our knowledge, no study has been conducted on the role of Smoc2 in HCC.

Thus, the present study was designed to investigate the effect of Smoc2 on proliferation of HCC cells and the impact on cell cycle. Our study showed that Smoc2 promoted proliferation of HCC cells and accelerated cell cycle progression. The results suggested that Smoc2 might act as an anti-cancer therapeutic target for HCC treatment.

MATERIALS AND METHODS
Clinical samples
A total of 20 pairs of HCC tissues and corresponding non-tumor liver (CNL) tissues were obtained from the Liver Surgery Department of the corresponding hospital. All the human liver tissues were obtained with informed consent and the study was approved by the Ethical Committee of the corresponding hospital.

Immunohistochemistry
The paraffin-embedded human liver tissues were incised into 4-5 μm thickness slices and then dewaxed using xylene and ethanol, in a stepwise manner. After dewaxing, the slices were rehydrated for subsequent staining. For immunohistochemistry (IHC) staining, the slices were treated with hydrogen peroxide and boiled for 15 min in citrate solution for antigen retrieval. When the slices had cooled naturally to room temperature, we added goat serum for blocking of unrelated antigens. Afterwards, the slices were incubated with Smoc2 antibody (Abcam) at 4 ℃ overnight. The following day, the slices were washed three times with phosphate buffer and incubated with horseradish peroxidase-labelled secondary antibody for 1 h at room temperature. The slices were then developed using DAB substrate liquid (Thermo) and dehydrated by ethanol, in a stepwise manner, and finally sealed with neutral balsam.

Western blotting assay
The tissue protein was isolated from human liver tisseus using T-PER Tissue Protein Extraction Reagent

(Pierce Biotechnology) according to protocols provided by the manufacturer. The cell protein was lysed using lysis buffer that contained Tris-HCl, NaCl, Triton-X 100, $MgCl_2$, PMSF and so on. The cell lysate, which was used for cell signaling detection, was obtained by IP cell lysis solution (Beyotime Biotechnology). All the proteins were separated by SDS-PAGE and transferred to a nitrocellulose membrane under constant current condition. Then, the membrane was blocked using 5% bovine serum albumin at room temperature for 1 h. The nitrocellulose membrane was then incubated using antibodies for Smoc2 (Abcam), extracellular regulated protein kinase (ERK; Cell Signaling Technology, CST), phospho-ERK (CST), AKT (CST), phospho-AKT (CST), Src (CST), phospho-Src (CST), FAK (CST), phospho-FAK (CST) and GAPDH (Sigma) at 4 ℃ overnight. The next day, the nitrocellulose membrane was incubated with the fluorescence-conjugated secondary antibodies at room temperature for 1 h. All the fluorescence signals were captured and saved by the Odyssey imaging system (LI-COR). The images of western blots were analyzed using ImageJ software for gray value calculation.

Immunofluorescence staining

For cell staining, SMMC-7721 cells transfected with Smoc2 vector using lentivirus method were seeded on rounded slides in 24-well plates and incubated at 37 ℃ and a 5% CO_2 atmosphere overnight. The next day, cells were fixed using paraformaldehyde and washed three times by phosphate buffer before staining. Then, the sildes were incubated with Smoc2 antibody (Abcam) for 75 min at room temperature. After that, the slides were washed three times using phosphate buffer and incubated with Alexa Fluor 488nm-conjugated secondary antibody. The nuclei were stained using DAPI (Sigma) and the immunofluorescence stain images were record using fluorescence microscope (Carl Zeiss).

Real-time quantitative PCR assay

Total RNA was isolated from fresh liver tissues using the Trizol Reagent (Takara). The isolated RNA was then immediately applied to the reverse transcription reaction. Primers used for quantitative PCR reaction were: forward primer, 5'-GCTCACGTTCTTGAGAGTCG-3'; and reverse primer, 5'-TGTAGCTGTGACACTGGACC-3'. The PCR reaction conditions were 30 s at 94 ℃, 1 min at 57 ℃ and 1 min and 30 s at 72 ℃ for 35 cycles. The Amplitaq polymerase and related reagents were supplied by Takara.

Lentivirus transfection assay

The full-length cDNA cloning vector was purchased from GeneCopoeia and used for amplification template. The Smoc2 full-length cDNA was amplified with *Nhe*I and *BamH*I restriction sites using KOD-PLUS Neo polymerase. Then, the PCR-amplified cDNA was cloned

into the pcDNA3.1 vector using restriction enzymes and DNA ligase. The recombind vector was packed with VSV, REV and GAG vectors and transfected into 293T cells using the Lipofectamine 2000 Reagent (Invitrogen) to obtain virus supernatent. The virus supernatent was added into SMMC7721 cells and Huh7 cells with polybrene. The transfected SMMC7721 cells and Huh7 cells were cultured under puromycin condition and verified by western blot assay.

Small interfering (si)RNA interference assay

MHCC-97H cells and HCC-LM3 cells were transfected with siRNA duplexes against Smoc2 or with control RNA duplex oligonucleotides using Lipofectamine 2000, following the manufacturer's instructions. The Smoc2 interference target sequence was TTAAGAGGTTCCTGCGAAA.

CCK-8 cell proliferation assay

MHCC-97H and HCC-LM3 cells transfected with Smoc2-SiRNA were seeded into 96-well plates at density of 3000 cells per 100 μL and cultured for 5 d *in vitro*. In addition, the SMMC-7721 and HCC-LM3 cells transfected with Smoc2 lentivirus were seeded into 96-well plates at density of 2000 cells per 100 μL and cultured for 5 d at 37 ℃ and in 5% CO_2 atmosphere. The CCK-8 reagent was added to each well at 10 μL volume and reacted for 1 h in light-free condition, at 37 ℃, and in 5% CO_2 atmosphere. Then, the absorbance value was detected using a microplate reader. The absorbance value at 450 nm was recorded.

Cell cycle assay

The cells were seeded into 6-well plates and collected at logarithmic growth phase. The cells were cultured in serum-free condition overnight before collection. Liver cancer cells were collected and washed three times using phosphate buffer. Then, the cells were centrifuged at 1000 r/min for 5 min and resuspended in 1 mL phosphate buffer. Next, the cells were added to 9 mL 70% cool ethanol, slowly and carefully. The fixed cells were then stored at -20 ℃ for at least 24 h. For cell cycle assay, the fixed cells were centrifuged at 1000 r/min for 10 min and washed three times using cool phosphate buffer. Then, we added 0.5 mL RNase (50 μg/mL) to each tube and incubated for 20 min at 37 ℃. Lastly, we added propidium iodide at 50 μg/mL to each tube and stained on ice for 30 min in light-free condition. The stained cells were detected using flow cytometry (BD Biosciences).

Statistical analysis

We used SPSS 16.0 software to analyze the statistical significance of differences in our study. Statistic differences were calculated using two-tailed Student's *t*-test. $P < 0.05$ was considered statistically significant and $P < 0.01$ was considered very statistically significant.
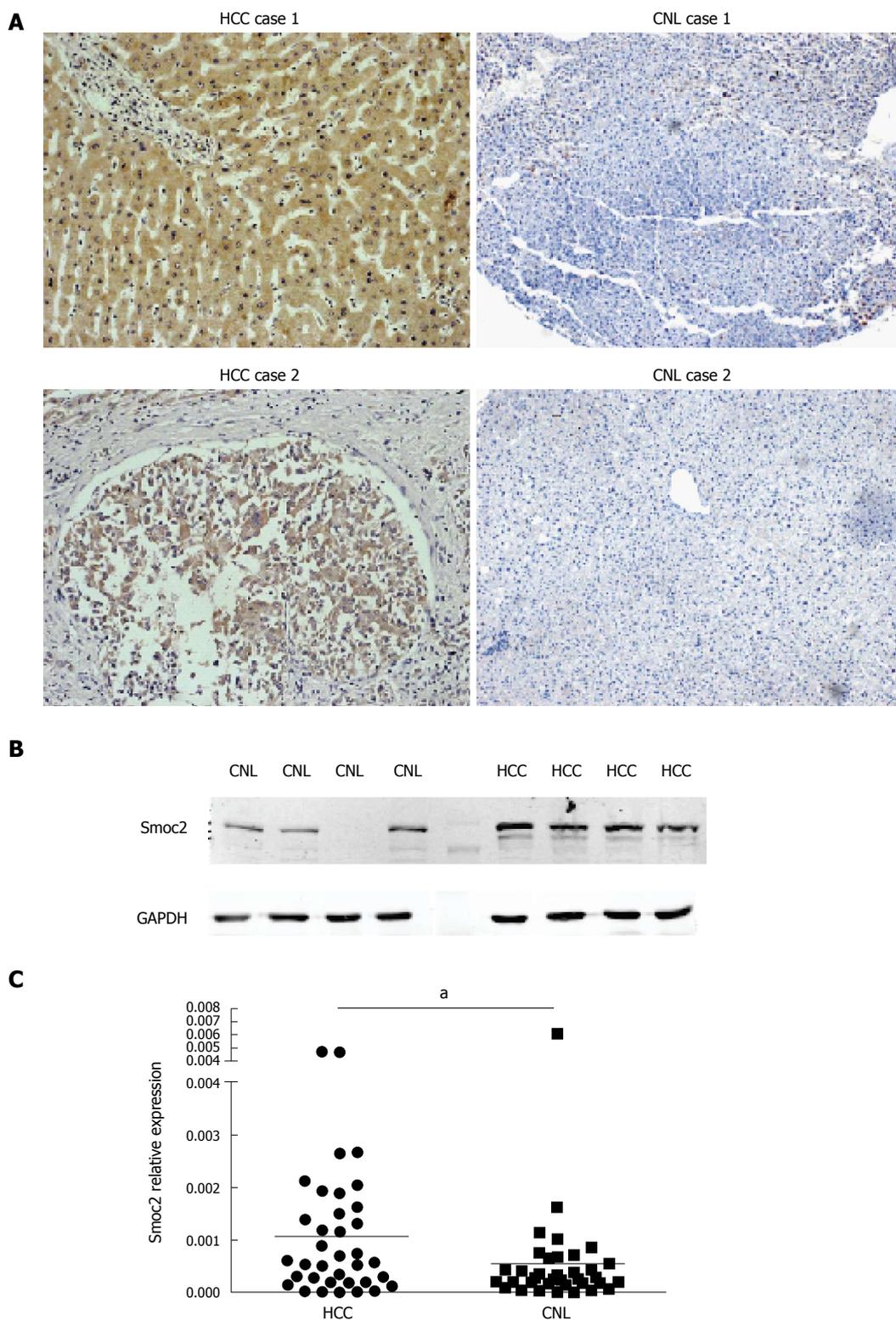
A

B

C

Figure 1 Smoc2 was up-regulated in hepatocellular carcinoma tissues compared with corresponding non-tumor liver tissues. A: Representative images of immunohistochemistry (IHC) staining assay; IHC images show that expression of Smoc2 was higher in hepatocellular carcinoma (HCC) tissues compared with corresponding corresponding non-tumor liver (CNL) tissues; B: Western blot assay show the expression of Smoc2 was higher in fresh HCC tissues than in CNL tissues; C: Quantitative real-time PCR assay showed that the relative expression of Smoc2 was higher in fresh HCC tissues than in CNL tissues. [a]$P < 0.05$.

RESULTS

Smoc2 was up-regulated in HCC tissues compared with CNL tissues

The expression of Smoc2 was significantly up-regulated in HCC tissues, compared to CNL tissues, as evidenced by IHC (Figure 1A). IHC results showed that expression of Smoc2 was mainly located in the cytoplasm of HCC cells and the extracellular lesion of liver tissues. Western blot assay showed that protein
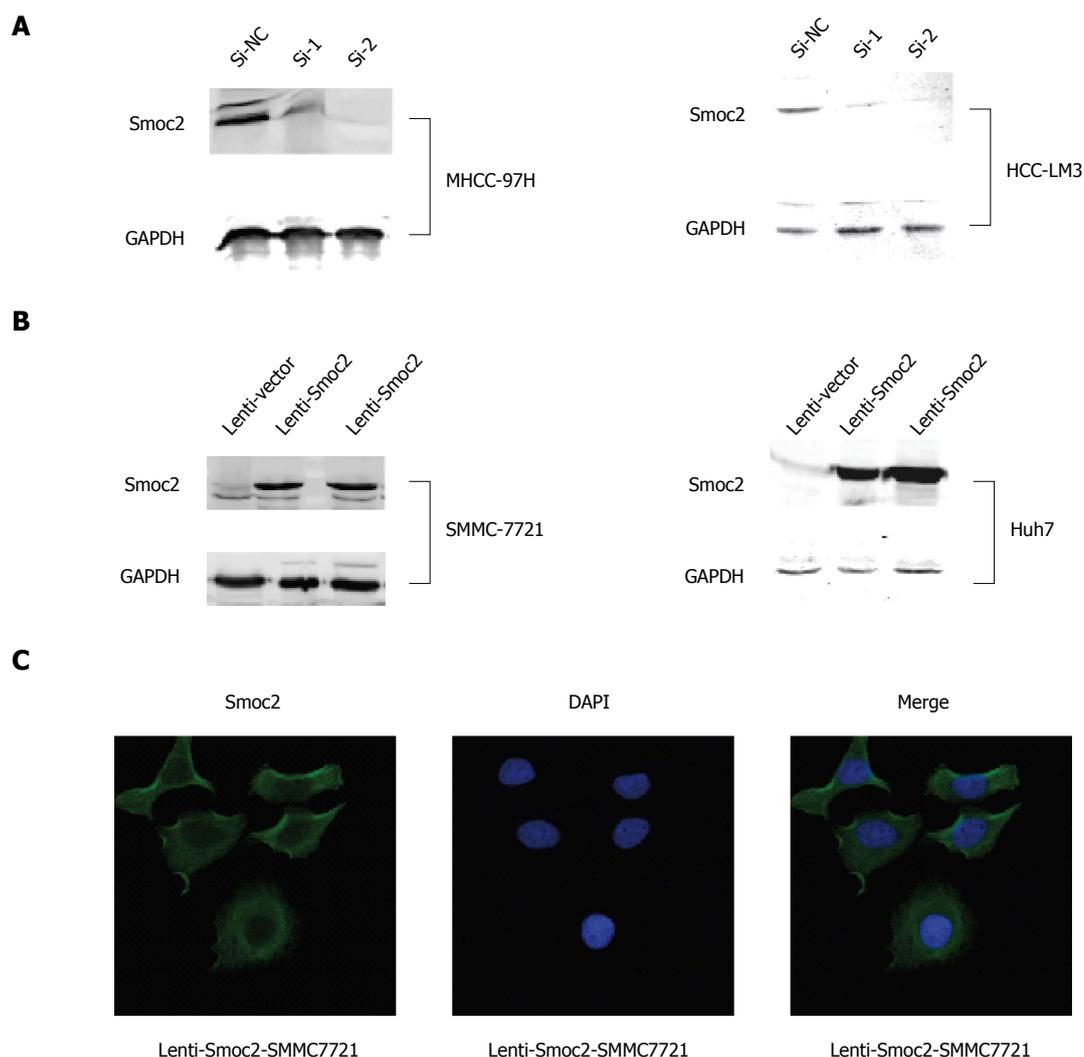
Figure 2 **Western blot assay.** A: Western blot assay showing that expression of Smoc2 in MHCC-97H cells and HCC-LM3 cells was inhibited by small interfering (si)RNA; B: Western blot assay showing that expression of Smoc2 in SMMC-7721 cells and Huh7 cells was significantly up-regulated by lentivirus transfection. Lenti-Smoc2 ha overexpression of Smoc2 by lentivirus transfection; lenti-vector was the negative control of lentivirus transfection; C: Immunofluorescence staining showing overexpression of Smoc2 in SMMC-7721 cells; DAPI was used for nucleus staining.

expression level of Smoc2 was significantly higher in human HCC tissues, compared to CNL tissues (Figure 1B). The real-time quantitative PCR result indicated that mRNA expression level of Smoc2 in HCC tissues was remarkably higher than in CNL tissues. All the results above revealed that expression of Smoc2 was up-regulated in HCC tissues, compared to CNL tissues, at both protein and mRNA levels (Figure 1C).

Silencing Smoc2 by siRNA transfection and overexpressing Smoc2 by lentivirus transfection assay

We carried out siRNA transfection for silencing of Smoc2 in MHCC-97H and HCC-LM3 cells, and verified the silencing effect using western blot assay (Figure 2A). We induced overexpression of Smoc2 in SMMC-7721 and Huh7 cells using the lentivirus transfection method and identified the overexpressing effect using western blot assay (Figure 2B). The immunofluorescence stain results showed that expression of Smoc2 induced by

lentivirus transfection can be found in cytoplasm of SMMC-7721 cells (Figure 2C).

Silencing of Smoc2 inhibited HCC cell proliferation and overexpression of Smoc2 promoted HCC cell proliferation in vitro

CCK-8 assay directly reflects cell viability and can be used for evaluating cell proliferation. We collected MHCC-97H and HCC-LM3 cells transfected with Smoc2-siRNA at 48 h and detected the cell viability for 5 d *in vitro* using CCK-8 assay. The results showed that the proliferation capacity of MHCC-97H and HCC-LM3 cells transfected with Smoc2-siRNA were significantly worse than that in the control group (Figure 3A). Besides, the proliferative capacity in Smoc2 overexpressing SMMC-7721 and Huh7 cells was remarkably stronger than in control groups *in vitro* (Figure 3B). These results suggested that Smoc2 plays an important role in promoting HCC cells proliferation *in vitro*.
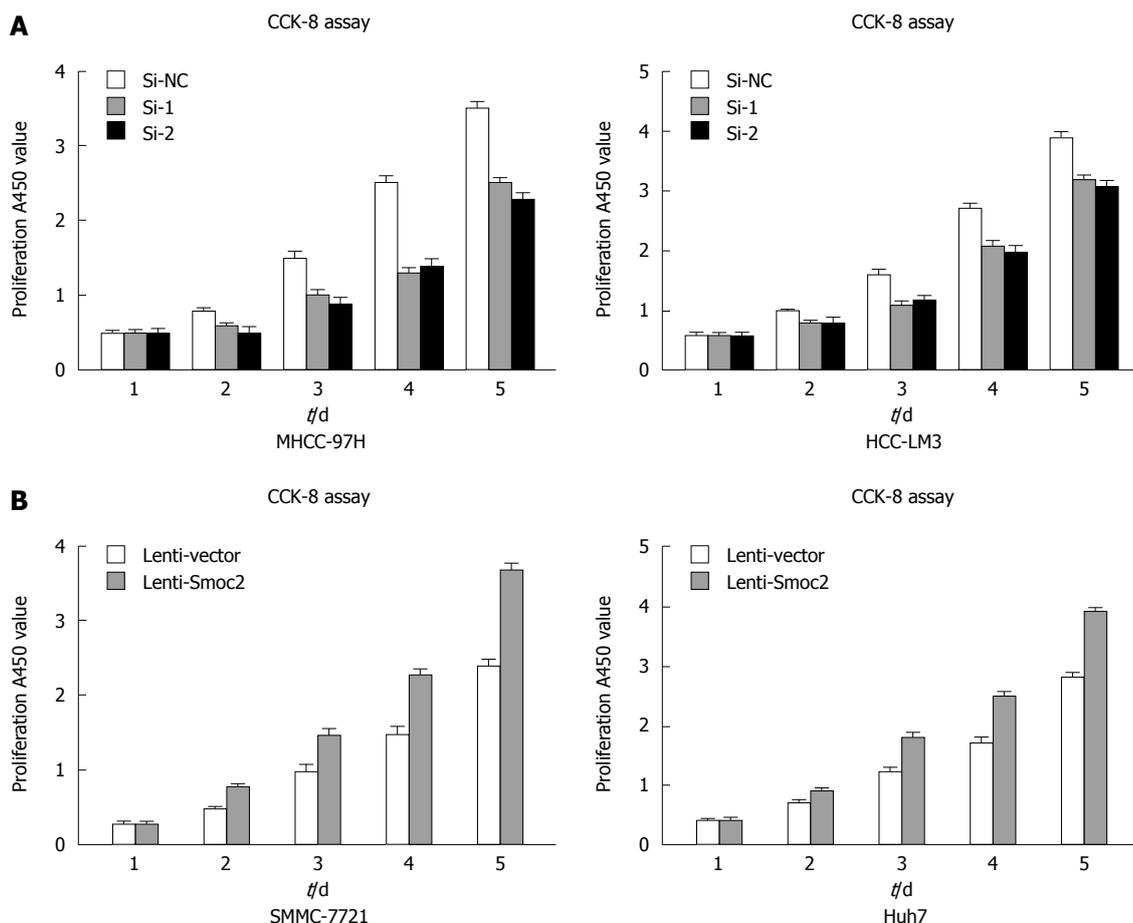
Figure 3 CCK-8 assay. A: CCK-8 assay was used for cell viability detection and reflected cell proliferation. The results show that the proliferation ability was inhibited by small interfering (si)RNA in MHCC-97H cells and HCC-LM3 cells; B: CCK-8 assay results show that the cell proliferation ability in SMMC-7721 cells and Huh7 cells was enhanced by lenti-Smoc2-virus transfection.

Overexpression of Smoc2 accelerated cell cycle progression by increasing the proportion of S phase cells

Cell cycle directly reflects cell proliferation capacity and is composed of G0/G1 phase, S phase and G2/M phase. The cell proliferation capacity can be reflected by proliferation index (PI) and S phase cell fraction (SPF). PI equates to the percentage of sum of S phase and G2/M phase cells in total cell cycle cells. SPF equates to the percentage of S phase cells in total cell cycle (G0/G1 + S + G2/M). Our results showed that both PI and SPF values in overexpressing Smoc2 cells were higher than those in the control group, which indicated the promotive role of Smoc2 in cell cycle progression and cell proliferation (Figure 4A and B; Figure 5A and B).

Smoc2 potentiated ERK, AKT and FAK signaling in HCC cells

The ERKs consist of ERK1 and ERK2, having molecular weights of 44 kDa and 42 kDa respectively[12]. Phosphorylation of ERK allows translocation from the cytoplasm to the nucleus, and mediates transcriptional activation of Elk-1, ATF, NF-κB, Ap-1, c-fos and c-Jun. Activation of ERK signaling participates in regulating cell proliferation and differentiation, construction of the cell skeleton, and the processes of cell apoptosis and cell canceration[13]. Our results showed that phosphorylation of ERK was up-regulated in Smoc2 overexpressed cells and down-regulated in Smoc2 siRNA interfered cells (Figure 6).

Signaling by AKT, also known as protein kinase B, plays an important role in regulating cell survival and apoptosis[14]. Our results indicated that phosphorylation of AKT was up-regulated in Smoc2 overexpressed cells and down-regulated in Smoc2 siRNA interfered cells (Figure 6).

FAK, also known as focal adhesion kinase, and its activation is related to cell proliferation and cell cycle[15]. Inhibition of FAK signaling can cause decrease of S phase cell ratio in cell cycle. Thus, inhibition of FAK signaling lead to inhibition of cell proliferation and enhancement of cell apoptosis. Our results illustrated that down-regulation of Smoc2 by siRNA transfection caused inhibition of FAK phosphorylation (Figure 6).

DISCUSSION

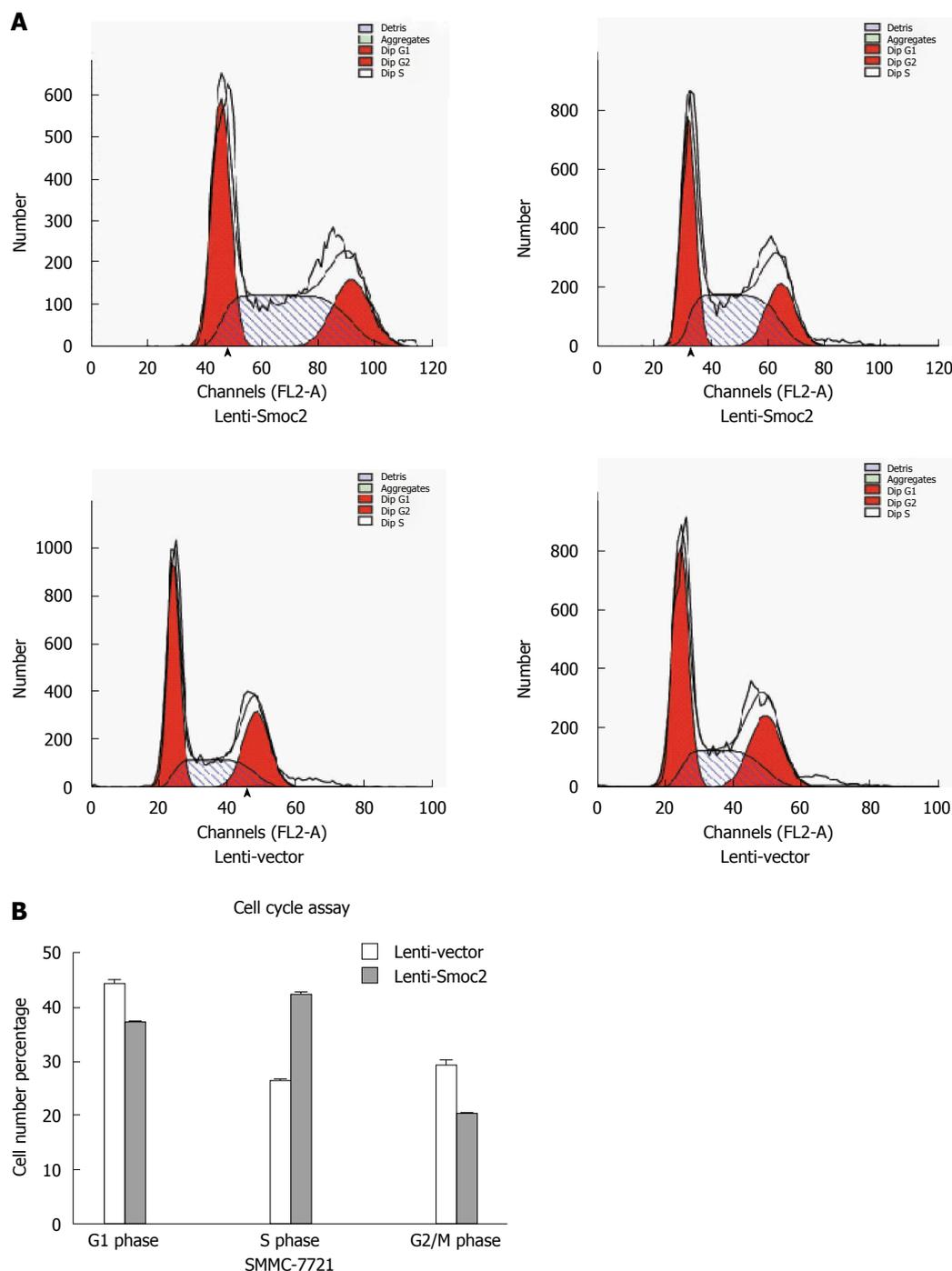As one of the most frequent human cancers worldwide,

A

B

Figure 4 SMMC-7721 cells. A: Fluorescence-activated cell sorting assay for cell cycle detection. All the SMMC-7721 cells were stained by propidium iodide, which can be detected at 488 nm; B: The overexpression of Smoc2 caused increase of S stage cell proportion in SMMC-7721 cells, which indicated promotion of cell cycle progression.

the incidence and progression of HCC are affected by oncogenes and tumor microenvironment[16,17]. Cytokines, chemokines, pH value, hypoxia, fibroblast and extracellular matrix proteins are important components of the tumor microenvironment[18-21]. The tumor microenvironment plays an important role in regulating tumor progression[22,23]. It has been reported that many extracellular matrix proteins exert significant influence on tumor progression. Thus, we focused on finding a new secretory protein which can

cause tumor development.

The *Smoc2* gene, encoding a protein belonging to the SPARC family and identified as an extracellular matrix protein, is up-regulated during embryogenesis and wound healing[24]. The *Smoc2* gene encoded protein promotes extracellular matrix assembly and potentiates endothelial cell proliferation and migration[25], and has pro-angiogenic activity[26]. It was reported that the *Smoc2* gene is indispensable for VEGF-induced vascular endothelial cells mitogenesis
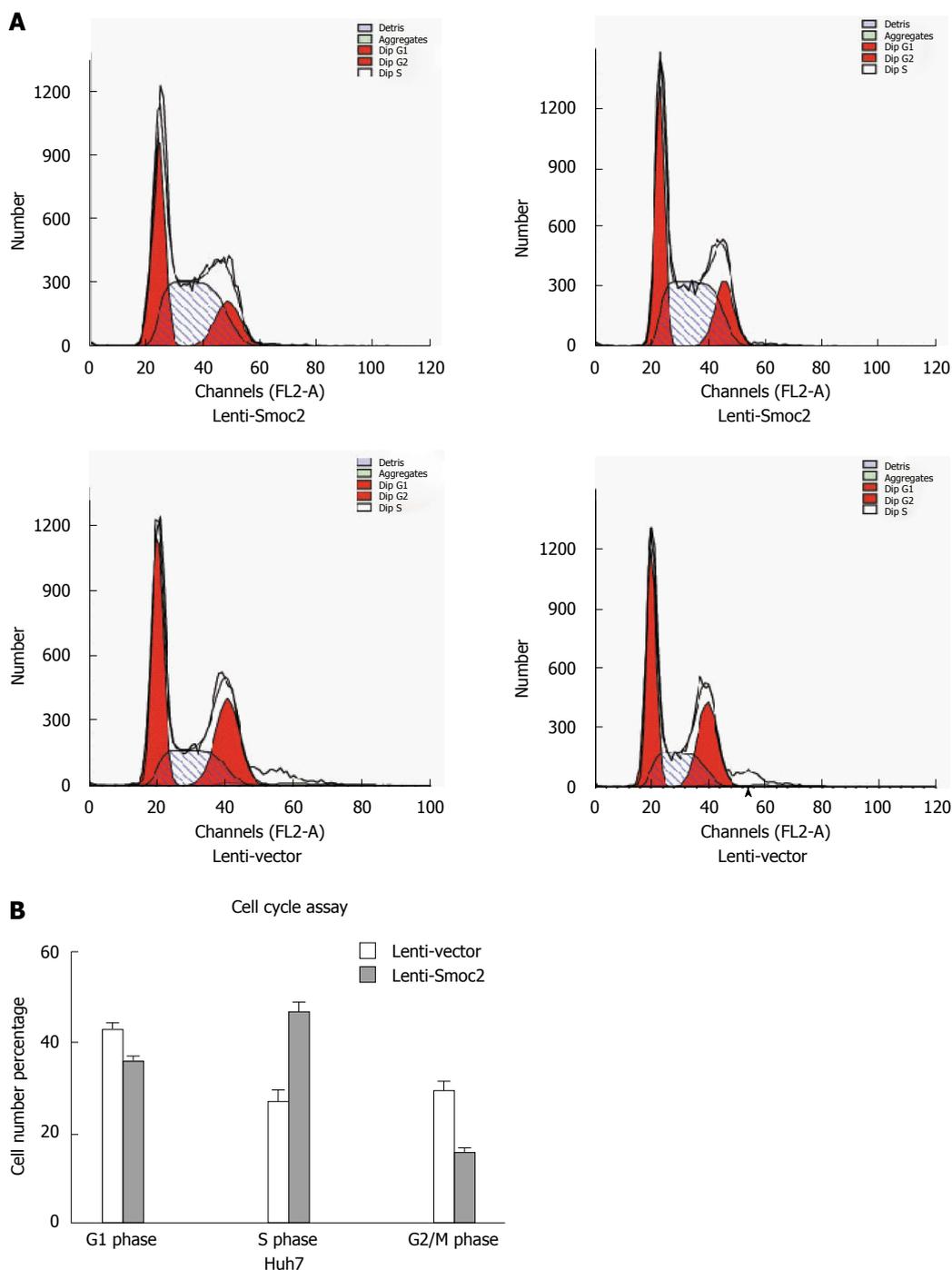
A

B

Figure 5 Huh7 cells. A: Fluorescence-activated cell sorting assay for cell cycle detection. All the Huh7 cells were stained by propidium iodide, which can be detected at 488 nm; B: The overexpression of Smoc2 caused increase of S stage cell proportion in Huh7 cells, which indicated the promotion of cell cycle progression.

and tube formation[27,28]. Due to its promotive effect on angiogenesis, the *Smoc2* gene may represent a useful target for inhibition of tumor progress.

The cell cycle consists of G0/G1 phase, S phase and G2/M phase in mammalian cells[29,30]. The cell cycle is mainly regulated by various kinds of growth factors, such as platelet-derived growth factor[31,32]. Cyclin D1 expression is induced by mitogens and is crucial for G1 progression[33]. Previous results showed that Smoc2 plays an important role in promoting G1/S progression through participating in the maintenance

of cyclin D1 expression[34-36]. In other words, cyclin D1 can be considered as an effector of Smoc2-induced DNA synthesis[37,38].

It was also demonstrated that Smoc2 was necessary for DNA synthesis in response to PDGF and other growth factors during the cell cycle[39-41]. Our study demonstrated that Smoc2 had a promotive effect on HCC cell G1/S phase progression, as well as on cell proliferation. The S phase cell ratio was significantly elevated in Smoc2 overexpressing HCC cells, and this result was in accordance with those from a previous
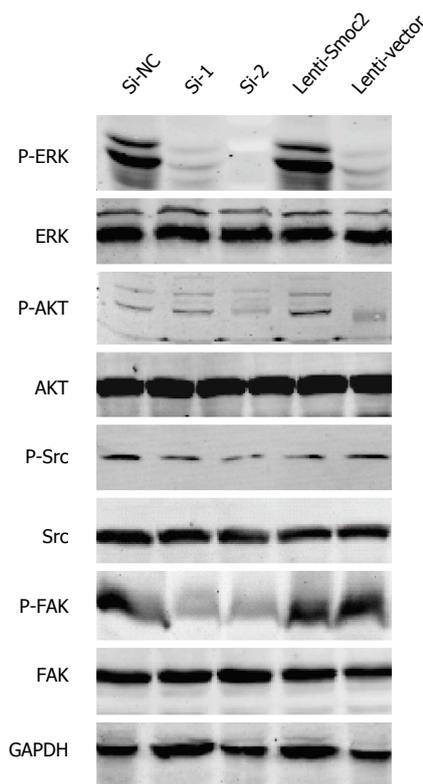
Figure 6 Phosphorylation of ERK up-regulated in Smoc2 overexpressing cells and down-regulated in cells with Smoc2 small interfering (si)RNA. Western blot assay showed the change of phospho-ERK, Src, FAK and AKT expression by Smoc2 interference or overexpression.

study using human umbilical vein endothelial cells transduced with Ad-Smoc2[42,43].

Compared with Swiss 3T3 cells transfected with Sicontrol, the cells transfected with SiSmoc2 oligonucleotide duplexes showed no significant change in phospho-MAPK and phospho-AKT levels[44-47]. Unlike the results in Swiss 3T3 cells, our results indicated that Smoc2 had promotive influence on MAPK/ERK and AKT signaling pathways in HCC cells. MAPK/ERK and AKT are identified as PDGF β receptor effector kinases and positively regulate the expression of cyclin D1[48]. Therefore, we speculated that Smoc2 could potentiate PDGF-induced mitogenesis and the subsequent cell proliferation. Nevertheless, another study based on Swiss 3T3 cells showed that the promotion of Smoc2 in DNA synthesis was independent of PDGF-binding activity but dependent on integrin-activated protein kinase[49].

Previous studies revealed that Smoc2 promotes growth factor-induced DNA synthesis and differs from SPARC, which was identified as an anti-mitogenic factor. It was reported that SPARC could bind various kinds of mitogens (such as VEGF, PDGF, FGF and so on) but inhibit the signaling mediated by these growth factors. Unlike SPARC, Smoc2 could facilitate growth factor-induced DNA synthesis[50]. The exact mechanism of how Smoc2 promotes growth factor-induced DNA synthesis in HCC cells remains unclear and needs to be

studied further.

Our research focused on the proliferation promotion effect of Smoc2 in HCC cells. However, Smoc2 also has influence on cell migration. It has been reported that extracellular matrix protein Smoc2 can promote keratinocyte migration *in vitro*. Accordingly, the role of Smoc2 on HCC cell migration and invasion need to be further studied. The influences of Smoc2 on HCC cell biological behaviors may be extensive.

In conclusion, our results demonstrated that Smoc2 is an important regulator of cell mitogenesis, and plays a promotive role in growth factor signaling and cell cycle progression in HCC. Based on our study, Smoc2 might represent a promising therapeutic target for HCC treatment.

COMMENTS

Background
The secreted modular calcium-binding protein-2 (*Smoc2*) gene, belonging to the secreted protein acidic and rich in cysteine (SPARC) family, was identified as encoding an extracellular matrix protein and as being up-regulated during embryogenesis and wound healing. The *Smoc2* gene encoded protein promotes extracellular matrix assembly and potentiates endothelial cell proliferation and migration, as well as has pro-angiogenic activity. Due to the promotive effect on angiogenesis, the *Smoc2* gene can be chosen as a target for inhibition of tumor progression.

Research frontiers
Smoc2 is a novel member of the SPARC family. Current studies have confirmed that Smoc2 can promote cell cycle progression by inducing the expression of transcripts required for the cell cycle. Other studies have shown that Smoc2 is necessary for DNA synthesis in the cell cycle and is likely to impact cell growth *in vitro* and *in vivo*. These studies also revealed that Smoc2 plays an important role in regulating the cell cycle. However, to the best of our knowledge, no study has investigated the role of Smoc2 in hepatocellular carcinoma (HCC).

Innovations and breakthroughs
The expression of Smoc2 was significantly higher in HCC tissues, as compared to CNL tissues. Overexpression of Smoc2 promoted HCC cell proliferation and cell cycle progression. Down-regulation of Smoc2 led to inhibition of cell proliferation and cell cycle progression. Smoc2 had a positive effect on ERK and AKT signaling.

Applications
According to our studies, Smoc2 was identified as pro-proliferative gene in HCC. Besides, blockage of Smoc2 could result in inhibition of liver cancer cell proliferation. Therefore, Smoc2 might be chosen as a target gene for preventing HCC progression. In the future, a neutralizing antibody for Smoc2 could be produced and used in animal experiments to further test its efficacy for preventing HCC proliferation. Blockage of Smoc2, by using neutralizing antibody or another approach, may contribute to HCC treatment and improve therapeutic efficacy.

Terminology
The SPARC family was recognized as extracellular matrix proteins. SPARC has been found to be up-regulated in several solid tumor tissues and to facilitate tumor metastasis. Smoc2, a novel member of the SPARC family, was identified as an extracellular matrix protein.

Peer-review
This study is very interesting. The authors determined the influence of Smoc2 on HCC cell proliferation and aimed to find a possible new therapeutic target for preventing HCC progression. The authors detected expression of Smoc2

in HCC tissues and corresponding non-tumor liver (CNL) tissues using PCR, western blot, immunohistochemistry methods and down-regulation and up-regulation of Smoc2 expression using small interfering RNA and lentivirus transfection assay respectively. The effect of Smoc2 on cell proliferation and cell cycle was identified using CCK-8 and flow cytometry respectively. The common cell growth signaling influenced by Smoc2 was detected. The authors found that Smoc2 promotes the proliferation of HCC cells through accelerating cell cycle progression and might act as an anti-cancer therapeutic target in the future. Overall, this study is well designed and the manuscript is well written.

REFERENCES

1   de Castro Brás LE, Toba H, Baicu CF, Zile MR, Weintraub ST, Lindsey ML, Bradshaw AD. Age and SPARC change the extracellular matrix composition of the left ventricle. *Biomed Res Int* 2014; **2014**: 810562 [PMID: 24783223 DOI: 10.1155/2014/810562]

2   Zhu GC, Gao L, He J, Long Y, Liao S, Wang H, Li X, Yi W, Pei Z, Wu M, Xiang J, Peng S, Ma J, Zhou M, Zeng Z, Xiang B, Xiong W, Tang K, Cao L, Li X, Li G, Zhou Y. CD90 is upregulated in gastric cancer tissues and inhibits gastric cancer cell apoptosis by modulating the expression level of SPARC protein. *Oncol Rep* 2015; **34**: 2497-2506 [PMID: 26329007 DOI: 10.3892/or.2015.4243]

3   Gao YY, Han RB, Wang X, Ge SH, Li HL, Deng T, Liu R, Bai M, Zhou LK, Zhang XY, Ba Y, Huang DZ. Change of SPARC expression after chemotherapy in gastric cancer. *Cancer Biol Med* 2015; **12**: 33-40 [PMID: 25859409 DOI: 10.7497/j.issn.2095-3941.2014.0023]

4   Yang L, Rong W, Xiao T, Zhang Y, Xu B, Liu Y, Wang L, Wu F, Qi J, Zhao X, Wang H, Han N, Guo S, Wu J, Gao Y, Cheng S. Secretory/releasing proteome-based identification of plasma biomarkers in HBV-associated hepatocellular carcinoma. *Sci China Life Sci* 2013; **56**: 638-646 [PMID: 23749381 DOI: 10.1007/s11427-013-4497-x]

5   Won C, Kim BH, Yi EH, Choi KJ, Kim EK, Jeong JM, Lee JH, Jang JJ, Yoon JH, Jeong WI, Park IC, Kim TW, Bae SS, Factor VM, Ma S, Thorgeirsson SS, Lee YH, Ye SK. Signal transducer and activator of transcription 3-mediated CD133 up-regulation contributes to promotion of hepatocellular carcinoma. *Hepatology* 2015; **62**: 1160-1173 [PMID: 26154152 DOI: 10.1002/hep.27968]

6   Deng B, Qu L, Li J, Fang J, Yang S, Cao Z, Mei Z, Sun X. MiRNA-211 suppresses cell proliferation, migration and invasion by targeting SPARC in human hepatocellular carcinoma. *Sci Rep* 2016; **6**: 26679 [PMID: 27230656 DOI: 10.1038/srep26679]

7   Lindner JL, Loibl S, Denkert C, Ataseven B, Fasching PA, Pfitzner BM, Gerber B, Gade S, Darb-Esfahani S, Sinn BV, Huober J, Engels K, Tesch H, Karn T, Pommerenke F, Liedtke C, Untch M, Müller V, Rack B, Schem C, von Minckwitz G. Expression of secreted protein acidic and rich in cysteine (SPARC) in breast cancer and response to neoadjuvant chemotherapy. *Ann Oncol* 2015; **26**: 95-100 [PMID: 25355716 DOI: 10.1093/annonc/mdu487]

8   Nian Q, Zhang Z, Wei C, Kuang X, Wang X, Wang L. Gene expression profiling in myelodysplastic syndrome after SPARC overexpression associated with Ara-C. *Oncol Rep* 2015; **34**: 2072-2082 [PMID: 26238482 DOI: 10.3892/or.2015.4139]

9   Pazin DE, Albrecht KH. Developmental expression of Smoc1 and Smoc2 suggests potential roles in fetal gonad and reproductive tract differentiation. *Dev Dyn* 2009; **238**: 2877-2890 [PMID: 19842175 DOI: 10.1002/dvdy.22124]

10 Liu P, Pazin DE, Merson RR, Albrecht KH, Vaziri C. The developmentally-regulated Smoc2 gene is repressed by Aryl-hydrocarbon receptor (Ahr) signaling. *Gene* 2009; **433**: 72-80 [PMID: 19146932 DOI: 10.1016/j.gene.2008.12.010]

11 Plentz RR, Malek NP. Early Detection of Hepatocellular Carcinoma: How to Screen and Follow up Patients with Liver Cirrhosis According to the GERMAN S3 Guideline? *Diagnostics* (Basel) 2015; **5**: 497-503 [PMID: 26854167 DOI: 10.3390/diagnostics5040497]

12 Birlea SA, Gowan K, Fain PR, Spritz RA. Genome-wide association study of generalized vitiligo in an isolated European founder population identifies SMOC2, in close proximity to IDDM8. *J Invest Dermatol* 2010; **130**: 798-803 [PMID: 19890347 DOI: 10.1038/jid.2009.347]

13 Lin CY, Hu CT, Cheng CC, Lee MC, Pan SM, Lin TY, Wu WS. Oxidation of heat shock protein 60 and protein disulfide isomerase activates ERK and migration of human hepatocellular carcinoma HepG2. *Oncotarget* 2016; **7**: 11067-11082 [PMID: 26840563 DOI: 10.18632/oncotarget.7093]

14 Wen S, Liu Y, Yang M, Yang K, Huang J, Feng D. Increased NEK2 in hepatocellular carcinoma promotes cancer progression and drug resistance by promoting PP1/Akt and Wnt activation. *Oncol Rep* 2016; **36**: 2193-2199 [PMID: 27509921 DOI: 10.3892/or.2016.5009]

15 Mattos AA, Marcon Pdos S, Araújo FS, Coral GP, Tovo CV. Hepatocellular carcinoma in a non-cirrhotic patient with sustained virological response after hepatitis c treatment. *Rev Inst Med Trop Sao Paulo* 2015; **57**: 519-522 [PMID: 27049708 DOI: 10.1590/S0036-46652015000600011]

16 Liu D, Staveley-O'Carroll KF, Li G. Immune-based Therapy Clinical Trials in Hepatocellular Carcinoma. *J Clin Cell Immunol* 2015; **6**: [PMID: 26877890 DOI: 10.4172/2155-9899.1000376]

17 He M, Qin H, Poon TC, Sze SC, Ding X, Co NN, Ngai SM, Chan TF, Wong N. Hepatocellular carcinoma-derived exosomes promote motility of immortalized hepatocyte through transfer of oncogenic proteins and RNAs. *Carcinogenesis* 2015; **36**: 1008-1018 [PMID: 26054723 DOI: 10.1093/carcin/bgv081]

18 Shahab J, Baratta C, Scuric B, Godt D, Venken KJ, Ringuette MJ. Loss of SPARC dysregulates basal lamina assembly to disrupt larval fat body homeostasis in Drosophila melanogaster. *Dev Dyn* 2015; **244**: 540-552 [PMID: 25529377 DOI: 10.1002/dvdy.24243]

19 Ehninger A, Boch T, Medyouf H, Müdder K, Orend G, Trumpp A. Loss of SPARC protects hematopoietic stem cells from chemotherapy toxicity by accelerating their return to quiescence. *Blood* 2014; **123**: 4054-4063 [PMID: 24833352 DOI: 10.1182/blood-2013-10-533711]

20 Qiu F, Sun R, Deng N, Guo T, Cao Y, Yu Y, Wang X, Zou B, Zhang S, Jing T, Ling T, Xie J, Zhang Q. miR-29a/b enhances cell migration and invasion in nasopharyngeal carcinoma progression by regulating SPARC and COL3A1 gene expression. *PLoS One* 2015; **10**: e0120969 [PMID: 25786138 DOI: 10.1371/journal.pone.0120969]

21 Nagaraju GP, Dontula R, El-Rayes BF, Lakka SS. Molecular mechanisms underlying the divergent roles of SPARC in human carcinogenesis. *Carcinogenesis* 2014; **35**: 967-973 [PMID: 24675529 DOI: 10.1093/carcin/bgu072]

22 Liao CY, Lee CC, Tsai CC, Hsueh CW, Wang CC, Chen IH, Tsai MK, Liu MY, Hsieh AT, Su KJ, Wu HM, Huang SC, Wang YC, Wang CY, Huang SF, Yeh YC, Ben RJ, Chien ST, Hsu CW, Kuo WH. Novel Investigations of Flavonoids as Chemopreventive Agents for Hepatocellular Carcinoma. *Biomed Res Int* 2015; **2015**: 840542 [PMID: 26858957 DOI: 10.1155/2015/840542]

23 Aseer KR, Kim SW, Choi MS, Yun JW. Opposite Expression of SPARC between the Liver and Pancreas in Streptozotocin-Induced Diabetic Rats. *PLoS One* 2015; **10**: e0131189 [PMID: 26110898 DOI: 10.1371/journal.pone.0131189]

24 Shi D, Jiang K, Fu Y, Fang R, Liu XI, Chen J. Overexpression of SPARC correlates with poor prognosis in patients with cervical carcinoma and regulates cancer cell epithelial-mesenchymal transition. *Oncol Lett* 2016; **11**: 3251-3258 [PMID: 27123099 DOI: 10.3892/ol.2016.4399]

25 Cheng CT, Chu YY, Yeh CN, Huang SC, Chen MH, Wang SY, Tsai CY, Chiang KC, Chen YY, Ma MC, Liu CT, Chen TW, Yeh TS. Peritumoral SPARC expression and patient outcome with resectable intrahepatic cholangiocarcinoma. *Onco Targets Ther* 2015; **8**: 1899-1907 [PMID: 26251613 DOI: 10.2147/OTT.S78728]

26 Villarreal G, Chatterjee A, Oh SS, Oh DJ, Rhee DJ. Pharma-

cological regulation of SPARC by lovastatin in human trabecular meshwork cells. *Invest Ophthalmol Vis Sci* 2014; **55**: 1657-1665 [PMID: 24474275 DOI: 10.1167/iovs.13-12712]

27 **Stenzel A**, Goss KU, Endo S. Prediction of partition coefficients for complex environmental contaminants: Validation of COSMOtherm, ABSOLV, and SPARC. *Environ Toxicol Chem* 2014; **33**: 1537-1543 [PMID: 24668883 DOI: 10.1002/etc.2587]

28 **Nakashima S**, Kobayashi S, Sakai D, Tomokuni A, Tomimaru Y, Hama N, Wada H, Kawamoto K, Marubashi S, Eguchi H, Matsuura N, Doki Y, Mori M, Nagano H. Prognostic impact of tumoral and/or peri-tumoral stromal SPARC expressions after surgery in patients with biliary tract cancer. *J Surg Oncol* 2014; **110**: 1016-1022 [PMID: 25155283 DOI: 10.1002/jso.23767]

29 **Han W**, Cao F, Chen MB, Lu RZ, Wang HB, Yu M, Shi CT, Ding HZ. Prognostic Value of SPARC in Patients with Pancreatic Cancer: A Systematic Review and Meta-Analysis. *PLoS One* 2016; **11**: e0145803 [PMID: 26731428 DOI: 10.1371/journal.pone.0145803]

30 **Joo I**, Lee JM. Recent Advances in the Imaging Diagnosis of Hepatocellular Carcinoma: Value of Gadoxetic Acid-Enhanced MRI. *Liver Cancer* 2016; **5**: 67-87 [PMID: 26989660 DOI: 10.1159/000367750]

31 **Alfawaz S**, Fong F, Plagnol V, Wong FS, Fearne J, Kelsell DP. Recessive oligodontia linked to a homozygous loss-of-function mutation in the SMOC2 gene. *Arch Oral Biol* 2013; **58**: 462-466 [PMID: 23317772 DOI: 10.1016/j.archoralbio.2012.12.008]

32 **Hua HW**, Jiang F, Huang Q, Liao ZJ, Ding G. Re-sensitization of 5-FU resistance by SPARC through negative regulation of glucose metabolism in hepatocellular carcinoma. *Tumour Biol* 2015; **36**: 303-313 [PMID: 25252848 DOI: 10.1007/s13277-014-2633-2]

33 **Bradshaw AD**. The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: Does expression of SPARC by macrophages influence outcomes? *J Mol Cell Cardiol* 2016; **93**: 156-161 [PMID: 26582465 DOI: 10.1016/j.yjmcc.2015.11.014]

34 **Morgan SS**, Nagle RB, Cranmer LD. Serum protein acidic and rich in cysteine (SPARC) as a prognostic marker in soft tissue sarcomas. *Clin Sarcoma Res* 2014; **4**: 2 [PMID: 24484617 DOI: 10.1186/2045-3329-4-2]

35 **Alkhateeb A**, Al-Dain Marzouka N, Qarqaz F. SMOC2 gene variant and the risk of vitiligo in Jordanian Arabs. *Eur J Dermatol* 2010; **20**: 701-704 [PMID: 20965805 DOI: 10.1684/ejd.2010.1095]

36 **Mommaerts H**, Esguerra CV, Hartmann U, Luyten FP, Tylzanowski P. Smoc2 modulates embryonic myelopoiesis during zebrafish development. *Dev Dyn* 2014; **243**: 1375-1390 [PMID: 25044883 DOI: 10.1002/dvdy.24164]

37 **Peixoto E**, Atorrasagasti C, Aquino JB, Militello R, Bayo J, Fiore E, Piccioni F, Salvatierra E, Alaniz L, García MG, Bataller R, Corrales F, Gidekel M, Podhajcer O, Colombo MI, Mazzolini G. SPARC (secreted protein acidic and rich in cysteine) knockdown protects mice from acute liver injury by reducing vascular endothelial cell damage. *Gene Ther* 2015; **22**: 9-19 [PMID: 25410742 DOI: 10.1038/gt.2014.102]

38 **Salvatierra E**, Alvarez MJ, Leishman CC, Rivas Baquero E, Lutzky VP, Chuluyan HE, Podhajcer OL. SPARC Controls Melanoma Cell Plasticity through Rac1. *PLoS One* 2015; **10**: e0134714 [PMID: 26248315 DOI: 10.1371/journal.pone.0134714]

39 **Sinn M**, Sinn BV, Striefler JK, Lindner JL, Stieler JM, Lohneis P, Bischoff S, Bläker H, Pelzer U, Bahra M, Dietel M, Dörken B, Oettle H, Riess H, Denkert C. SPARC expression in resected pancreatic cancer patients treated with gemcitabine: results from the CONKO-001 study. *Ann Oncol* 2014; **25**: 1025-1032 [PMID: 24562449 DOI: 10.1093/annonc/mdu084]

40 **Zhang J**, Wang P, Zhu J, Wang W, Yin J, Zhang C, Chen Z, Sun L, Wan Y, Wang X, Chen G, Liu Y. SPARC expression is negatively correlated with clinicopathological factors of gastric cancer and inhibits malignancy of gastric cancer cells. *Oncol Rep* 2014; **31**: 2312-2320 [PMID: 24676680 DOI: 10.3892/or.2014.3118]

41 **Peixoto E**, Atorrasagasti C, Malvicini M, Fiore E, Rodriguez M, Garcia M, Finocchieto P, Poderoso JJ, Corrales F, Mazzolini G. SPARC gene deletion protects against toxic liver injury and is associated to an enhanced proliferative capacity and reduced oxidative stress response. *Oncotarget* 2016; Epub ahead of print [PMID: 27213596 DOI: 10.18632/oncotarget.9456]

42 **Rossi MK**, Gnanamony M, Gondi CS. The 'SPARC' of life: Analysis of the role of osteonectin/SPARC in pancreatic cancer (Review). *Int J Oncol* 2016; **48**: 1765-1771 [PMID: 26983777 DOI: 10.3892/ijo.2016.3417]

43 **Morrissey MA**, Jayadev R, Miley GR, Blebea CA, Chi Q, Ihara S, Sherwood DR. SPARC Promotes Cell Invasion In Vivo by Decreasing Type IV Collagen Levels in the Basement Membrane. *PLoS Genet* 2016; **12**: e1005905 [PMID: 26926673 DOI: 10.1371/journal.pgen.1005905]

44 **Alachkar H**, Santhanam R, Maharry K, Metzeler KH, Huang X, Kohlschmidt J, Mendler JH, Benito JM, Hickey C, Neviani P, Dorrance AM, Anghelina M, Khalife J, Tarighat SS, Volinia S, Whitman SP, Paschka P, Hoellerbauer P, Wu YZ, Han L, Bolon BN, Blum W, Mrózek K, Carroll AJ, Perrotti D, Andreeff M, Caligiuri MA, Konopleva M, Garzon R, Bloomfield CD, Marcucci G. SPARC promotes leukemic cell growth and predicts acute myeloid leukemia outcome. *J Clin Invest* 2014; **124**: 1512-1524 [PMID: 24590286 DOI: 10.1172/JCI70921]

45 **Nian Q**, Xiao Q, Wang L, Luo J, Chen LP, Yang ZS, Liu L. SPARC silencing inhibits the growth of acute myeloid leukemia transformed from myelodysplastic syndrome via induction of cell cycle arrest and apoptosis. *Int J Mol Med* 2014; **33**: 856-862 [PMID: 24535175 DOI: 10.3892/ijmm.2014.1648]

46 **López-Murcia FJ**, Terni B, Llobet A. SPARC triggers a cell-autonomous program of synapse elimination. *Proc Natl Acad Sci U S A* 2015; **112**: 13366-13371 [PMID: 26420865 DOI: 10.1073/pnas.1512202112]

47 **Vaz J**, Ansari D, Sasor A, Andersson R. SPARC: A Potential Prognostic and Therapeutic Target in Pancreatic Cancer. *Pancreas* 2015; **44**: 1024-1035 [PMID: 26335014 DOI: 10.1097/MPA.0000000000000409]

48 **Hartley PS**, Motamedchaboki K, Bodmer R, Ocorr K. SPARC-Dependent Cardiomyopathy in Drosophila. *Circ Cardiovasc Genet* 2016; **9**: 119-129 [PMID: 26839388 DOI: 10.1161/CIRCGENETICS.115.001254]

49 **Rayne S**, Forest K. Use of the SPARC software program to calculate hydrolysis rate constants for the polymeric brominated flame retardants BC-58 and FR-1025. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2016; **51**: 509-513 [PMID: 26889790 DOI: 10.1080/10934529.2015.1128749]

50 **Alkhateeb A**, Marzouka NA, Tashtoush R. Variants in PTPN22 and SMOC2 genes and the risk of thyroid disease in the Jordanian Arab population. *Endocrine* 2013; **44**: 702-709 [PMID: 23463390 DOI: 10.1007/s12020-013-9908-z]