STMN2 overexpression promotes cell proliferation and EMT in pancreatic cancer mediated by WNT/β-catenin signaling

Mingrui Shao
The First Hospital of China Medical University Department of Surgical Oncology
Li Wang
The First Hospital of China Medical University Department of Surgical Oncology
Qi Zhang
The First Hospital of China Medical University Department of Surgical Oncology
Tianlong Wang
The First Hospital of China Medical University Department of Surgical Oncology
Shiyang Wang (cmu_wangshiyang@163.com)
The First Affiliated Hospital of China Medical University Department of Surgical Oncology
https://orcid.org/0000-0001-8920-6221

Research Article

Keywords: STMN2, WNT/β-catenin signaling, epithelial to mesenchymal transition, pancreatic cancer

Posted Date: March 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1298753/v1

Abstract

Background: STMN2, as a key regulator in microtubule disassembly and dynamics, has been recently reported to participate in cancer development. However, the corresponding role in pancreatic ductal adenocarcinoma (PC), to our knowledge has not been reported.

Methods: We investigate the potential role of STMN2 in the development of PC in vitro and vivo.

Results: Overexpression of STMN2 was prevalently observed in human PC tissues compared with that in paired pancreas (44/81,54.3% vs 15/81, 18.5%, P<0.01), which was positively with multiple advanced stage of PC patients (tumor size, T stage, lymph-node metastasis and the poor survival). Meanwhile, a close correlation between high STMN2 and cytoplasmic/nuclear β-catenin expression (P=0.007) was observed in PC tissues and cell lines. STMN2 overexpression induced EMT and cell proliferation in vitro, which stimulated EMT-like cellular morphology, cell motility and proliferation, and the change of EMT (Snail1, E-cad and Vimentin) and Cyclin D1 signaling. However, XAV939 inhibited STMN2 overexpression-enhanced EMT and proliferation. Conversely, KY19382 reversed STMN2 silencing- inhibited EMT and cell proliferation in vitro. In addition, STMN2 promoted subcutaneous tumor growth with the overexpression of EMT and Cyclin D1 signaling. Finally, activated STMN2 and β-catenin were co-localized in cytoplasm/nuclear in vitro.

Conclusions: β-catenin/TCF-mediated the transcription of STMN2. STMN2 overexpression promotes aggressive clinical stage of PC patients and promotes EMT and cell proliferation in vitro and vivo mediated by WNT/β-catenin signaling.

Background

Pancreatic ductal adenocarcinoma (PC) is one of the most fatal digestive cancers, with a 5-year survival rate of less than 10% [1]. It would overtake breast cancer as the third leading cause of cancer death by 2025 in Europe [2] and will become the 2nd most cause of cancer-related death in the US by 2030 [3]. Intense invasion and rapid metastase contribute to the unfavorable outcomes of PC patients. One of a critical driving factor is epithelial-to-mesenchymal transition (EMT). EMT provides cancer cells with a dramatic cytoskeleton rearrangement and metastatic phenotype characterized by the loss of the epithelial phenotype (E-cadherin) and the gain of mesenchymal properties (N-cadherin and Vimentin), playing a key role in the aggressive progression of PC [4]. Thus, it is urgent to explore the molecular regulation mechanism target EMT during tumor development.

STMN2, a neuronal growth-associated protein of Stathmin family [5], plays a significant role in neuronal growth, microtubule dynamics, cell motility and signaling pathway regulation [6–9]. Decreased STMN2 have been associated with Down's syndrome and Alzheimer's diseases [10], whereas increased STMN2 participated in the progression of hepatocellular [11], neuroblastoma [12] and ovarian cancer [13]. However, its potential role and related signal transduction in PC, to our knowledge, has not been reported yet.

The WNT/β-catenin signaling pathway, is a classic and conserved signal pathway participating in multiple physiological processes, including cell proliferation, differentiation, apoptosis, polarity, mobility and homeostasis [14]. Dysregulation of the WNT/β-catenin pathway is implicated in many human diseases, including various cancers. Meanwhile, the WNT/β-catenin signaling is an indispensable component to drive EMT in cancer development [15]. Previous study showed that STMN2 was a novel target of β-catenin/TCF-mediated transcription in human hepatoma cells [16, 17]. Taken together, we systematically investigated the potential role of STMN2 in regulating malignant behavior of PC in vitro and vivo in combination with WNT/β-catenin pathway, which supplies a novel gene targeted therapy for PC.

Methods

Clinical human samples and PC cell lines

This study was approved by the academic committee at the First hospital of China Medical University with the agreement of specimen consent signed by each patient. The study methodology has been admitted by the ethics committee from the same institution. 81 PC and paired adjacent pancreas were picked up from postoperative patients from 2010 to 2020 which were pathologically diagnosed as pancreatic ductal adenocarcinoma. Patients with endocrine carcinoma, acinar cell carcinoma and invasive intraductal papillary mucinous carcinoma were excluded from this study. PANC-1, BxPC-3, and SW1990 cells were purchased from the cell culture collection in Chinese Academy of Sciences. Capan-2 cells were purchased from the American Type Culture Collection

Immunohistochemistry

According to previous studies under IHC protocol [18, 19], PC sections were deparaffinized, dehydrated and next incubated with H2O2, subjected to high microwave repair and blocked with goat serum. Sections were incubated with anti-STMN2 (Abcam, Cambridge, UK), β-catenin (Proteintech, Chicago, IL), E-cadherin (E-cad, Abcam, dilution: 1:500), Vimentin (Proteintech), Cyclin D1(Abcam) overnight. Slices were next covered with the secondary antibody, detected with 3, 3'-diaminobenzidine (DAB), stained with haematoxylin and evaluated by pathologists. The final staining scores were evaluated according the staining area and intensity.

Western blot

As our previous study showed [19], proteins from tissues and cell lines extracted from whole-cell lysates were inserted into 10–12% SDS-polyacrylamide gels, transmitted to wet transfer, blocked with 5% BAS and incubated with STMN2 (Abcam), β-catenin (Proteintech), E-cad (Abcam), N-cadherin (Proteintech, dilution), Vimentin (Proteintech), Snail1 (Proteintech), and GAPDH (Proteintech) antibodies. All bands were detected with the ECL instrument (Bio-Rad, California, USA) following the incubation of secondary antibodies (Proteintech). WB was conducted in triple experiment.

Real-time quantitative PCR (qRT-PCR)

As our previous study showed [19], the condition of qRT-PCR from SYBR Premix Ex TaqTM (DRR420A) was as below: 95^{o}C for 30s and 40 cycles of 95^{o}C for 10s and 55^{o}C for 30s. The primers were used as follow: STMN2, 5'- GCAATGGCCTACAAGGAAAA-3' (sense) and 5'-ATAGAAGGCTGCGGAATTGT-3'(antisense); β-catenin, 5'- GCTTTCAGTTGAGCTGACCA – 3' (sense) and 5'-AAGTCCAAGATCAGCAGTCTCA – 3'(antisense).Amplification products was calculatedn following the△△Ct method.

siRNA and lentivirus vector mediated STMN2 overexpression

Two effective sequence (UTR'3) of STMN2siRNA were as followed:

1. AGAAUCUAUAGAGUCUCAA; 2. CUGUGAGCUGGUUGUUGCA. Oligofectamine-3000 (Invitrogen, USA) were used for siRNA transfections under the corresponding protocol. Lentivirus vector mediated STMN2 overexpression (STMN2-GFP) and empty vector (GFP) were purchased from Genechem (Shanghai, China). PANC-1/Capan-2 cells and BxPC-3/SW1990 cells were available for STMN2 silencing and overexpressing construct, respectively according to the distinguished expression of STMN2 in vitro as indicated in result sections.

EMT construction

In order to enhance EMT induction, STMN2-GFP and GFP transfected PANC-1 and Capan-2 cells were pre-cultured with medium containing 1%FBS for 24h. Then cells were pretreated with XAV939 (20uM, Selleckchem, USA) for 12h. Similarly, STMN2 silencing BxPC-3 and SW1990 cells were pretreated with KY19382 (1uM, MedChemExpress, USA) for 24h. 1% DMSO was used as the vehicle. We evaluated EMT model from three aspects: EMT-like cellular morphology, cell motility and the change of EMT signaling.

Transwell assays

Based on our previous study [19], STMN2-GFP and GFP transfected PANC-1 and Capan-2 cells were pretreated with XAV939 (20uM, Selleckchem, USA) for 12h, while STMN2 silencing BxPC-3 and SW1990 cells were pretreated with KY19382 (1uM, MedChemExpress, USA) for 24h. Cells were implanted into membrane inserts (BD Biosciences) covered with 10%matrigel with free serum medium. Medium containing 10%FBS was put at the bottom. The crossed cells were calculated in at least 5 random fields/well (x200). The migration assay was conducted in the similar way without matrigel. Transwell was repeated in triplicates.

MTT assay

MTT was used to investigate the effect of STMN2 silencing or overexpressing PC cells in regulating cell proliferation with different time points combining with XAV939 (20uM for 12h repeated 3 times) or KY19382 (1uM for 24h repeated twice) treatments. PC cells (the density of 5,000 viable cells per well) were seeded into 96-well plates and incubated for 1 to 5 days. 15 μl of MTT (5 mg/ml in PBS, Sigma) and

100μl of DMSO were successively added to each well. 96-well plates was finally measured at a wavelength of 570 nm in an ELISA 96-well microtiter plate reader (BIORAD680, USA).

Immunofluorescence (IF) staining

BxPC-3 cells pretreated with KY19382 were implanted into 24-well culture plates, fixed in 4% paraformal dehyde, permeabilized with Triton X-100 (0.1%), incubated with 5% BSA, and then stained with the primary antibodies: STMN2 (Abcam) combining with β-catenin (Proteintech) following with the different origins of secondary antibodies (rabbit-TRITC and mouse-FITC). Hoechest33258 (Proteintech) were used for nuclear visualizing. IF was repeated in triplicates.

Chromatin immunoprecipitation (ChIP) assay

ChIP assay was performed in BxPC-3 cells under the protocol of the ChIP Assay Kit (Sigma) and previous study [17]. Briefly, BxPC-3 cells cultured in a 75 cm^2 plate were pretreated with KY19382 (1uM) for 24h. Then the cells were fixed with formaldehyde, lysed in the lysis buffer, and sonicated to extract approximately 800-bp chromatin fragments. Following dilution with IP dilution buffer, the lysate was incubated at 4°C overnight with β-catenin antibody (Proteintech), and the antibody-bound chromatin complex was precipitated by salmon sperm DNA/protein A–agarose. Finally, DNA was isolated from the immunoprecipitated chromatin. The corresponding PCR-amplified primer pairs flanking consensus TCF sites in STMN2 promoter was as below: F1-R1: 5’- TATTTCCAGACCCTGCCAAC-3’ (sense) and 5’-TGCTGAATCATGGGGAAAAT-3’(antisense); F2-R2: 5’- TGATTGGACAGAAAGCTGCTAA-3’ (sense) and 5’-AATTGCTAATTCCGACGTTTG-3’(antisense). All the PCR was carried out for 30 cycles with the primers annealed at 58°C, and the PCR products were resolved on a 2% agarose gel in TBE buffer.

In vivo xenograft model

Animals were kept according to the Animal Care Committee of China Medical University. The 8-week-old nude mice (BALB/c, female, Beijing Vital River Laboratory Animal Technology Co., Ltd. China) were acclimatized for a week and randomly assigned in each group (n = 5/group). STMN2-GFP and GPF transfected Capan-2 (5x10^6) cells were subcutaneously transplanted into the subcutaneous axillas, respectively. A cotton swab was used to avoid leakage from the injection site. Mice were treated with carbon dioxide for euthanasia 3 weeks later. The following formula was used to calculate tumor size: length x width x height x 0.52 in millimeters. The final samples were extracted for late hematoxylin and eosin (HE) and IHC staining shown in result section.

Statistical analysis

Based on our previous study [19], non-parametric paired, chi-squared and spearman testes were used to analyze the statistical data in IHC assays. The Kaplan–Meier curve in univariate analysis and Cox regression tests in multivariate analysis were used to analyze the survival data. The difference of WB, qRT-PCR, transwell and tumor size were represented as means ± standard deviation and were compared via independent *t*-test. P-value is regarded statistically significant as: *: $P < 0.05$; **= $P < 0.01$.

Results

Overexpression of STMN2 was closely associated with the clinicopathological characters of PC patients

STMN2 was localized in cytoplasm and nuclear in PC and adjacent pancreas (Fig. 1A) detected by IHC. STMN2 was overexpressed in human PC specimens compared with that in the paired pancreas (44/81,54.3% vs 15/81, 18.5%, P < 0.01) (Fig. 1A). STMN2 was defined as low (#8) and high expression (#15) for the late clinical data analyze (Fig. 1A). Interestingly, PC patients with SMTN2 overexpression was accompanied with cytoplasmic and nuclear expression of β-catenin. β-catenin showed membrane expression in normal pancreas (#3) and some cases of PC samples (#7), while most PC patients showed β-catenin cytoplasmic and nuclear expression (#25) (Fig. 1B). According to previous study [20], β-catenin membrane and negative expression was regarded as normal expression, whereas β-catenin cytoplasmic and nuclear expression was identified as abnormal expression. PC samples with STMN2 overexpression was associated with β-catenin abnormal expression (#7) in most serial sample slices (Fig. 1B), and vice versa (#25) (Fig. 1C) (Table 1).

Table 1
A positive relationship between STMN2 high and β-catenin abnormal expression in clinical samples

Parameters		STMN2		*r*	*P*
		Low	High		
β-catenin	Normal	20	11	0.298	0.007
	Abnormal	17	33		

STMN2 overexpression was associated with tumor size (P = 0.015), T stage (P = 0.008), lymph node metastasis (P = 0.017) and the poor survival (P = 0.004) of PC patients, but had no relationship with the other clinical characters (Table 2) (Fig. 1D). In multivariate model, STMN2 was an independent unfavorable prognostic indicator (P = 0.046) (Table 3). Interestingly, though β-catenin expression had no association with the prognosis (P = 0.138), patients with both high STMN2 and abnormal β-catenin expression showed much worse postoperative survival (P = 0.002) (Fig. 1E and F). Combination of STMN2 and β-catenin contributed to the advance clinical stage of PC patients.

Table 2. Relationship between clinicopathological features and STMN2

expression in clinical PC samples

Parameters	No. of patients	STMN2		*P*
		Low	High	
Cases	81	37	44	
Age(years)				
≤60	50	20	30	0.193
>60	31	17	14	
Gender				
Male	52	24	28	0.909
Female	29	13	16	
Tumor size(cm)				
<3	28	18	10	0.015
≥3	53	19	34	
Tumor location				
Head	58	27	31	0.802
Body-tail	23	10	13	
Differentiation				
Well	39	21	18	0.155
Moderate to Poor	42	16	26	
T stage[a]				
T1+T2	51	29	22	0.008
T3	30	8	22	
Lymph node metastasis[a]				
N0(negative)	60	33	27	0.017
N1(1-3)	14	3	11	
N2(≥4)	7	1	6	
UICC stage[a]				
I stage	24	13	11	0.176
II stage	50	23	27	

III stage	7	1	6	
Vascular permeation				
Absent	48	25	23	0.163
Present	33	12	21	
Pre-therapeutic CA19-9 level				
<37 U/ml	23	13	10	0.217
≥37 U/ml	58	24	34	

1. According to the 8th edition of AJCC staging system

Table 3
Univariate and Multivariate analysis in survival time

Parameters	**Median survival (days)**	**Univariate analysis *P* (log rank)**	**Multivariate analysis hazard ratio (95% CI)**	***P***
Lymph nodes metastasis[a] (N0/N1/N2)	981/632/601	0.001	1.685(0.818–3.471)	0.157
Vascular permeation (absent/present)	988/679	0.004	2.058(0.950–4.461)	0.067
UICC stage[a]	1066/786/632	0.002	2.029(1.05–3.924)	0.035
I/II/III				
STMN2 (high/low)	703/1036	0.004	2.009(1.012–3.988)	0.046
a. According to the 8th edition of AJCC staging system.				

In relative to high STMN2 protein expression in PC tissues, its mRNA level was also much higher in PC specimens in contrast with paired adjacent pancreas by qRT-PCR ($P < 0.01$) (Fig. 2A). In 4 PC cell lines, both STMN2 and β-catenin protein and mRNA were significantly higher in BxPC-3 and SW1990 cells than that the other 2 cells (Fig. 2B and C). It is well known that Nuclear β-catenin is a key inducer of EMT [21]. The tight relationship between STMN2 and β-catenin in human PC tissues and cell lines drive us focus on the potential function of STMN2 in regulating EMT in vitro and vivo.

Based on above results, PANC-1 and Capan-2 cells with low STMN2 expression was construct for STMN2 overexpressing stable cell lines, while BxPC-3 and SW1990 cells were used for STMN2 silencing experiment. WB showed that STMN2 protein level was significantly decreased in si1-STMN2 and si2-STMN2 transfected BxPC-3 and SW1990 cells, respectively (Fig. 2D). Conversely, STMN2 was overexpressed in STMN2-GFP transfected PANC-1 and Capan-2 cells in comparison to GFP groups (Fig. 2D).

STMN2 promoted EMT in vitro mediated by WNT/β-catenin signaling

STMN2 overexpression promoted EMT-like cellular morphology in PANC-1 cells: most cells (75–80%) exhibited a spindle-shaped/fibroblast-like morphology (Fig. 3A). However, XAV939, as a specific WNT/β-catenin signaling inhibitor, reversed STMN2 overexpression-stimulated EMT-like cellular morphology in vitro. Only 25–35% of spindle-shaped/fibroblast-like cellular morphology was observed in STMN2-GFP plus XAV939 group in contrast with STMN2-GFP group (Fig. 3A). The similar experiment was also repeated in STMN2 overexpressing Capan-2 cells (Fig. 3B).

A hallmark of EMT is its remarkable stimulation of cancer invasion [22]. In present study, cell invasion and migration were obviously enhanced in STMN2-GFP group in contrast with GFP group in PANC-1 (Fig. 4A and B) and Capan-2 cells (Fig. 4C and D). However, XAV939 significantly inhibited STMN2 overexpression-enhanced cell motility in vitro (Fig. 4A-D). Conversely, cell invasion and migration were significantly decreased in si2-STMN2 group in contrast with GFP group in PANC-1 and Capan-2 cells (Fig. 4A-D). However, KY19382 (a specific WNT/β-catenin signaling activator) significantly reversed STMN2 silencing- decreased cell motility in BxPC-3 (Fig. 4E and F) and SW1990 (Fig. 4G and H) cells. Taken together, STMN2 promoted cell motility mediated by WNT/β-catenin signaling.

STMN2 promoted cell proliferation in vitro mediated by WNT/β-catenin signaling

We next investigated the potential role of STMN2 in cell proliferation in vitro. MTT showed that STMN2 overexpression promoted cell proliferation in PANC-1 cells in time-dependent manner, especially in 4 to 5 cultured days (Fig. 5A). However, XAV939 reversed STMN2 overexpression-promoted cell proliferation in vitro in corresponding culturing time (Fig. 5A). The similar experiment was also repeated in STMN2 overexpressing Capan-2 cells (Fig. 5B). Conversely, STMN2 silencing inhibited cell proliferation in BxPC-3 cells in the same cultured time, which was reversed by KY19382 (Fig. 5C). The similar experiment was also repeated in STMN2 silencing Capan-2 cells (Fig. 5D). Taken together, STMN2 promoted cell proliferation in PC vitro mediated by WNT/β-catenin signaling.

STMN2 regulating EMT and Cyclin D1 signaling mediated by WNT/β-catenin signaling

We next investigated the potential mechanism of STMN2 in regulating EMT and cell proliferation in vitro. WB showed that STMN2 overexpression upregulated Vimentin, Snail1 and Cyclin D1, but downregulated E-cad expression in PANC-1 (Fig. 6A) and Capan-2 (Fig. 6B) cells. β-catenin and N-cadherin expression was unchanged. XAV939 not only specially inhibited β-catenin and STMN2 expression, but also reversed STMN2 overexpression-induced the change of EMT and Cyclin D1 expression (Fig. 6A and B). Conversely, STMN2 silencing downregulated Vimentin, Snail1 and Cyclin D1, but upregulated E-cad expression in BxPC-3 (Fig. 6C) and SW1990 cells (Fig. 6D). KY19382 not only specially activated β-catenin and STMN2 expression, but also reversed STMN2 silencing-inhibited the change of EMT and Cyclin D1 expression (Fig. 6C and D). Meanwhile, upon KY19382, activated β-catenin and STMN2 showed co-localization in the cytoplasm and nuclear in BxPC-3 cells by IF (Fig. 7A). To observe whether β-catenin directly interacts with STMN2 promoter, ChIP assays were conducted using an antibody against β-catenin in BxPC-3 cells pretreated with KY19382. The result of PCR on immunoprecipitated DNA using the primer pairs representing each of the three potential TCF binding sites (F1-R1, F2-R2 and F3-R3) in STMN2 promoter (Fig. 7B). Upon immunoprecipitation with anti-β-catenin, the DNA fragment containing the F1-R1 TCF site was amplified at a significantly higher level from the chromatin of KY19382 activated BxPC-3 cells. However, the other two primer pairs (F2-R2 and F3-R3) did not show any increased amplification upon β-catenin activation (Fig. 7C). Above results supported that the TCF binding site at -1816 to -1822 is crucial for the regulation of STMN2 expression by β-catenin/TCF.

STMN2 promoted subcutaneous tumor size in vitro

The subcutaneous tumor size in STMN2-GFP transfected Capan-2 cells was significantly increased in contrast with GFP group ($P < 0.05$) (Fig. 8A, B and C). IHC further showed that STMN2, Vimentin and Cyclin D1 expression were obviously upregulated in STMN2-GFP group in contrast with the scramble GFP group (Fig. 8D, E and F). β-catenin showed abnormal (cytoplasm and nuclear) and normal (membrane) expression in STMN2-GFP and GFP group, respectively (Fig. 8E and F). Taken together, a tight relationship of STMN2 with EMT and Cyclin D1 signaling were prevalently existed in clinical PC samples, in vitro and vivo.

Discussion

Previous studies mainly focused on the function of STMN1 in several cancers, including hepatocellular, gastric, colon, pancreatic and lung cancer [23–27]. However, STMN2, as a novel discovered oncogene, is

poorly understood in cancer, especially in PC. In current study, we first identified STMN2 as a novel target of β-catenin/TCF-mediated transcription in PC cells. Overexpression of STMN2 contributes to the aggressive clinical stage of PC patients in coordination with WNT/β-catenin signaling. Meanwhile, STMN2 promotes cell proliferation and EMT in PC via activating WNT/β-catenin mediated EMT and Cyclin D1 signaling, which has not been studied yet.

We first found that STMN2 was overexpressed in PC patients, which was positively associated with tumor size, T stage, lymph node metastasis and the poor survival of PC patients. STMN2 was also overexpressed in hepatocellular, neuroblastoma and ovarian cancer [11–13]. STMN2 overexpression was associated with advanced clinical characters and bad prognosis in hepatocellular cancer [11]. Meanwhile, it was an independent unfavorable prognostic factor in ovarian cancer [13]. Thus, STMN2 act as a potential oncogene based on previous and current studies. Interestingly, the combination of high SMTN2 and cytoplasmic/nuclear expression of β-catenin contributed to the much worse survival of PC patients. Meanwhile, the parallel expression of STMN2 and β-catenin were observed in both PC tissue and cell lines. It is well known that WNT/β-catenin signaling pathway closely correlates with the characteristic of EMT and potency of proliferation in cancer development [15, 28], which drive us to investigate the cooperative interaction of STMN2 and WNT/β-catenin signaling in regulating EMT and cell proliferation of PC.

In current study, STMN2 overexpression promoted EMT and cell proliferation in PC cells. EMT-like cell morphology, cell mobility and proliferation were significantly enhanced in STMN2 overexpression PC cells. However, the specific inhibitor (XAV939) of WNT/β-catenin signaling reversed STMN2 overexpression- induced function in vitro. Conversely, the specific activator (KY19382) of WNT/β-catenin signaling reversed STMN2 silencing- inhibited EMT and cell proliferation. Only one study reports the function of STMN2 in the malignant behavior of cancer that STMN2 promotes cell migration, invasion and metastasis in vitro and in hepatocellular cancer by triggers EMT [11]. Taken together, STMN2, act as an oncogene, promotes the development of cancers partially by triggers EMT.

Further potential mechanism showed that STMN2 overexpression upregulated Snail1, Vimentin, Cyclin D1 and downregulated E-cad in vitro. XAV939 not only inhibited STMN2 expression, but also reversed STMN2 overexpression- induced EMT and Cyclin D1 signaling. Conversely, KY19382 reversed STMN2 silencing- induced EMT and Cyclin D1 signaling in vitro. Snail1, as a critical EMT stimulator, induced EMT by repressing E-cadherin and claudins with concomitant upregulation of Vimentin [29]. Thus, STMN2 induced EMT by regulating Snail1. It is well known that Cyclin D1 plays a critical role in regulating proliferation, involving the extracellular signaling environment to cell cycle progression [30]. High Cyclin D1 expression drives unchecked cellular proliferation promoting tumor growth [31]. Therefore, STMN2 promoted cell proliferation by activating Cyclin D1 signaling. STMN2 also mediates nuclear translocation of Smad2/3 and enhances TGFβ signaling by destabilizing microtubules to promote EMT in hepatocellular cancer [11].

Previous study showed that STMN2 was a novel target of β-catenin/TCF-mediated transcription in human hepatoma cells [16, 17]. Similarly, the oncogenic function of STMN2 in PC was mediated by WNT/β-catenin signaling in current study. Activating β-catenin and STMN2 showed co-localization in the cytoplasm and nuclear in BxPC-3 cells. ChIP assays further showed that TCF binding site at -1816 to -1822 is crucial for the regulation of STMN2 expression by β-catenin/TCF. Taken together, overexpression of STMN2 promotes cell proliferation and EMT in PC mediated by WNT/β-catenin signaling.

Finally, STMN2 overexpression promoted subcutaneous tumors formation in vivo with the overexpression of EMT and Cyclin D1 signaling, which was consistent with the results in vitro.

Conclusion

In conclusion, we first identified STMN2 as a novel target of β-catenin/TCF-mediated transcription in PC cells. Overexpression of STMN2 contributes to the advanced clinical stage of PC patients in coordination with WNT/β-catenin signaling. Meanwhile, STMN2 promotes cell proliferation and EMT in PC via activating WNT/β-catenin- mediated EMT and Cyclin D1 signaling. STMN2 could serve as a promising prognostic biomarker and potential therapeutic gene target for PC.

Abbreviations

STMN2
Stathmin 2
PC
pancreatic ductal adenocarcinoma

Declarations

Ethics approval and informed consent

The present study was approved by the Ethics Committee of the first hospital of China Medical University. The processing of clinical

tissue samples is in strict compliance with the ethical standards of the

Declaration of Helsinki. All patients signed written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available

from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest related to this study.

Funding

Funding information is not available.

AUTHOR CONTRIBUTIONS:

Conception and design: Mingrui S and Shiyang W; acquisition of data: Li W, Qi Z, and Tianlong W; analysis and interpretation of data: Mingrui S, Li W, Qi Z, and Tianlong W. writing, review, and revision of the manuscript: Mingrui S and Shiyang W.

Acknowledgements

Thanks for the technical supports from both central laboratory and general laboratory at the First Hospital of China Medical University.

References

1. Survival Rates for Pancreatic Cancer. Available online: https://www.cancer.org/cancer/pancreatic-cancer/detection-diagnosisstaging/survival-rates.html (accessed on 15 March 2021).
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2021, 70.
3. Khalaf N, El-Serag HB, Abrams HR, Thrift AP. Burden of Pancreatic Cancer: From Epidemiology to Practice. Clin Gastroenterol Hepatol. 2020;19:876–84.
4. Luu T. Epithelial-Mesenchymal Transition and Its Regulation Mechanisms in Pancreatic Cancer. Front Oncol. 2021 Apr;13:11:646399.
5. Grenningloh G, Soehrman S, Bondallaz P, Ruchti E, Cadas H. Role of the microtubule destabilizing proteins SCG10 and stathmin in neuronal growth. J Neurobiol. 2004;58:60–9.
6. Conde C, Caceres A. Microtubule assembly, organization and dynamics in axons and dendrites. Nat Rev Neurosci. 2009;10:319–32.
7. Riederer BM, Pellier V, Antonsson B, Di Paolo G, Stimpson SA, Lutjens R, Catsicas S, Grenningloh G, Regulation of microtubule dynamics by the neuronal growth-associated protein SCG10, Proc. Natl. Acad. Sci. U. S. A. 94 (1997)741–745.
8. Bieche I, Maucuer A, Laurendeau I, Lachkar S, Spano AJ, Frankfurter A, Levy P, Manceau V, Sobel A, Vidaud M, Curmi PA. Expression of stathmin family genes in human tissues: non-neural-restricted expression for SCLIP. Genomics. 2003;81:400–10.

9. Paradis V, Dargere D, Bieche Y, Asselah T, Marcellin P, Vidaud M, Bedossa P. SCG10 expression on activation of hepatic stellate cells promotes cell motility through interference with microtubules. Am J Pathol. 2010;177:1791–7.
10. Liu Z, Chatterjee TK, Fisher RA. RGS6 interacts with SCG10 and promotes neuronal differentiation. Role of the G gamma subunit-like (GGL) domain of RGS6. J Biol Chem. 2002 Oct 4;277(40):37832–9.
11. Zhong FJ, Sun B, Cao MM, Xu C, Li YM, Yang LY. STMN2 mediates nuclear translocation of Smad2/3 and enhances TGFβ signaling by destabilizing microtubules to promote epithelial-mesenchymal transition in hepatocellular carcinoma. Cancer Lett. 2021 May 28;506:128–141.
12. Rogers DA, Schor NF. Kidins220/ARMS depletion is associated with the neural-to Schwann-like transition in a human neuroblastoma cell line model. Exp Cell Res. 2013 Mar 10;319(5):660–9.
13. Prudencio M, Humphrey J, Pickles S, Brown AL, Hill SE, Kachergus JM, Shi J, et al. Truncated stathmin-2 is a marker of TDP-43 pathology in frontotemporal dementia. J Clin Invest. 2020 Nov 2;130(11):6080–6092.
14. Maleki Dana P, Sadoughi F, Mansournia MA, Mirzaei H, Asemi Z, Yousefı B. Targeting Wnt signaling pathway by polyphenols: implication for aging and age-related diseases. Biogerontology. 2021 Sep 3. doi:10.1007/s10522-021-09934-x.
15. LarueL BellacosaA. Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3' kinase/AKT pathways. Oncogene. 2005;24:7443–54.
16. LEE HS, LEE D C, PARK M H, et al. STMN2 is a novel target of betacatenin/TCF-mediated transcription in human hepatoma cells[J]. Biochem Biophys Res Commun. 2006;345(3):1059–67.
17. LEE H S, PARK M H, YANG SJ, et al. Novel candidate targets of Wnt/betacatenin signaling in hepatoma cells[J]. Life Sci. 2007;80(7):690–8.
18. Sheng W, Wang G, Tang J, Shi X, Cao R, Sun J, Lin YH, Jia C, Chen C, Zhou J, Dong M. Calreticulin promotes EMT in pancreatic cancer via mediating Ca2 + dependent acute and chronic endoplasmic reticulum stress. J Exp Clin Cancer Res. 2020 Oct 7;39(1):209.
19. Wang S, Wang T, Wang L, Zhong L, Li K. Overexpression of RNF126 Promotes the Development of Colorectal Cancer via Enhancing p53 Ubiquitination and Degradation. Onco Targets Ther. 2020 Oct;28:13:10917–29.
20. Saukkonen K, Hagström J, Mustonen H, Juuti A, Nordling S, Kallio P, Alitalo K, Seppänen H, Haglund C. PROX1 and β-catenin are prognostic markers in pancreatic ductal adenocarcinoma. BMC Cancer. 2016 Jul 13;16:472.
21. Kim K, Lu Z, Hay ED. Direct evidence for a role of beta-catenin/LEF-1 signaling pathway in induction of EMT. Cell Biol Int. 2002;26(5):463–76.
22. Huang L, Chen S, Fan H, Ji D, Chen C, Sheng W. GINS2 promotes EMT in pancreatic cancer via specifically stimulating ERK/MAPK signaling. Cancer Gene Ther. 2020 Aug 3. doi:10.1038/s41417-020-0206-7.

23. Zhang R, Gao X, Zuo J, Hu B, Yang J, Zhao J, Chen J. STMN1 upregulation mediates hepatocellular carcinoma and hepatic stellate cell crosstalk to aggravate cancer by triggering the MET pathway. Cancer Sci. 2020 Feb;111(2):406–17.
24. 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 May;20(10):2038–44.
25. Suzuki K, Watanabe A, Araki K, Yokobori T, Harimoto N, Gantumur D, Hagiwara K, Yamanaka T, Ishii N, Tsukagoshi M, Igarashi T, Kubo N, Gombodorj N, Nishiyama M, Hosouchi Y, Kuwano H, Shirabe K. High STMN1 Expression Is Associated with Tumor Differentiation and Metastasis in Clinical Patients with Pancreatic Cancer. Anticancer Res. 2018 Feb;38(2):939–44.
26. Bai T, Yokobori T, Altan B, Ide M, Mochiki E, Yanai M, Kimura A, Kogure N, Yanoma T, Suzuki M, Bao P, Kaira K, Asao T, Katayama A, Handa T, Gombodorj N, Nishiyama M, Oyama T, Ogata K, Kuwano H. High STMN1 level is associated with chemo-resistance and poor prognosis in gastric cancer patients. Br J Cancer. 2017 Apr;25(9):1177–85. 116(.
27. Bao P, Yokobori T, Altan B, Iijima M, Azuma Y, Onozato R, Yajima T, Watanabe A, Mogi A, Shimizu K, Nagashima T, Ohtaki Y, Obayashi K, Nakazawa S, Bai T, Kawabata-Iwakawa R, Asao T, Kaira K, Nishiyama M, Kuwano H. High STMN1 Expression is Associated with Cancer Progression and Chemo-Resistance in Lung Squamous Cell Carcinoma. Ann Surg Oncol. 2017 Dec;24(13):4017–24.
28. Jiang YG, Luo Y, He DL, Li X, Zhang LL, Peng T, Li MC, Lin YH. Role of Wnt/beta-catenin signaling pathway in epithelial-mesenchymal transition of human prostate cancer induced by hypoxia-inducible factor-1alpha. Int J Urol. 2007 Nov;14(11):1034–9.
29. Kaufhold S, Bonavida B. Central role of Snail1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. J Exp Clin Cancer Res. 2014 Aug 2;33(1):62.
30. Stacey DW. Cyclin D1 serves as a cell cycle regulatory switch in actively proliferating cells. Curr Opin Cell Biol. 2003 Apr;15(2):158–63.
31. Montalto FI, De Amicis F. Cyclin D1 in Cancer: A Molecular Connection for Cell Cycle Control, Adhesion and Invasion in Tumor and Stroma. Cells. 2020 Dec;9(12):2648. 9(.

Figures

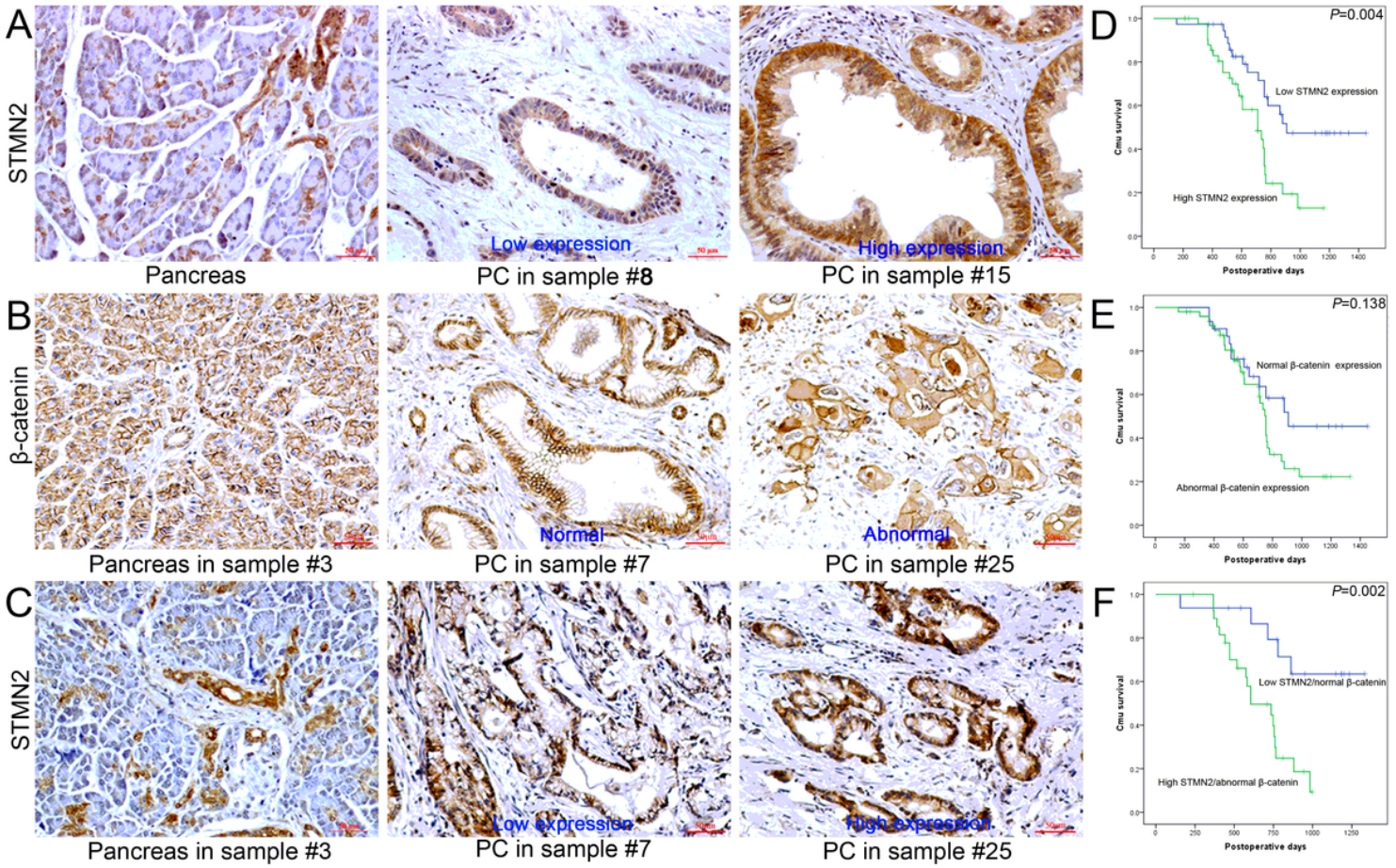

Figure 1

The expression of STMN2 and β-catenin in human PC and adjacent pancreas with the prognosis of PC patients. **A.** STMN2 expression in paired pancreas and PC specimens (#8 and #15). **B.** β-catenin expression in paired pancreas (#3) and PC specimens (#7 and #25). **C.** STMN2 expression in paired pancreas (#3) and PC specimens (#7 and #25). **D.** High (+) and low (-) expression of GINS2 against prognosis. **E.** Normal and abnormal expression of β-catenin against prognosis. **F.** Combination of STMN2 and β-catenin against prognosis.

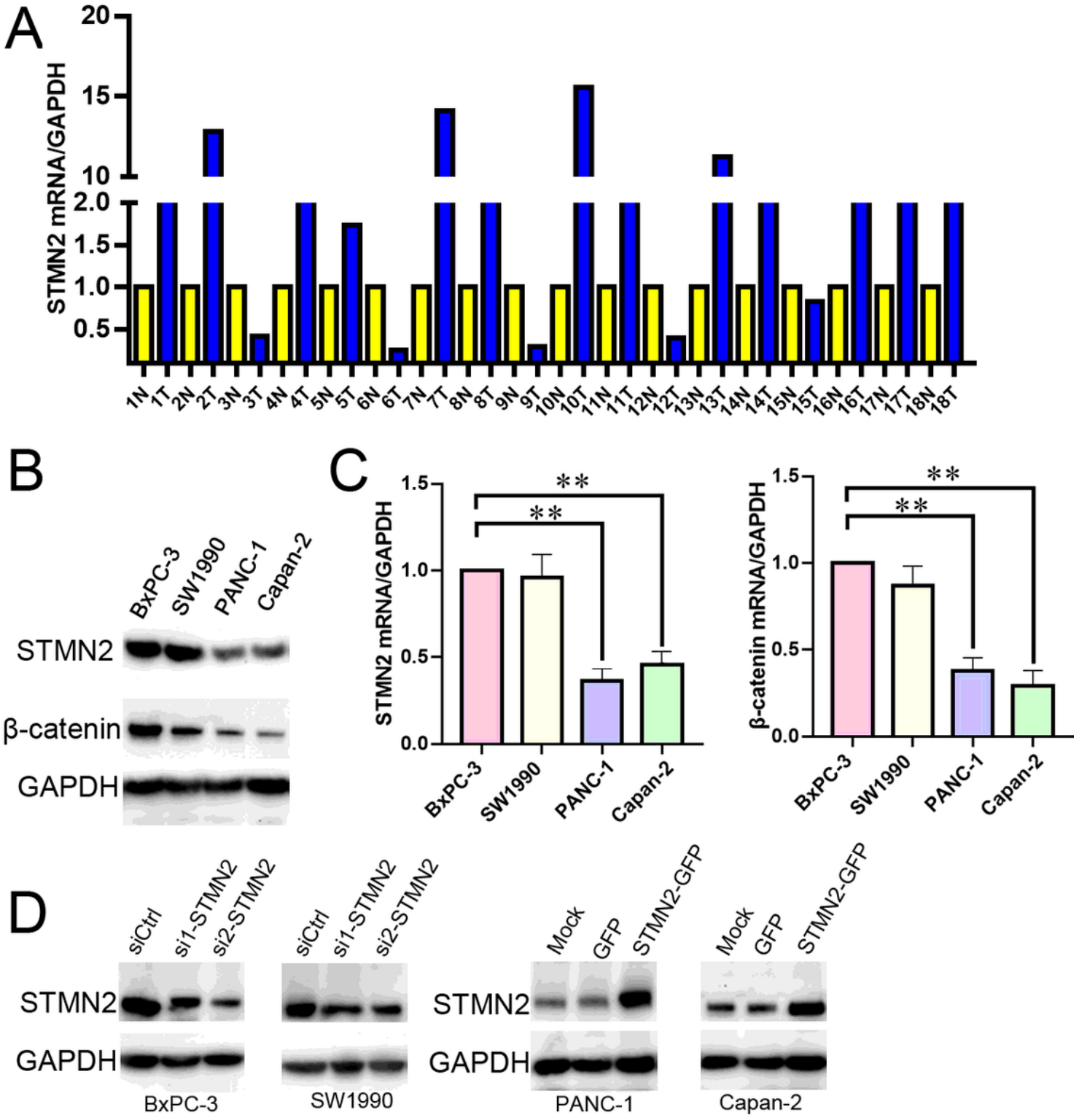

Figure 2

The expression of STMN2 in PC specimens and cell lines and the silencing and overexpressing effect of STMN2 in vitro.

A. STMN2 mRNA level in 18 PC and paired pancreas (T: PC; N: paired pancreas). **B and C.** STMN2 and β-catenin protein (B) and mRNA (C) levels in PC cell lines. **D.** the silencing (siCtrl vs si1-STMN2/si2-STMN2)

and overexpressing (Mock/GFP vs STMN2-GFP) effect of STMN2 in vitro by WB. Bars indicate ± S.E.*, *P* <0.05; **, *P* <0.01 compared with the control.

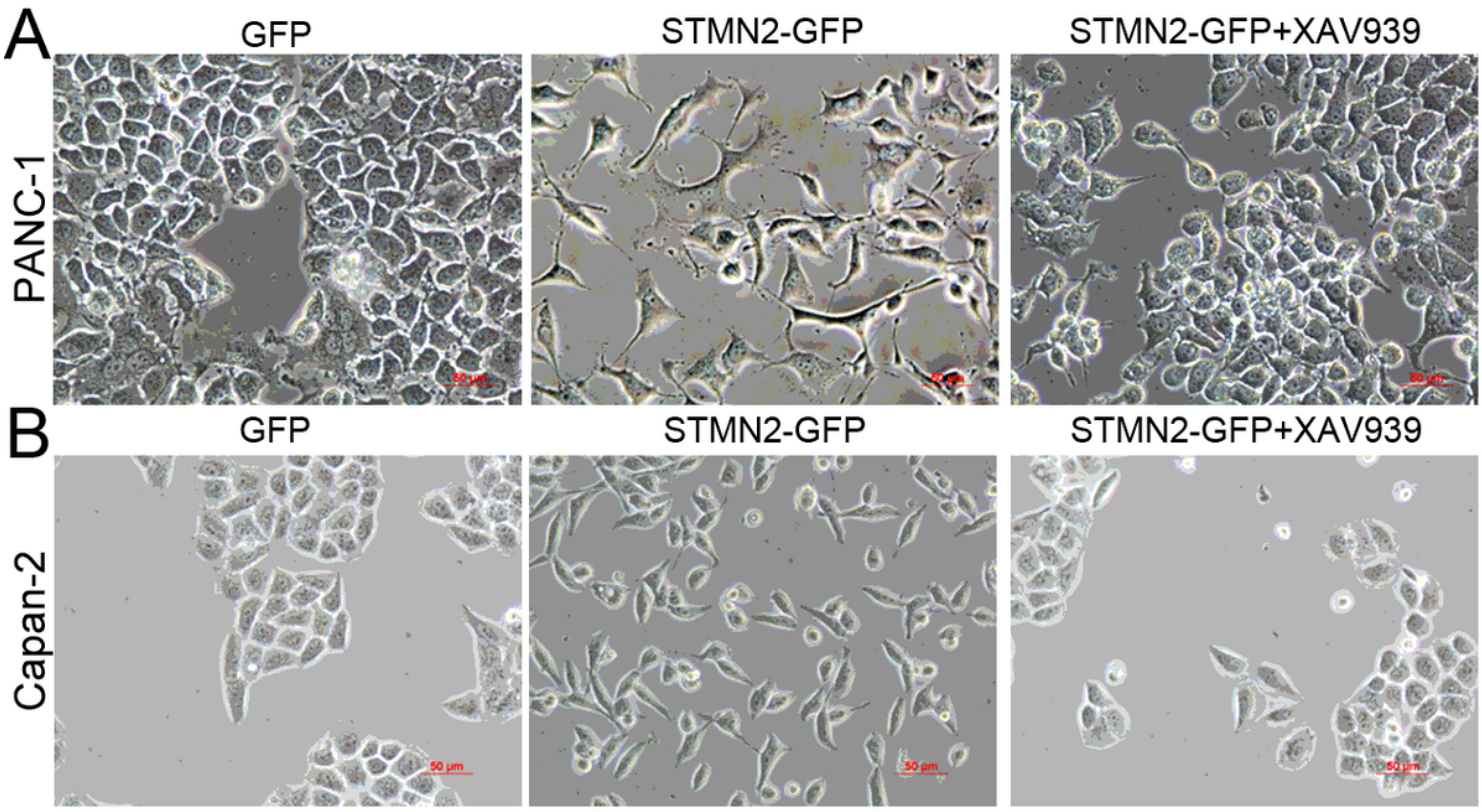

Figure 3

Cellular morphology (x100 magnification) in vitro. **A.** Cellular morphology in GFP, STMN2-GFP and STMN2-GFP plus XAV939 groups in PANC-1 cells. **B.** Cellular morphology in GFP, STMN2-GFP and STMN2-GFP plus XAV939 groups in Capan-2 cells.

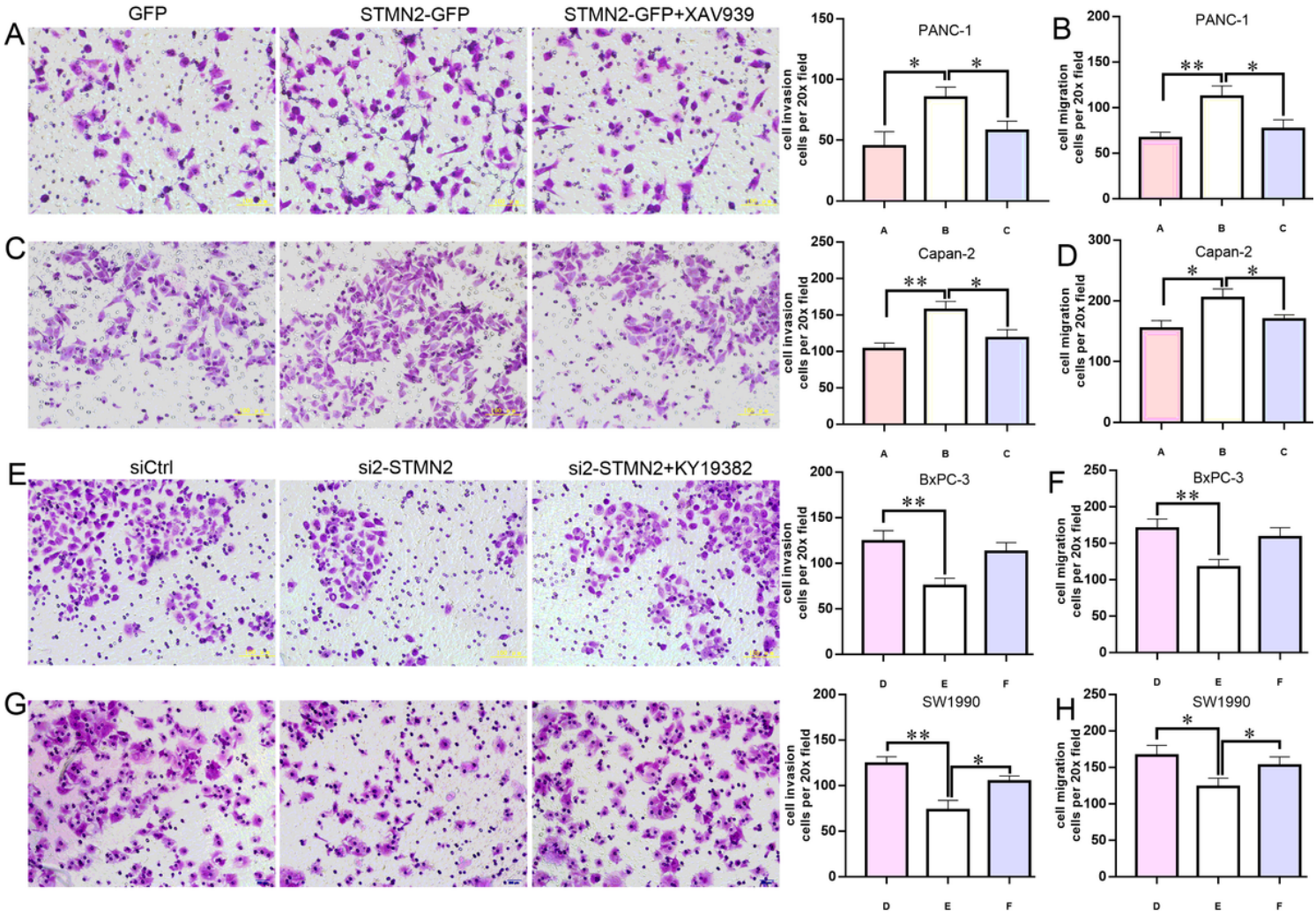

Figure 4

STMN2 promoted mobility in vitro mediated by WNT/β-catenin signaling. **A-D.** Cell invasion and migration in GFP, STMN2-GFP and STMN2-GFP plus XAV939 groups in PANC-1 (A and B) and Capan-2 cells (C and D). **E-H.** Cell invasion and migration in siCtrl, si2-STMN2, and si2-STMN2 plus KV19382 groups in BxPC-3 (E and F) and SW1990 (G and H) cells. A. GFP group; B. STMN2-GFP group; C. STMN2-GFP plus XAV939 group. D. siCtrl group; E. si2-STMN2 group; F. si2-STMN2 plus KV19382 group. Bars indicate ± S.E.*, P <0.05; **, P <0.01 in contrast with the control.

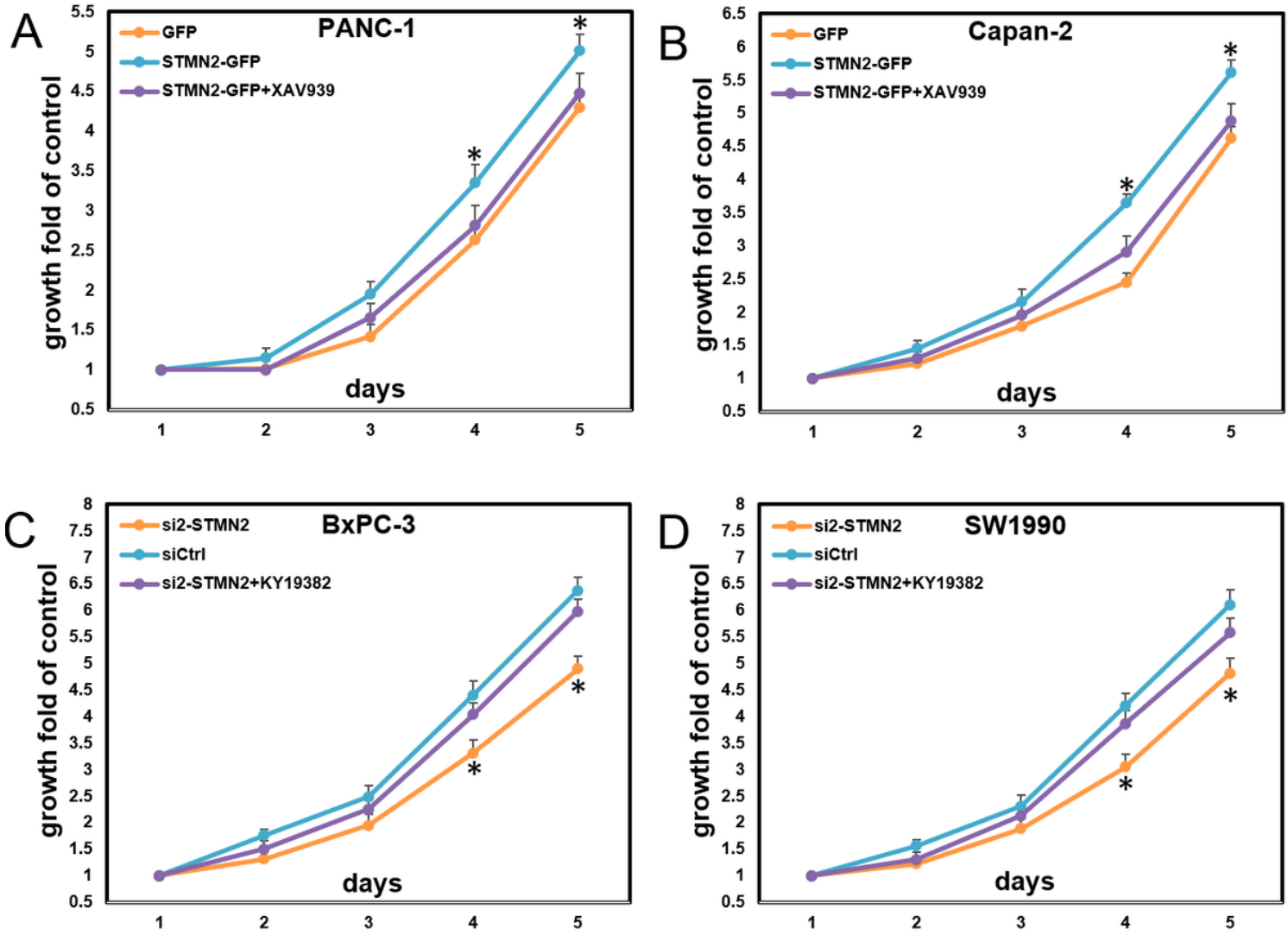

Figure 5

STMN2 promoted cell proliferation in vitro mediated by WNT/β-catenin signaling. **A and B**. MTT assays in GFP, STMN2-GFP and STMN2-GFP plus XAV939 groups of PANC-1 (A) and Capan-2 cells (B) culturing within 5 days. **C and D.** MTT assays in siCtrl, si2-STMN2, and si2-STMN2 plus KV19382 groups of BxPC-3 (C) and SW1990 (D) cells culturing within 5 days. Bars indicate ± S.E.*, P <0.05; **, P <0.01 compared with the control.

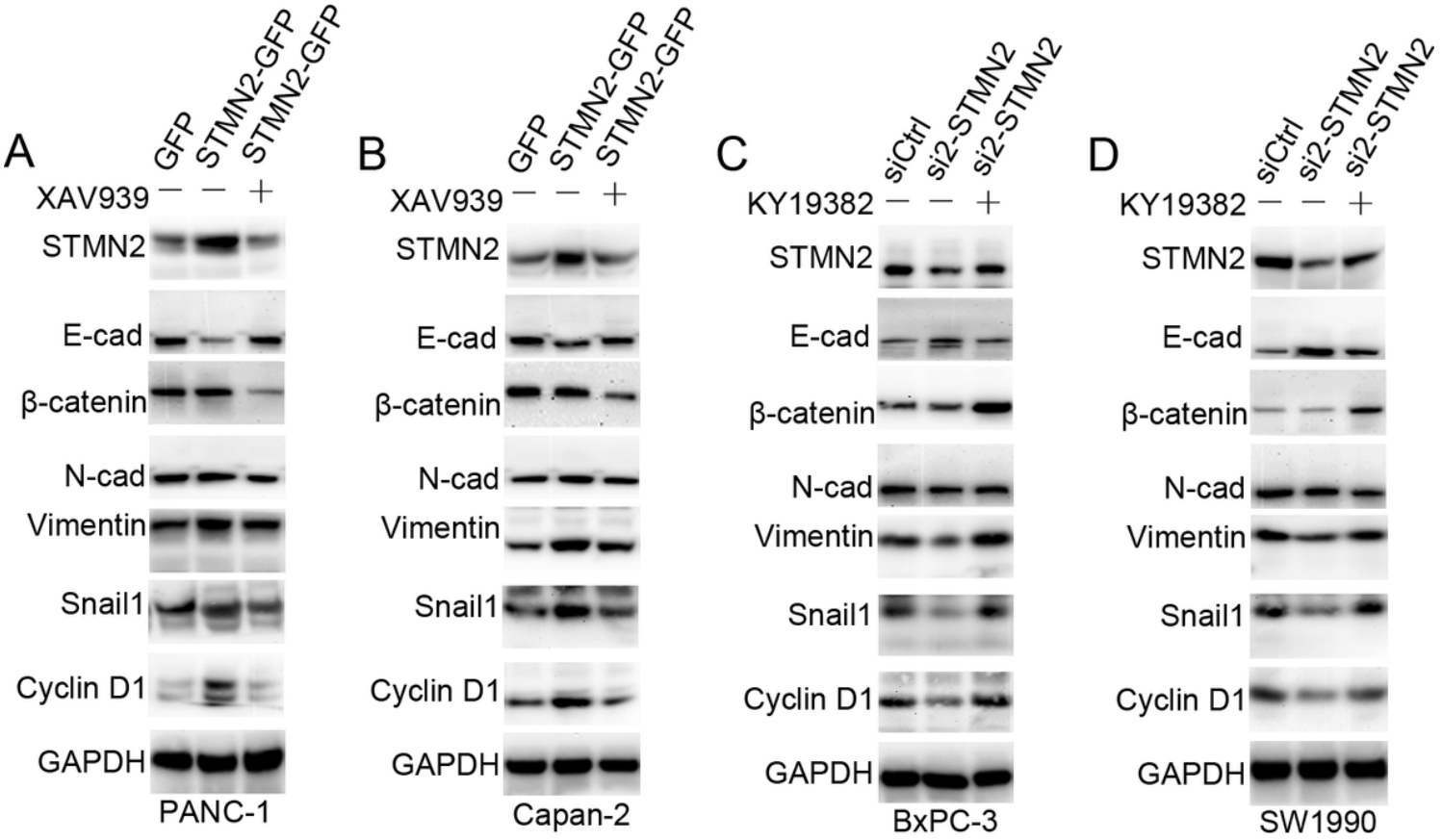

Figure 6

STMN2 promoted EMT and Cyclin D1 signaling mediated by WNT/β-catenin signaling. **A and B.** The protein level of STMN2, E-cad, β-catenin, N-cad, Vimentin, Snail1 and Cyclin D1 in GFP, STMN2-GFP and STMN2-GFP plus XAV939 groups of PANC-1 (A) and Capan-2 cells (B). **C and D.** The protein level of STMN2, E-cad, β-catenin, N-cad, Vimentin, Snail1 and Cyclin D1 in siCtrl, si2-STMN2, and si2-STMN2 plus KV19382 groups of BxPC-3 (C) and SW1990 (D) cells. Bars indicate ± S.E.*, $P<0.05$; **, $P<0.01$ in contrast with the control.

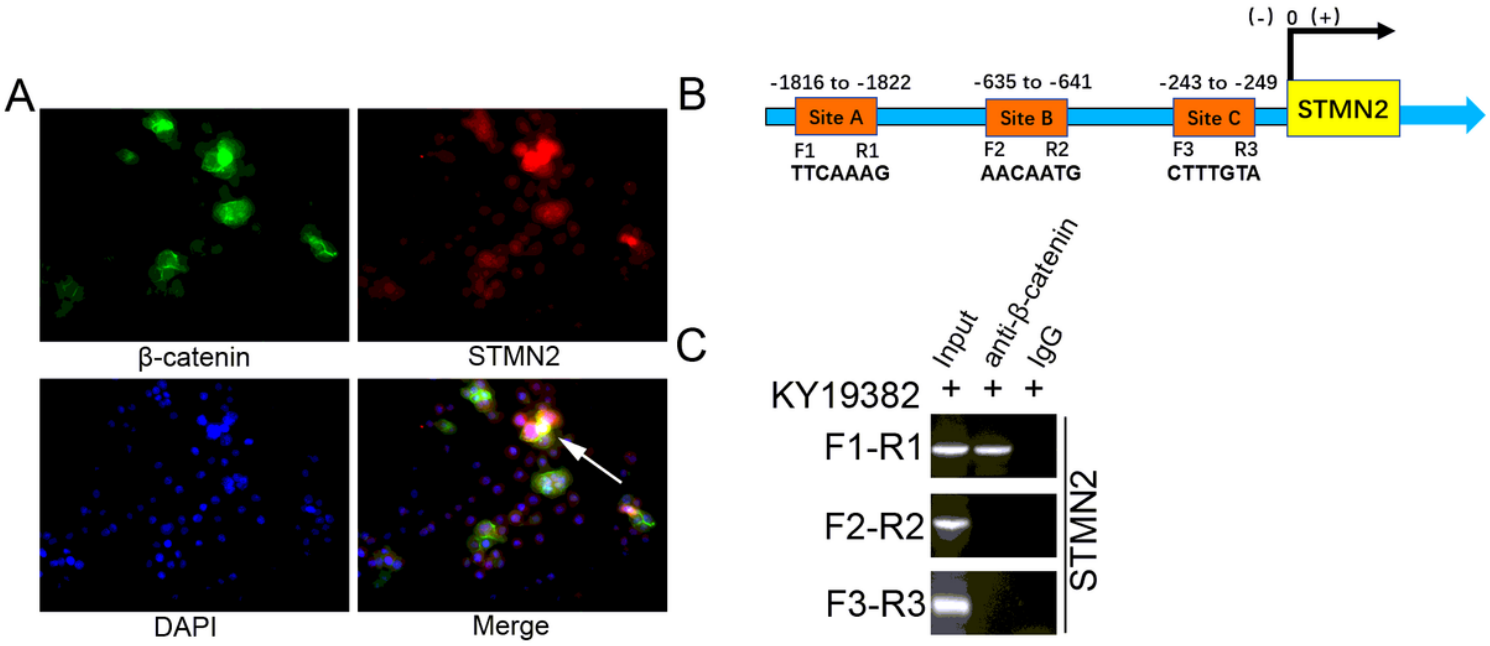

Figure 7

IF and Chip assays. **A.** IF staining of KV19382 activated STMN2 combing β-catenin in BxPC-3 cells. **B.** The potential three potential TCF binding sites of STMN2 promoter. **C.** ChIP assays in BxPC-3 cells.

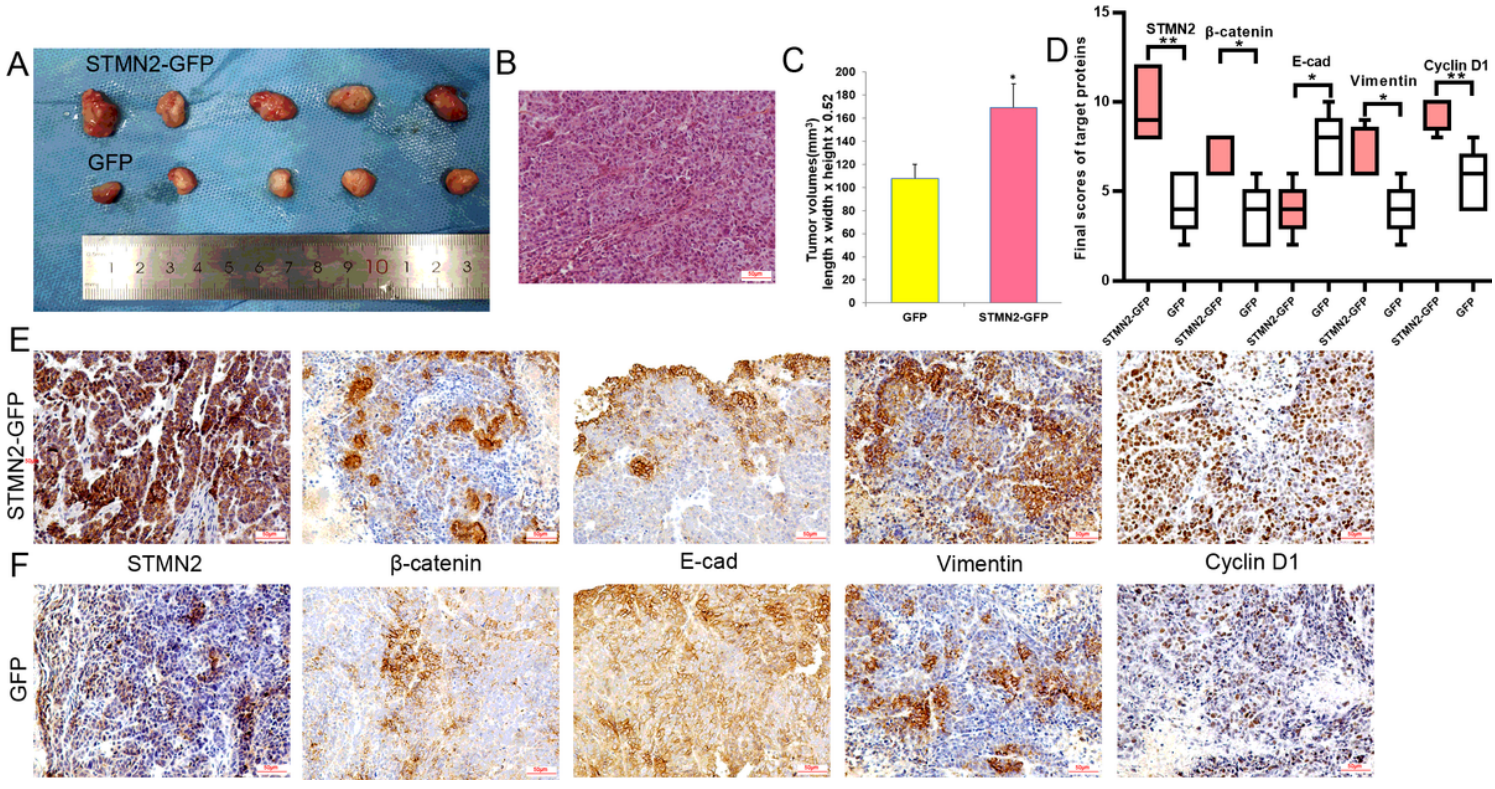

Figure 8

STMN2 promoted subcutaneous tumor size in vivo. **A, B and C.** The representative images (A), HE staining (B) and statistical comparison (C) of tumor volumes between STMN2-GFP and GFP groups in

nude mice. **D, E and F** The statistical comparison (D) and representative IHC images (E and F) of STMN2, β-catenin, E-cad, Vimentin and Cyclin D1 expression in subcutaneous tumor between STMN2-GFP and GFP groups. Bars indicate ± S.E.*, P <0.05; **, P <0.01 in contrast with the control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Checklist.pdf