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Stathmin 1 promotes the progression of liver cancer through interacting with YAP1

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Abstract. – OBJECTIVE: The present study aimed to explore whether Hippo/YAP signaling pathway was involved in STMN1 (stathmin 1)-mediated liver cancer progression.

PATIENTS AND METHODS: STMN1 expression patterns were determined by Real Time-Polymerase Chain Reaction (PCR) assay (RT-PCR) and Western blotting. The relationship between STMN1 expression levels and the clinical features and the prognosis of patients with liver cancer were evaluated by χ^2-test and student's t-test. Cell Counting Kit-8 (CCK-8), flow cytometry and in vivo tumor formation assays were used to assess cell proliferation, apoptosis and tumorigenesis, respectively. Interaction between STMN1 and Yes associated protein (YAP1) was determined by immunoprecipitation (IP) and immunofluorescence technologies.

RESULTS: The results showed that STMN1 expression in liver cancer tissues was significantly higher than that in the adjacent normal tissues and increased STMN1 expression predicted an advanced clinical process and short overall survival of patients. Cell proliferation was increased and apoptosis was decreased when STMN1 was upregulated in HepG2 and SNU-398 cells. Besides, our results demonstrated that the overexpression of STMN1 enhanced the tumorigenesis of liver cancer cells through upregulating YAP1.

CONCLUSIONS: The current study demonstrates that STMN1 upregulation promotes the occurrence and development of liver cancer *via* activating YAP1 signaling. STMN1 overexpression may be an early event of liver carcinogenesis and it may be served as a marker for the diagnosis and treatment of liver cancer.

Key Words:
STMN1, Hippo/YAP, Liver cancer, Proliferation, Tumorigenesis.

Introduction

Primary liver cancer is the third leading cause of cancer-related death in the world[1] with hepatocellular carcinoma (HCC) as the most common type. Although serum alpha-fetoprotein (AFP) has long time been considered as a useful biomarker for diagnosis, low serum AFP is not specific for certain HCC patients after surgery[2]. In addition, liver cancer is a significantly heterogeneous entity at the molecular and clinical levels. Therefore, it is essential to further elucidate the pathogenetic mechanisms underlying liver cancer, aiming to find appropriate diagnostic and therapeutic targets.

STMN1 (stathmin 1 or oncoprotein-18) is a gene which belongs to a microtubule-destabilizing protein family[3-5] and is transcriptionally repressed by functional p53, a tumor suppressive protein[6]. STMN1 was first identified as a cellular phosphoprotein and was overexpressed in leukemia in 1983[7]. Nowadays, STMN1 has been documented to be highly expressed in many kinds of cancers, including liver cancer[8,9]. The high expression of STMN1 closely associates several clinicopathological features of liver cancer[9], and significantly enhances liver cancer cell invasion[2-9]. However, the roles and mechanisms of STMN1 in liver cancer progression still remain largely unclear.

The Hippo-YAP network is one of the signaling pathways which play important roles in cell proliferation and survival[10-12]. The transcriptional co-activators Yes associated protein (YAP) and the highly similar protein TAZ are the major downstream effectors of this pathway and are under the negative control of a core cascade of proteins, including LATS1/2, NF2, and MST1/2. In the presence of Hippo pathway, both YAP and TAZ are directly phosphorylated by LATS1/2 and kept in cytoplasm and subsequently degraded *via* proteasomal pathway[13-15]. However, YAP/TAZ will translocate into the nucleus and interact with the transcription factors, such as TEAD and CREB family once getting out of the supervision of Hippo pathway, leading to the activation

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of genetic programs[16]. Recently, the deregulated Hippo/YAP pathway is detected in the majority kinds of cancers, including liver cancer. Perra et al[17] and Yimlamai et al[18] illustrated that the high expression of YAP was an early event in liver cancer and it had the potential to be served as a therapeutic target for liver cancer.

The present study focused on exploration of the roles and mechanisms of STMN1 in liver cancer progression. Our results revealed that STMN1 was overexpressed in liver cancer tissues and functioned as an oncogene by promoting YAP1 expression.

Patients and Methods

Liver Tissue Samples

Twenty-six matched liver cancer tissues and normal tissues were obtained from patients with liver cancer. All patients received hepatectomy as the first treatment mean and signed informed consent before the study. Protocols involved in human tissue samples were carried out according with the Helsinki Declaration guide and were approved by the Ethical Committee of No. 1 People's Hospital of Jining City, Shandong Province.

Cell Lines and Culture Conditions

Human liver cancer HepG2 and SNU-398 cells were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). HepG2 cells were cultured in the complete medium, which was made up of 90% of F-12K medium (Gibco, Rockville, MD, USA) and 10% of fetal bovine serum (FBS; Gibco, Rockville, MD, USA). SNU-398 cells were maintained in Roswell Park Memorial Institute-1640 (RPMI-1640) medium, supplemented with 10% fetal bovine serum (FBS).

Modification of the Expression of STMN1/YAP1

Lentivirus vector used to upregulate STMN1 (OE-STMN1) and its negative control vector (OE-NC) were purchased from OriGene (Beijing, China). Small interfering RNAs (siRNAs) used to silence STMN1 (si-STMN1), and the short hairpin RNAs (shRNAs) used to knock down YAP1 (sh-YAP1), as well as their negative control vectors (si-NC and sh-NC) were obtained from GenePharma (Shanghai, China). To construct stable cell lines, cells infected with OE-STMN1 or OE-STMN1 + sh-YAP1 were selected with 8 μg/ml puromycin, or 8 μg/ml puromycin + 100 μg/ml G418 for 14 days, respectively.

Real Time-Polymerase chain reaction (PCR) assay (RT-PCR)

Total mRNA was extracted from cells and tissues by using the Total RNA Extraction TRIzol reagent (Baiaolaibo Co., Ltd., Beijing, China) according to the manufacture's protocols. Then, a total of 1 μg mRNA was reversely transcribed into the first-strand cDNA using the First Strand cDNA Synthesis Kit (Sangon Biotechnology, Shanghai, China), following by RT-PCR procures with TransStart Green qPCR SuperMix (Transgen Biotechnology, Beijing, China) on DA7600 Real-Time Nucleic Acid Amplification Fluorescence Detection System (Bio Rad, Hercules, CA, USA). Melting-curve was conducted to analyze the reaction specificity. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was served as an internal reference. Primers for STMN1, Ankyrin repeat domain 1 (ANKRD1), Hepatocellular carcinoma upregulated long non-coding RNA (HULC), Cellular communication network factor 2 (CTGF/CCN2) and GAPDH were obtained from Sangon Biotechnology (Shanghai, China) and were listed as follows: STMN1: sense-5'-GTACTTCTGGACTCACGGGC-3', STMN1: antisense-5'-AGGATCCTCTTGGCCAGACT-3'; ANKRD1: sense-5'-AGAACTGTGCTGGGAAGACG-3' ANKRD1: antisense-5'- GCCATGCCTTCAAAATGCCA-3'; HULC: sense-5'-ACTCTGAAGTAAAGGCCGGA-3', HULC: antisense-5'-TGCCAGGAAACTTCTTGCTTG-3'; CTGF: sense-5'-CACCCGGGTTACCAATGACA-3', CTGF: antisense-5'- TCCGGGACAGTTGTAATGGC-3'; GAPDH: sense-5'-ACTAGGCGCTCACTGTTCTCTC-3', GAPDH: antisense-5'-CATGGTTCACACCCATGACG-3'.

Western Blot Assay

Total protein was isolated from tissues and cells using RIPA lysis referring to the instructions. The proteins were then centrifuged at a 12,000×g speed at 4°C for 25 min. After concentrations was determined by bicinchoninic acid (BCA) analysis (Millipore, Billerica, MA, USA), 20 μg protein from each sample was separated by 10% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). Then, the proteins were transformed onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were then immersed into 5% nonfat milk for 1 hour at room temperature and incubated

with the following primary antibodies at 4°C overnight: anti-STMN1 antibody (No. ab52630), anti-YAP1 antibody (No. ab52771), anti-GAPDH antibody (No. ab181602), all obtained from Abcam (Cambridge, MA, USA). On the next day, the membranes were incubated with the corresponding secondary antibodies (Thermo Fisher Scientific, Waltham, MA, USA) at room temperature for 1 hour. After washing with Tris-HCl Buffer Solution-Tween 20 (TBST) for 4×5 min, the complexes were measured with enhanced chemiluminescence substrate (ECL; Millipore, Billerica, MA, USA) and analyzed by Image J software.

Immunoprecipitation (IP)

Liver cancer HepG2 and SNU-398 cells were lysed in RIPA buffer with protease (Roche, Shanghai, China). Then, a total of 1 mg proteins was precleared with protein A/G-Sepharose beads (Sigma-Aldrich, St. Louis, MO, USA) at 4°C for 30 min. Then, the proteins were immunoprecipitated with anti-STMN1 antibody overnight at 4°C and protein A/G-Sepharose beads in succession. Subsequently, the immune complex was subjected to Western blot assay with anti-YAP1 antibody after being washed with RIPA buffer for three times.

Immunofluorescence Assay

After 24 hours of cell treatments with OE-STMN1, OE-NC, si-STMN1 or si-NC, HepG2 cells were seeded onto the cover glasses in 24-well plates and cultured at 37°C for 36 hours. The cells were then fixed in 4% paraformaldehyde for 10 min, and were permeabilized with 0.25% Triton X-100 (Solarbio, Beijing, China) for 10 min. Next, 5% goat serum was used to block the nonspecific binding for 1 hour at room temperature, and the cells were then incubated with anti-YAP1 antibody (No. ab52771, Abcam, Cambridge, MA, USA), anti-CREB antibody (No. ab168928, Abcam, Cambridge, MA, USA) or anti-TEAD antibody (No. ab58310, Abcam, Cambridge, MA, USA) in a humidified chamber at 4°C overnight. After washing with PBS for three times, the cells were incubated with DyLight® 650/488 secondary antibody (Abcam, Cambridge, MA, USA) at room temperature for 1 hour in the dark. 4, 6-diamidino-2-phenylindole (DAPI) was used for cell nuclei dyeing. The content and subcellular location of the measured proteins were determined by confocal laser scanning microscopy (Olympus, Tokyo, Japan).

Cell Growth Assessment

Cell Counting Kit-8 (CCK-8) assay was carried out to investigate cell proliferation under altered STMN1 expression conditions. In brief, transfected HepG2 and SNU-398 cells were seeded into the 96-well plate with 3000 cells in each well. Subsequently, 10 µl CCK-8 solution (Beyotime Biotechnology, Shanghai, China) were added into per well and incubated for further 4 hours at 37°C. The optical density (OD) values at 450 nm were measured.

Cell Apoptosis Detection

HepG2 and SNU-398 cells were inoculated into 12-well plate and cultured at 37°C overnight. Then, si-NC, si-STMN1, OE-NC and OE-STMN1 were given to cells. After 48 hours, the cells were resuspended with Annexin-binding buffer which was mixed with 5 µl Annexin V and 5 µl PI solution (BD Biosciences, Franklin Lakes, NJ, USA) and incubated at room temperature for 15 min in the dark. The apoptotic percentages of cells with different treatments were detected by flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed by FlowJo software.

In Vivo Tumor Bearing Models

Protocols involving animals were performed in accordance with the principles and procedures of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethical Committee of No. 1 People's Hospital of Jining City Shandong Province. Briefly, HepG2 or SNU-398 cells (5×10^6) with various STMN1/YAP1 expressing patterns were injected into the armpit area of BALB/c nude mice at 4-week-old (5 mice/group). 28 days post-transplantation, the mice were sacrificed and the tumors were collected for weighing.

Statistical Analysis

Data are presented as means ± standard deviation (SD) and analyzed with Graph Pad Prism software 6.0 with Student's *t*-tests or One-way analysis of variance (ANOVA) followed by Students-Newman-Keuls (S-N-K) test. The association between STMN1 expressions and the clinical features was determined by χ^2-test. Kaplan-Meier was carried out to analyze the effect of STMN1 expressions on the overall survival of patients with liver cancer. $p<0.05$ was regarded as a statistically significant difference.

Results

STMN1 Is Overexpressed in Liver Cancer Tissues

To investigate the effects of STMN1 in the progression of liver cancer, we first compared its expression profiles between liver cancer tissues and the normal adjacent liver tissues. The results showed that STMN1 expression was significantly elevated in liver cancer tissues as compared to that in the normal tissues at both mRNA (Figure 1A) and protein (Figure 1B) levels, suggesting that the high expression of STMN1 might play a role in the occurrence and development of liver cancer.

STMN1 Has Vital Clinical Significances in Liver Cancer

Next, we explored the clinical significance of different STMN1 expression patterns in patients with liver cancer. As shown in Table I, we concluded that the expression of STMN1 was positively related to tumor diameter (p=0.017), tumor number (p=0.043), TNM stage (p=0.018), the incidence of vascular invasion (p=0.09) and distant metastasis (p=0.02). Moreover, the high expression of STMN1 predicted a shorter overall survival in patients with liver cancer (Figure 2). Overall, these results indicate a crucial clinical value of STMN1 in liver cancer.

Overexpression of STMN1 Promotes Cell Growth and Inhibits Cell Apoptosis in Liver Cancer

To determine the effects of STMN1 in the progression of liver cancer, loss-of-function and gain-of-function assays were carried out in liver cancer HepG2 and SNU-398 cells. Compared with the control group, the mRNA and protein expression levels of STMN1 in OE-STMN1 group were significantly increased while they were reduced in si-STMN1 group (Figure 3A-3B). Upregulation of STMN1 significantly increased cell proliferation (Figure 3C-3D) and decreased cell apoptosis (Figure 3E), while the deletion of STMN1 expression caused the opposite results. These results suggest that STMN1 serves as an oncogene in liver cancer.

Upregulation of STMN1 Activates YAP1 Signaling in Liver Cancer

Then, we explored the effects of STMN1 on the activation of YAP signaling. IP assay showed that STMN1 protein could interact with YAP1 protein in liver cancer HepG2 and SNU-398 cells (Figure 4A). Besides, overexpression of STMN1 increased YAP1 expression (Figure 4B) and promoted its nuclear location (Figure 4C), while STMN1 downregulation caused the opposite results (Figure 4B-4C). Moreover, STMN1 upregulation enhanced the interactions between YAP1 protein and the transcription factors TEAD and CREB in HepG2 cells (Figure 5A), and increased the mRNA expression levels of CTGF, ANKRD1, HULC and MCAM (Figure 5B). Together, the above findings demonstrate that STMN1 can induce the activation of YAP1 signaling.

STMN1 Enhances Cell Tumorigenesis Via Upregulating YAP1 in Liver Cancer

Furthermore, we explored whether YAP1 took part in STMN1-mediated liver cancer progression *via* recruiting the sh-YAP1 to downregulate YAP1 expression in HepG2 and SNU-398 cells.

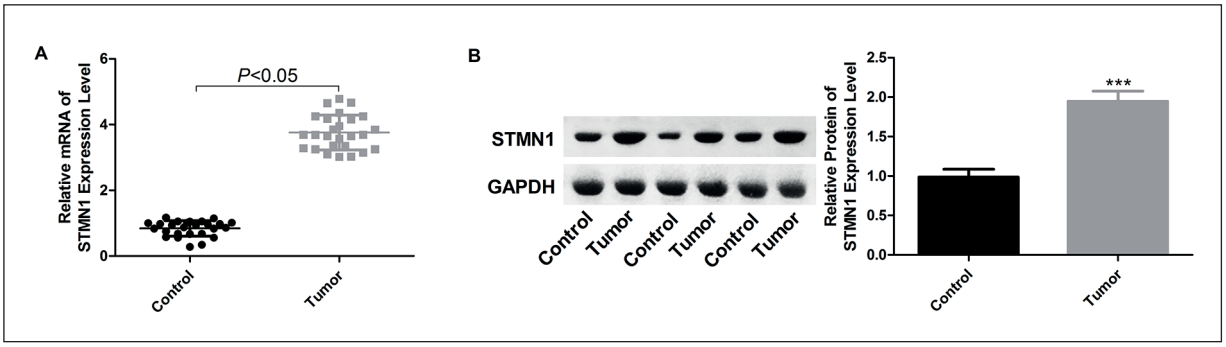

Figure 1. Evaluation of STMN1 expression patterns in liver cancer tissues and normal liver tissues. **A,** The mRNA expression levels of STMN1 in 26 matched liver cancer tissues and normal tissues were detected by RT-PCR technology. **B,** The protein expression profiles of STMN1 were determined by western blot assay in 26 matched liver cancer tissues and normal tissues, and the representative image was shown. (***p<0.001, Tumor group *vs.* Control group).

Table I. Analysis of the clinical significance of STMN1 in liver cancer.

Variable	Number	Low expression	High expression	*p*-value
Age (years)				0.682
< 50	48	22	26	
≥ 50	55	23	32	
Gender				0.755
Male	88	39	49	
Female	15	6	9	
HBV				0.124
Negative	26	8	18	
Positive	77	37	40	
Tumor diameter (cm)				0.017
< 5	36	10	26	
≥ 5	67	35	32	
Tumor number				0.043
Single	85	41	44	
Multiple	18	4	14	
Liver cirrhosis				0.256
No	35	18	17	
Yes	68	27	41	
Serum AFP (μg/L)				0.061
< 400	39	19	20	
≥ 400	64	26	38	
TNM stage				0.018
I/II	41	26	15	
III/IV	62	19	43	
Vascular invasion				0.09
No	37	21	16	
Yes	66	26	40	
Distant metastasis				0.02
No	28	18	10	
Yes	75	29	46	

HBV: Hepatitis B Virus; AFP: alpha fetoprotein; TNM: Tumor lymph node metastasis stage.

The expression of YAP1 in sh-YAP1 stably transfected HepG2 and SNU-398 cells was decreased to 30-40% of that in cells transfected with sh-NC (Figure 6A-6B). STMN1 overexpression significantly increased cell proliferation (Figure 6C-6D) and decreased cell apoptosis (Figure 6E), as well enhanced cell tumorigenesis (Figure 6F). However, the knockdown of YAP1 rescued the above effects of OE-STMN1 in both HepG2 and SNU-398 cells (Figure 6C-6F), suggesting that YAP1 is strongly implicated in the enhancement of cell tumorigenesis induced by STMN1.

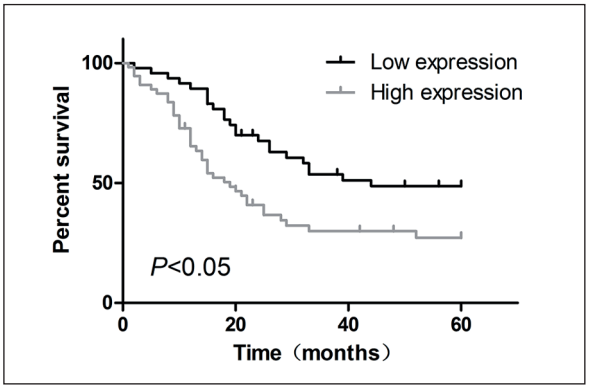

Figure 2. Evaluation of STMN1 expression in predicting the overall survival of patients with liver cancer. Differences in the overall survival of patients with STMN1 high/low expressions were analyzed by Kaplan-Meier analysis.

Discussion

STMN1 has been identified to be frequently amplified in a range of human cancers, such as breast cancer[19], gastric cancer[20], prostate cancer[21], esophageal cancer[22], cervical cancer[23], and ovarian cancer[24], as well as liver cancer[2-9]. Consistently with previous studies[2-9], the present study demonstrated that STMN1 was highly expressed in liver cancer tissues at both mRNA and protein levels, which was significantly associated with the tumor diameter (p=0.017), tumor number

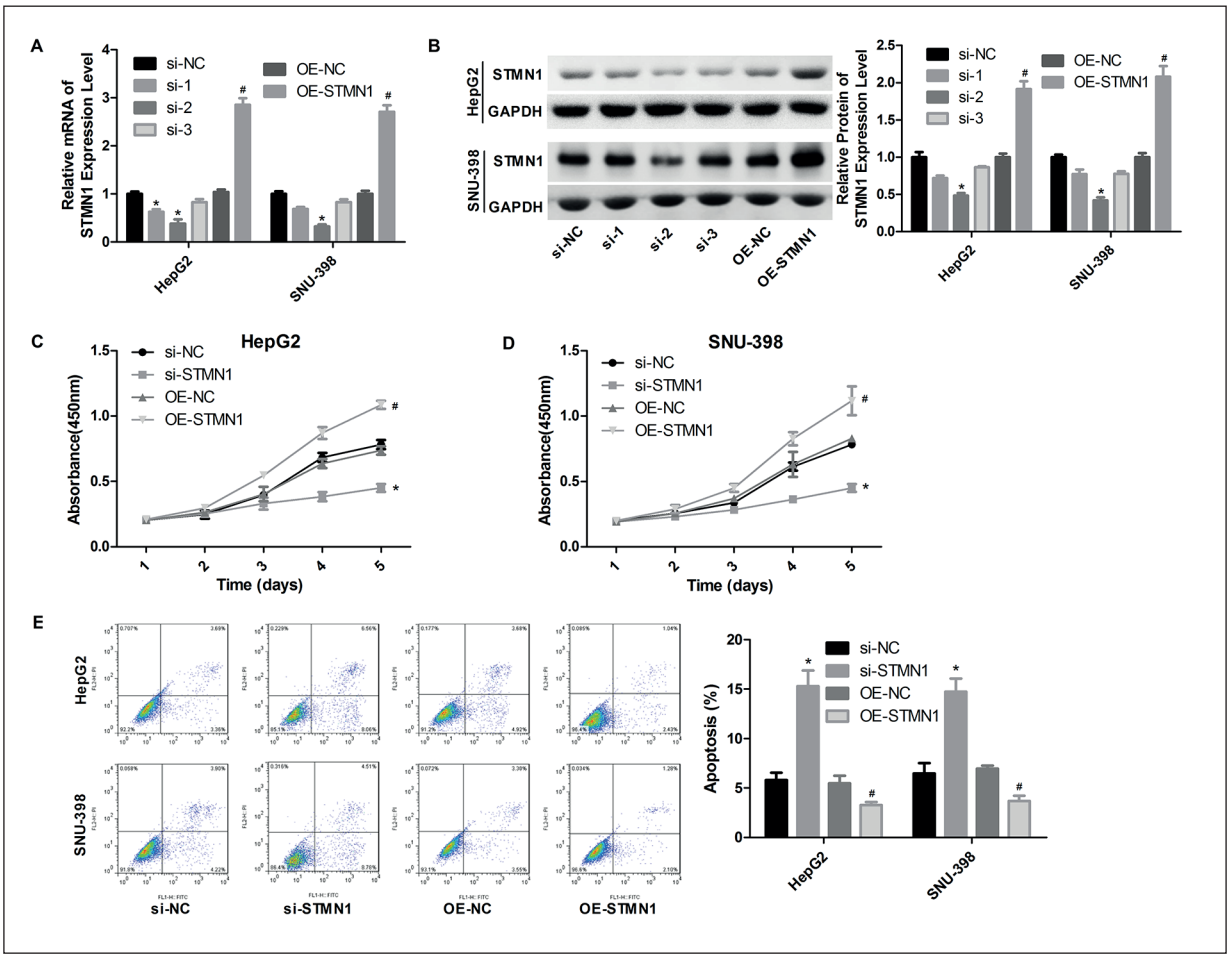

Figure 3. The effects of STMN1 on HepG2 and SNU-398 cell proliferation and apoptosis. HepG2 and SNU-398 cells were instantaneously transfected with si-STMN1, si-NC, OE-STMN1 or OE-STMN1, then the following experiments were carried out. **A-B,** STMN1 expressions in mRNA and protein levels were examined by RT-PCR and western blot assays, respectively. **C-D,** CCK-8 was used to detect the effects of STMN1 on cell proliferation, and results showed that STMN1 overexpression could enhance cell proliferation. **E,** Flow cytometry was carried out to detect cell apoptosis and results revealed that OE-STMN1 inhibited cell apoptosis rates. (*p<0.05, ***p<0.001, there were significant differences between si-STMN1 group and si-NC group; $^{\#\#}p$<0.01, there were significant differences between OE-STMN1 group and OE-NC group).

(p=0.043), TNM stage (p=0.018), the incidence of vascular invasion (p=0.09) and distant metastasis (p=0.02) in recruited Chinese liver cancer patients, as well as the shorter overall survival. Our results revealed a potential of STMN1 as a marker for the diagnosis of liver cancer and a potent target for liver cancer treatment again.

In this study, we explored STMN1 roles in liver cancer progression with both STMN1 silenced expression and ectopic expression. CCK-8 and flow cytometry assays showed that liver cancer HepG2 and SNU-398 cell proliferation was significantly enhanced while cell apoptosis proportion was reduced when STMN1 was overexpressed, suggesting that STMN1 serves as an oncogene in liver cancer. Hsieh et al[9] explored STMN1 effect on liver cancer Hep3B and HepG2 cell invasion, and they showed that STMN1 upregulation significantly enhanced cell invasion. Together, these findings indicate an important role of STMN1 in the development and progression of liver cancer. Consistently, Jeon et al[25] demonstrated that silencing STMN1 significantly inhibited the proliferation, migration, invasion and the *in vivo* growth of xenografts in nude mice in poorly differentiated gastric cancer cells. Chung et al[26] demonstrated that knockdown of STMN1 with siRNA transfection repressed the proliferation of prostate cancer cells. Kang et al[27] indicated that STMN1 downregulation induced by siRNA

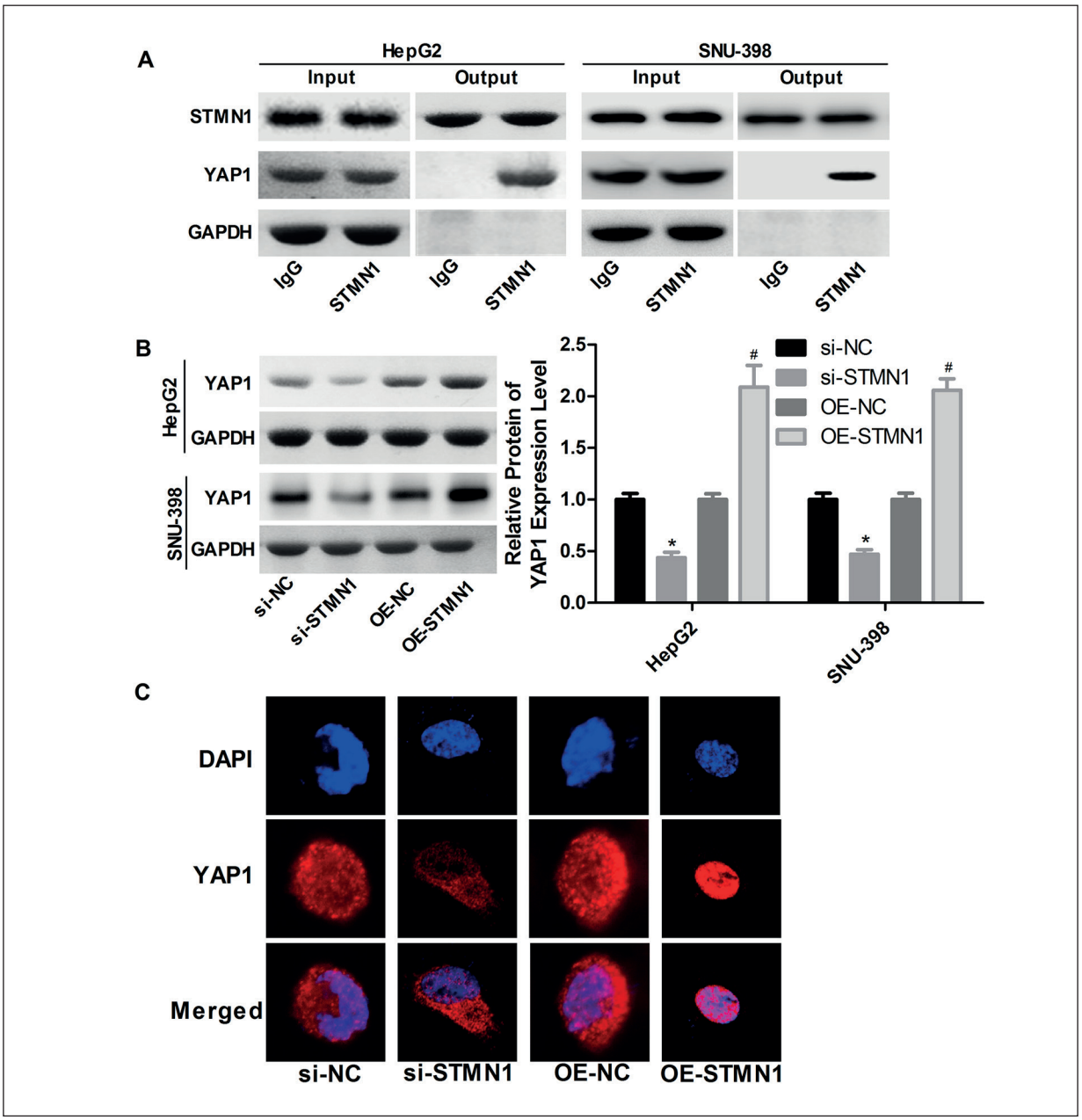

Figure 4. The effects of STMN1 expression profiles on YAP1 expression and subcellular location. **A,** IP assay was used to assess the interaction between YAP1 and STMN1 proteins in HepG2 and SNU-398 cells. **B,** Western blot assay was used to detect YAP1 expression after HepG2 and SNU-398 cells transfection with OE-STMN1, OE-NC, si-STMN1 or si-NC (***p<0.001, there were significant differences between si-STMN1 group and si-NC group; $^{\#}p$<0.05, there were significant differences between OE-STMN1 group and OE-NC group). **C,** Immunofluorescence assay was used to assess YAP1 location in HepG2 cells transfected with OE-STMN1 or OE-NC treatment (magnification: 100×).

transfection or microRNA-221 mimic apparently suppressed cell proliferation, monolayer colony formation, invasion, migration abilities and induced G1 phase arrest, as well as repressed tumor formation capacity of gastric cancer AGS and MKN7 cells. Moreover, scholars[28] also revealed that STMN1 overexpression induced radioresistance in non-small-cell lung cancer cells and strongly associated with the stem cell type of ovarian cancer[29].

Studies have shown that the Hippo-YAP signaling pathway is a major contributor to the tumorigenesis of liver cancer. Inhibition of the Hippo-signaling effector YAP by miR-375 could

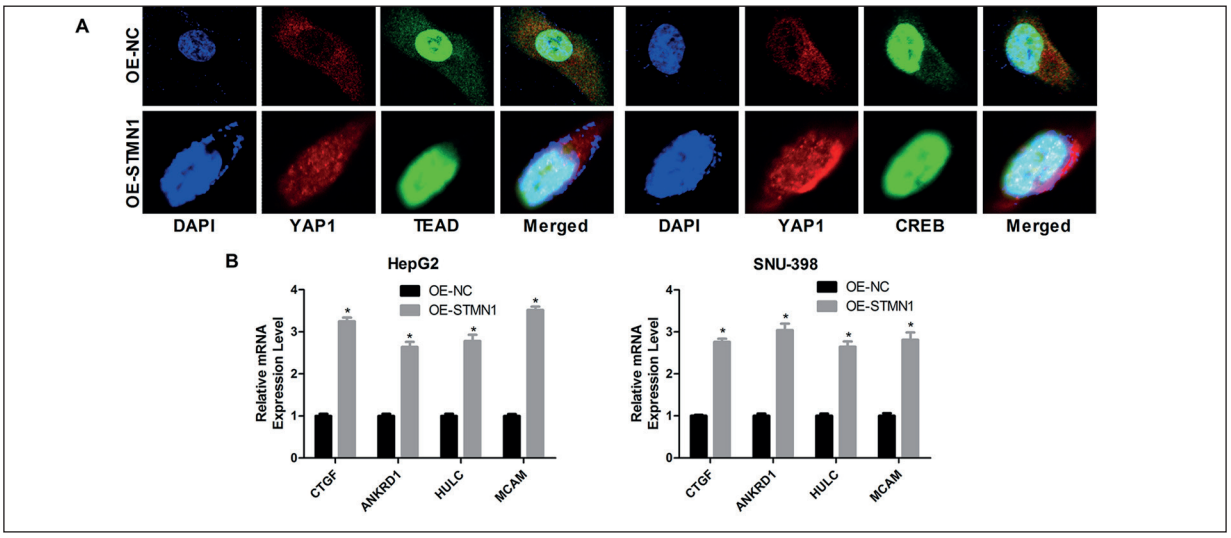

Figure 5. Evaluation of STMN1 overexpression on the activation of YAP1 signaling. **A,** HepG2 cells were transfected with OE-NC or OE-STMN1 for 48 hours, then immunofluorescence analysis was performed to determine the co-location of YAP1 and TEAD/CREB proteins (magnification: 100x). **B,** RT-PCR was used to detect the mRNA expressions of CTGF, ANKRD1, HULC and MCAM in HepG2 and SNU-398 cells; (***p<0.001, there were significant differences between OE-STMN1 group and OE-NC group).

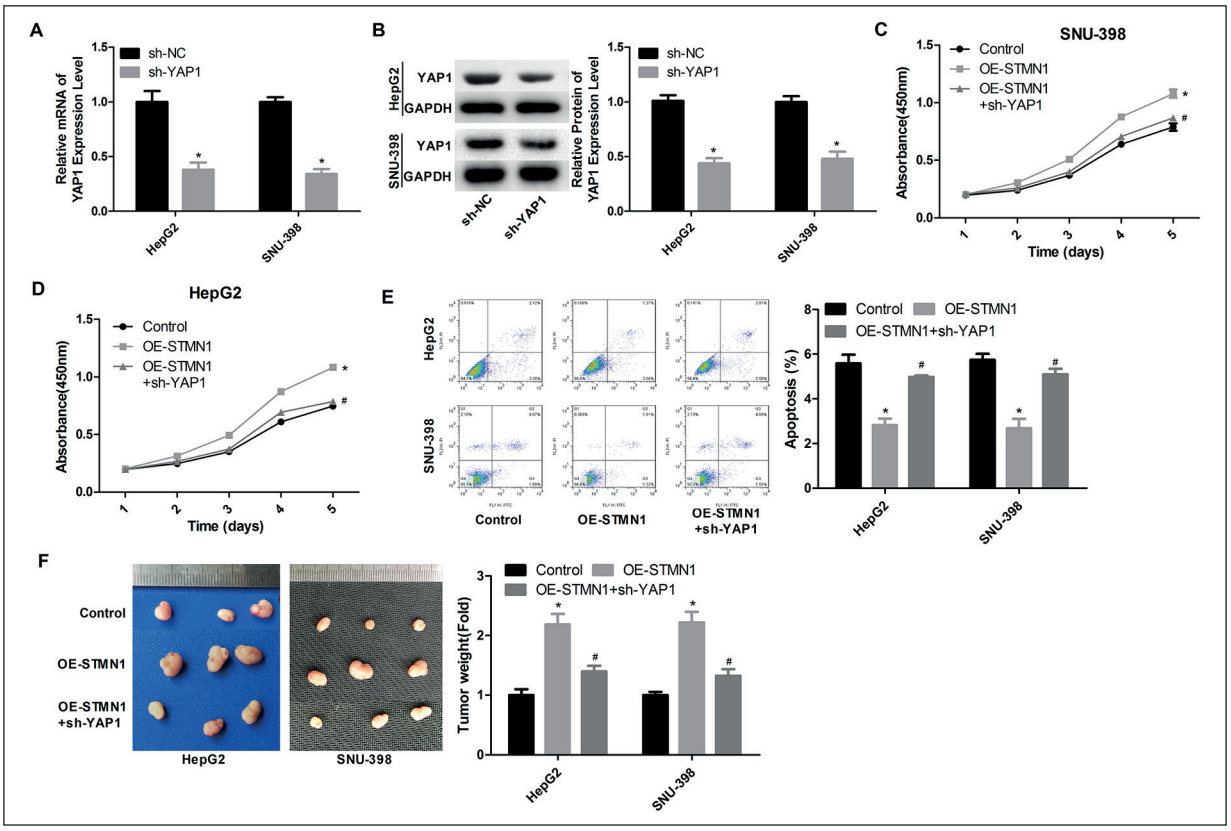

Figure 6. Assessment of the effects of STMN1/YAP1 axis on the tumorigenesis of HepG2 and SNU-398 cells. **A-B,** The mRNA and protein expression levels of STMN1 in YAP1 stable silenced HepG2 and SNU-398 cells were tested by RT-PCR and western blotting (***p<0.001, there were significant differences between sh-YAP1 group and sh-NC group). **C-D,** Cell proliferation was detected by CCK-8 assay. **E,** Cell apoptosis was detected by flow cytometry assay. **F,** Tumor sizes and weights in mice injected with HepG2 or SNU-398 cells in control, OE-STMN1 or OE-STMN1+sh-YAP1 group were determined by *in vivo* tumor-burdened experiments. (***p<0.001, there were significant differences between OE-STMN1 group and control group; $^{\#}p$<0.05, there were significant differences between OE-STMN1 + sh-YAP1 group and OE-STMN1 group).

significantly decreased HCC cell proliferation and invasion[30]. In addition, inhibition of YAP by ActinomycinD enhanced the anti-tumor role of Corosolic acid in liver cancer[31]. However, the enhanced expression and nuclear accumulation of YAP could significantly promote liver cancer progression[32,33]. All the findings support the opinion that YAP is the attractive therapeutic target for cancers[34]. In the present study, we demonstrated for the first time, that STMN1 could interact with YAP1 protein and significantly promote YAP1 expression and nuclear accumulation, and enhance the interaction between YAP1 and CREB and TEAD, as well as increased the expressions of CTGF, ANKRD1, HULC and MCAM, four YAP/TAZ-regulated genes. These results suggest that the activated YAP1 signaling might be involved in STMN1-mediated the malignant phenotypic transformation of liver cancer cells. To reveal YAP1 roles in STMN1-mediated liver cancer progression, we carried out *in vivo* tumor formation assays in nude mice. The results showed that YAP1 deletion impaired the role of STMN1 in tumor growth promotion, which demonstrated that STMN1 facilitated the progression of liver cancer in a YAP1-dependent manner.

Conclusions

The present study illustrates a functional link between STMN1 and Hippo/YAP signaling in the progression of liver cancer, which reveals that STMN1 not only promote YAP1 expression, but also functions as a co-activator of YAP to accelerate cancer progression. Collectively, this study demonstrates that STMN1 overexpression may be an early event of liver carcinogenesis and it can be served as a marker for the diagnosis and treatment of liver cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
2) Chen YL, Uen YH, Li CF, Horng KC, Chen LR, Wu WR, Tseng HY, Huang HY, Wu LC, Shiue YL. The E2F transcription factor 1 transactives stathmin 1 in hepatocellular carcinoma. Ann Surg Oncol 2013; 20: 4041-4054.
3) Holmfeldt P, Sellin ME, Gullberg M. Predominant regulators of tubulin monomer-polymer partitioning and their implication for cell polarization. Cell Mol Life Sci 2009; 66: 3263-3276.
4) Sellin ME, Holmfeldt P, Stenmark S, Gullberg M. Global regulation of the interphase microtubule system by abundantly expressed Op18/stathmin. Mol Biol Cell 2008; 19: 2897-2906.
5) Mistry SJ, Atweh GF. Role of stathmin in the regulation of the mitotic spindle: potential applications in cancer therapy. Mt Sinai J Med 2002; 69: 299-304.
6) Rubin CI, Atweh GF. The role of stathmin in the regulation of the cell cycle. J Cell Biochem 2004; 93: 242-250.
7) Sobel A, Tashjian AH Jr. Distinct patterns of cytoplasmic protein phosphorylation related to regulation of synthesis and release of prolactin by GH cells. J Biol Chem 1983; 258: 10312-10324.
8) Zhang HQ, Guo X, Guo SQ, Wang Q, Chen XQ, Li XN, Guo LS. STMN1 in colon cancer: expression and prognosis in Chinese patients. Eur Rev Med Pharmacol Sci 2016; 20: 2038-2044.
9) Hsieh SY, Huang SF, Yu MC, Yeh TS, Chen TC, Lin YJ, Chang CJ, Sung CM, Lee YL, Hsu CY. Stathmin1 overexpression associated with polyploidy, tumor-cell invasion, early recurrence, and poor prognosis in human hepatoma. Mol Carcinog 2010; 49: 476-487.
10) Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, Jaenisch R, Brummelkamp TR. YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol 2007; 17: 2054-2060.
11) Lu L, Li Y, Kim SM, Bossuyt W, Liu P, Qiu Q, Wang Y, Halder G, Finegold MJ, Lee JS, Johnson RL. Hippo signaling is a potent in vivo growth and tumor suppressor pathway in the mammalian liver. Proc Natl Acad Sci U S A 2010; 107: 1437-1442.
12) Shi Y, Liu B, Wang CS, Yang CS. MST1 down-regulation in decreasing apoptosis of aortic dissection smooth muscle cell apoptosis. Eur Rev Med Pharmacol Sci 2018; 22: 2044-2051.
13) Zhao B, Li L, Tumaneng K, Wang CY, Guan KL. A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCF(beta-TRCP). Genes Dev 2010; 24: 72-85.
14) Zhang J, Smolen GA, Haber DA. Negative regulation of YAP by LATS1 underscores evolutionary conservation of the Drosophila Hippo pathway. Cancer Res 2008; 68: 2789-2794.
15) Hao Y, Chun A, Cheung K, Rashidi B, Yang X. Tumor suppressor LATS1 is a negative regulator of oncogene YAP. J Biol Chem 2008; 283: 5496-5509.
16) Zhang X, George J, Deb S, Degoutin JL, Takano EA, Fox SB, Bowtell DD, Harvey KF. The Hippo pathway transcriptional co-activator, YAP, is an ovarian cancer oncogene. Oncogene 2011; 30: 2810-2822.
17) Perra A, Kowalik MA, Ghiso E, Ledda-Columbano GM, Di Tommaso L, Angioni MM, Raschioni C, Testore E, Roncalli M, Giordano S, Columbano A. YAP

activation is an early event and a potential therapeutic target in liver cancer development. J Hepatol 2014; 61: 1088-1096.

18) Yimlamai D, Fowl BH, Camargo FD. Emerging evidence on the role of the Hippo/YAP pathway in liver physiology and cancer. J Hepatol 2015; 63: 1491-1501.

19) Bieche I, Lachkar S, Becette V, Cifuentes-Diaz C, Sobel A, Lidereau R, Curmi PA. Overexpression of the stathmin gene in a subset of human breast cancer. Br J Cancer 1998; 78: 701-709.

20) Liu X, Liu H, Liang J, Yin B, Xiao J, Li J, Feng D, Li Y. Stathmin is a potential molecular marker and target for the treatment of gastric cancer. Int J Clin Exp Med 2015; 8: 6502-6509.

21) Ghosh R, Gu G, Tillman E, Yuan J, Wang Y, Fazli L, Rennie PS, Kasper S. Increased expression and differential phosphorylation of stathmin may promote prostate cancer progression. Prostate 2007; 67: 1038-1052.

22) Zhu HW, Jiang D, Xie ZY, Zhou MH, Sun DY, Zhao YG. Effects of stathmin 1 silencing by siRNA on sensitivity of esophageal cancer cells Eca-109 to paclitaxel. Genet Mol Res 2015; 14: 18695-18702.

23) Wang X, Ren JH, Lin F, Wei JX, Long M, Yan L, Zhang HZ. Stathmin is involved in arsenic trioxide-induced apoptosis in human cervical cancer cell lines via PI3K linked signal pathway. Cancer Biol Ther 2010; 10: 632-643.

24) Su D, Smith SM, Preti M, Schwartz P, Rutherford TJ, Menato G, Danese S, Ma S, Yu H, Katsaros D. Stathmin and tubulin expression and survival of ovarian cancer patients receiving platinum treatment with and without paclitaxel. Cancer 2009; 115: 2453-2463.

25) Jeon TY, Han ME, Lee YW, Lee YS, Kim GH, Song GA, Hur GY, Kim JY, Kim HJ, Yoon S, Baek SY, Kim BS, Kim JB, Oh SO. Overexpression of stathmin1 in the diffuse type of gastric cancer and its roles in proliferation and migration of gastric cancer cells. Br J Cancer 2010; 102: 710-718.

26) Chung MK, Kim HJ, Lee YS, Han ME, Yoon S, Baek SY, Kim BS, Kim JB, Oh SO. Hedgehog signaling regulates proliferation of prostate cancer cells via stathmin1. Clin Exp Med 2010; 10: 51-57.

27) Kang W, Tong JH, Chan AW, Lung RW, Chau SL, Wong QW, Wong N, Yu J, Cheng AS, To KF. Stathmin1 plays oncogenic role and is a target of microRNA-223 in gastric cancer. PLoS One 2012; 7: e33919.

28) Zhang X, Ji J, Yang Y, Zhang J, Shen L. Stathmin1 increases radioresistance by enhancing autophagy in non-small-cell lung cancer cells. Onco Targets Ther 2016; 9: 2565-2574.

29) Obayashi S, Horiguchi J, Higuchi T, Katayama A, Handa T, Altan B, Bai T, Bao P, Bao H, Yokobori T, Nishiyama M, Oyama T, Kuwano H. Stathmin1 expression is associated with aggressive phenotypes and cancer stem cell marker expression in breast cancer patients. Int J Oncol 2017; 51: 781-790.

30) Liu AM, Poon RT, Luk JM. MicroRNA-375 targets Hippo-signaling effector YAP in liver cancer and inhibits tumor properties. Biochem Biophys Res Commun 2010; 394: 623-627.

31) Xu Y, Zhao Y, Guan Y, Zhang X, Chen Y, Wu Q, Zhu G, Sun F, Wang J, Yu Y. Blocking inhibition to YAP by ActinomycinD enhances anti-tumor efficacy of Corosolic acid in treating liver cancer. Cell Signal 2017; 29: 209-217.

32) Li L, Wang J, Zhang Y, Ma L, Weng W, Qiao Y, Xiao W, Wang H, Yu W, Pan Q, He Y, Sun F. MEK1 promotes YAP and their interaction is critical for tumorigenesis in liver cancer. FEBS Lett 2013; 587: 3921-3927.

33) Kim Y, Jho EH. Deubiquitinase YOD1: the potent activator of YAP in hepatomegaly and liver cancer. BMB Rep 2017; 50: 281-282.

34) Zanconato F, Cordenonsi M, Piccolo S. YAP/TAZ at the roots of cancer. Cancer Cell 2016; 29: 783-803.