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SUFU reduced pancreatic cancer cell growth by Wnt/β-catenin signaling pathway

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Abstract

The aim of the present study was to investigate the function and mechanism of SUFU in treatment of pancreatic cancer. Patient with pancreatic cancer (n = 50) and normal volunteers (n = 30) were obtained from The Second Affiliated Hospital of Soochow University between May 2014 and April 2013. SUFU mRNA and protein expressions in patients with pancreatic cancer were reduced as compared to normal saline. OS and DFS of high expression of SUFU in pancreatic cancer were higher than those of low expression of SUFU in pancreatic cancer. The inhibition of SUFU promoted pancreatic cancer cell growth and migration; SUFU reduced pancreatic cancer cell growth and migration. SUFU suppressed Wnt/β-catenin signaling pathway in pancreatic cancer cell lines. SUFU reduced pancreatic cancer cell growth by Wnt/β-catenin signaling pathway, providing a novel possibility for an understanding of pancreatic cancer pathogenesis.

Keywords: SUFU; β-catenin; pancreatic cancer.

Practical Application: SUFU provide new possibilities for the study of the treatment and pathogenesis of pancreatic cancer.

1 Introduction

Pancreatic cancer is a highly malignant tumor of the digestive system, generally accompanied by acute complications and prone to metastasize (Gao et al., 2021). The mortality and morbidity of pancreatic cancer are almost equal (Ishida et al., 2020; El Azab & Mostafa, 2021). The incidence of pancreatic cancer has been rising in recent years. Studies have shown that less than 20% of patients are suitable for surgery after diagnosis (Lin et al., 2020). In addition, the poor efficacy of adjuvant treatment also causes a low survival rate of patients, with the 5-year survival rate less than 5% and 1-year survival rate less than 10% (Ansari et al., 2016). The median survival of patients with localized tumor and without metastasis is 6 to 10 months, which drops to 3-6 months in metastatic patients (Chu et al., 2017). At present, patients who can be treated surgically often have postoperative metastasis and recurrence, with a relatively high risk of postoperative death. The efficacy of radiochemotherapy is not ideal, causing potent toxic side effects (Chu et al., 2017). The efficacy of radiochemotherapy is not ideal, causing potent toxic side effects (Chu et al., 2017; Balthazar et al., 2021). The efficacy of immunotherapy and targeted therapy is not yet clear, which is difficult to prolong survival (Chu et al., 2017).

The Wnt/β-catenin signaling pathway plays an important role in embryonic development, while the abnormal activation of Wnt/β-catenin signaling pathway in normal adults can cause tumors (Javadinia et al., 2018). Wnt/β-catenin signaling pathway has been confirmed to be closely associated with the pathogenesis and development of pancreatic cancer (Liu et al., 2019). Activation of the Wnt/β-catenin signaling pathway can promote the proliferation, invasion and metastasis of pancreatic cancer tumor cells and inhibit apoptosis (Makena et al., 2019; Zhang et al., 2017).

Sonic Hedgehog (SHH) signaling pathway plays a vital role in embryonic development and maintaining homeostasis in adult tissue (Anger et al., 2019). As a negative regulator of the SHH signaling pathway, the tumor suppressor, suppressor of Fused (SUFU) plays an indispensable role in the process of SHH signal transduction (Gormley et al., 2015). In SUFU-deficient mice, the SHH signaling pathway was continuously activated and mice died 9.5 days after embryonic stage with an open neural tube. In addition, heterozygous SUFU knockout mice along with Tp53 deletion can spontaneously form medulloblastoma and rhabdomyosarcoma (Guerrini-Rousseau et al., 2018; Lightner et al., 2019). The aim of the present study was to investigate the function and mechanism of SUFU in treatment of pancreatic cancer.

2 Materials and methods

2.1 Clinical samples

Patient with pancreatic cancer (n = 50) and normal volunteers (n = 30) were obtained fromThe Second Affiliated Hospital of Soochow University between May 2014 and April 2013. Informed

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consent was obtained from each patient recruited, and the study protocol was approved by the institutional Ethics Committee of The Second Affiliated Hospital of Soochow University.

2.2 Quantitative Reverse-Transcriptase PCR (qRT-PCR)

Total RNA was extracted using Trizol Reagent (Life Sciences) and RNA was transcribed using the RevertAidTM First Stand cDNASynthesis kit (Life Sciences). PCR conditions were set as follows: 30 s at 95 °C for denaturation, 30 s at 55 °C for annealing, 30 s at 72 °C for extension. 40 cycles of amplifications were performed for each gene. Quantities of mRNA were normalized to mRNA quantities of β-actin.

2.3 Cell lines, transfection of miRNAs and siRNAs

Human pancreatic cancer cells (HPAC, CFPAC-1, Bxpc-3 and AsPC-1 cells) and human pancreatic normal cell line (HPDE6c7 cell) were provided by Cell Bank (Shanghai, China) of and cultured in Dulbecco's Modifed Eagle's Medium (DMEM; Gibco,) containing 10% fetal bovine serum (FBS, Gibco) at 37 °C and 5% CO2. Cell was transfected with SUFU (60 nM), siSUFU (60 nM), β-catenin (60 nM), siβ-catenin (60 nM) and negative mimics (60 nM) using LipofectamineTM 2000 (Invitrogen, Carlsbad, CA, USA). After 48 h of transfection, cell was incubated with 10−6 M angiotensin II (Sigma, Shanghai, China).

2.4 Cell migration assay

5×10^4 tumor cells were inoculated on the upper chamber with 8 μm pores, cultured with serum-free medium. 20% FBS growth medium (800 μL) was added to the lower chambers. Following 48 h incubation at 37 °C, cells that migrated to the pores were fixed with 4% paraformaldehyde for 20 min at room temperature. Cell was then stained with hematoxylin for 10 min.

2.5 Cell proliferation assay

MTT assay was performed to assess cell proliferation ability. Cells were transfected with SUFU inhibitors and subsequently cultured for 48 h. MTT assay was added into cell for 4 h at 37 °C and DMSO was added into cell for 20 min at 37 °C. The transfected cells were measured at 450 nm with a Microplate Reader (Molecular Devices, USA).

2.6 Western blotting

The total protein was extracted using RIPA assay. 50 μg of protein samples were loaded separated on 10% SDS-PAGE and transferred to nitrocellulose membranes (Millipore, USA). Membranes was blocked with 5% BSA for 60 min and incubated with primary antibodies against SUFU, β-catenin or β-actin overnight at 4°C. After washing in the TBS containing 0.1% Tween for 15 min, membranes were with goat anti-rabbit or goat anti-mouse secondary antibodies for 1 h at 37 °C. Membranes were visualized with enhanced chemiluminescence (ECL) method by Tanon-5200 Chemiluminescence Imager (Tanon, Shanghai, China).

2.7 Statistical analysis

A p value < 0.05 was considered significant. Data are presented as mean ± SD from three independent experiments. All statistical analyses were carried out using SPSS 21.0 software. Significance in two-condition experiments was evaluated by student's t-test or one-way analysis of variance (ANOVA) test

3 Results

3.1 SUFU expression in patients with pancreatic cancer or cell lines

To explain the expression level and biological effects of SUFU in pancreatic cancer, so we utilized qRT-PCR and microarray analysis to test the expression level of SUFU in pancreatic cancer. Significantly down-regulation of SUFU expressions was observed as compared to normal group (Figure 1A, B). SUFU protein expressions in patients with pancreatic cancer were reduced as compared to normal saline (Figure 1C, D). SUFU mRNA expressions in pancreatic cancer cell lines (HPAC, CFPAC-1, Bxpc-3 and AsPC-1 cell) were lower than of HPDE6c7 cell (Figure 1E). OS and DFS of high expression of SUFU in pancreatic cancer were higher than those of low expression of SUFU in pancreatic cancer (Figure 1F, G).

3.2 SUFU reduced pancreatic cancer cell growth and migration

To better understand the biological function of SUFU in pancreatic cancer cell lines, cell were transfected into CFPAC-1 with SUFU plasmids. SUFU plasmid increased the expression of SUFU mRNA, reduced cell migration, cell growth and Edu positive cells, and increased caspase-3/9 activity and LDH activity levels in vitro model (Figure 2).

The inhibition of SUFU promoted pancreatic cancer cell growth and migration

Next, we were transfected into AsPC-1 cells with siSUFU mimics. siSUFU mimics. As shown in Figure 3, siSUFU mimics reduced SUFU expression, promoted cell migration, cell growth and Edu positive cells, and inhibited caspase-3/9 activity and LDH activity levels in vitro model (Figure 3).

SUFU suppressed Wnt/β-catenin signaling pathway in pancreatic cancer cell lines

To explain that the mechanism of SUFU in pancreatic cancer, so we utilized microarray analysis to test the gene expression by SUFU in pancreatic cancer. Over-expression of SUFU in vitro model reduced β-catenin signaling pathway and β-catenin expression (Figure 4A, B). Over-expression of SUFU induced SUFU protein expression and β-catenin nucleus protein expression, and suppressed β-catenin cytoplasm protein expression in vitro model (Figure 4C, F). Down-regulation

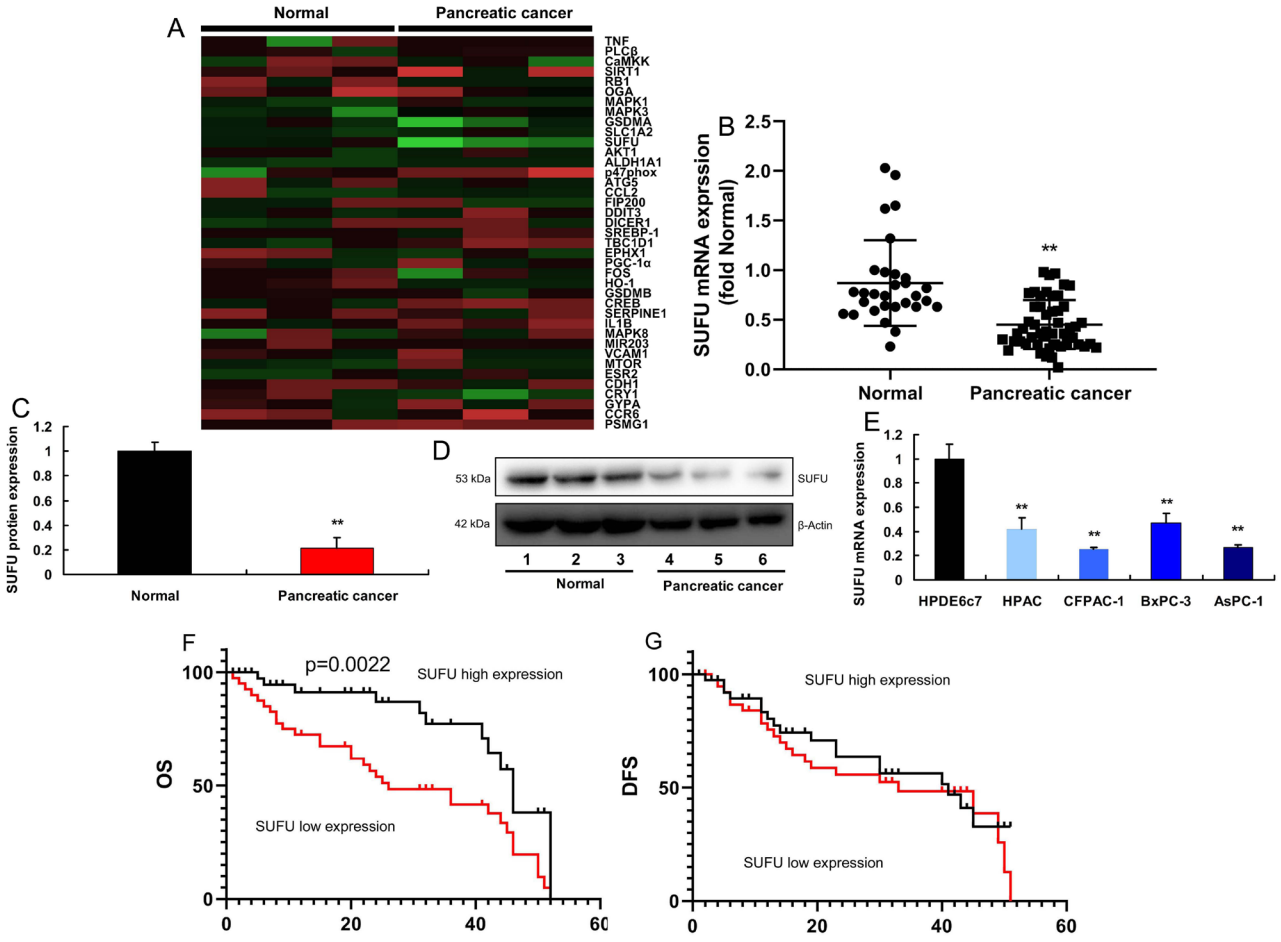

Figure 1. SUFU expression in patients with pancreatic cancer or cell lines. Heat map (A), SUFU mRNA expression (B) and protein expression (C, D) in patients with pancreatic cancer, SUFU mRNA expression in cell lines (E), OS and DFS of patients with pancreatic cancer (F, G). Normal, normal volunteers group; pancreatic cancer, pancreatic cancer group. ##p < 0.01 compared with normal volunteers group.

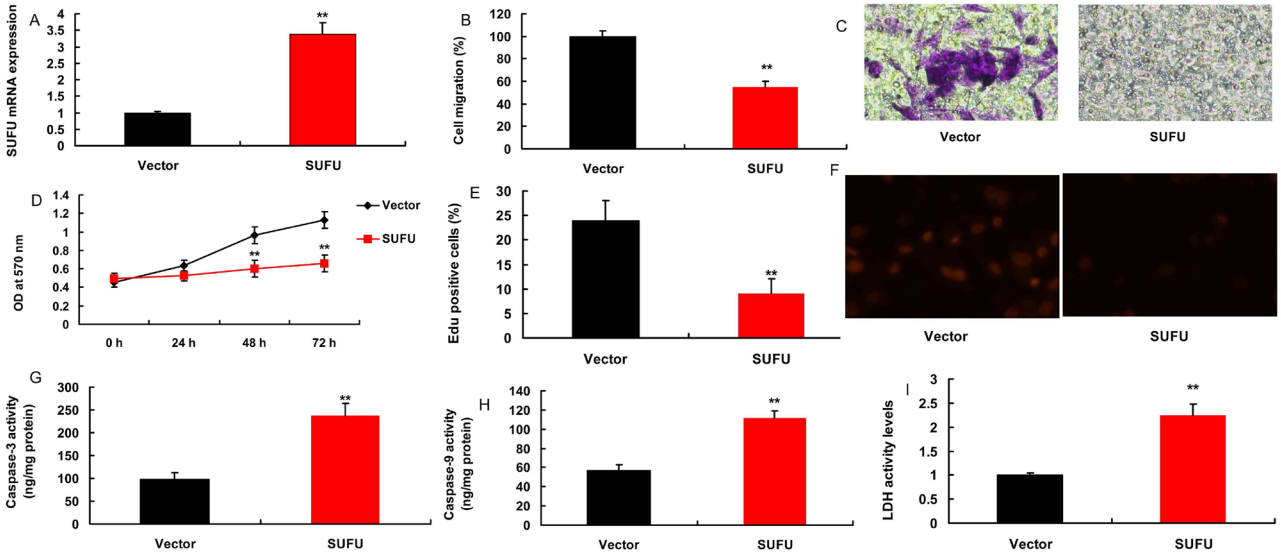

Figure 2. SUFU reduced pancreatic cancer cell growth. SUFU mRNA expression (A), cell migration (B, C), cell growth (D), Edu positive cells (E, F), caspase-3/9 activity and LDH activity levels (G-I). Negative, negative mimic group; SUFU, over-expression of SUFU group. ##p < 0.01 compared with negative mimic group.

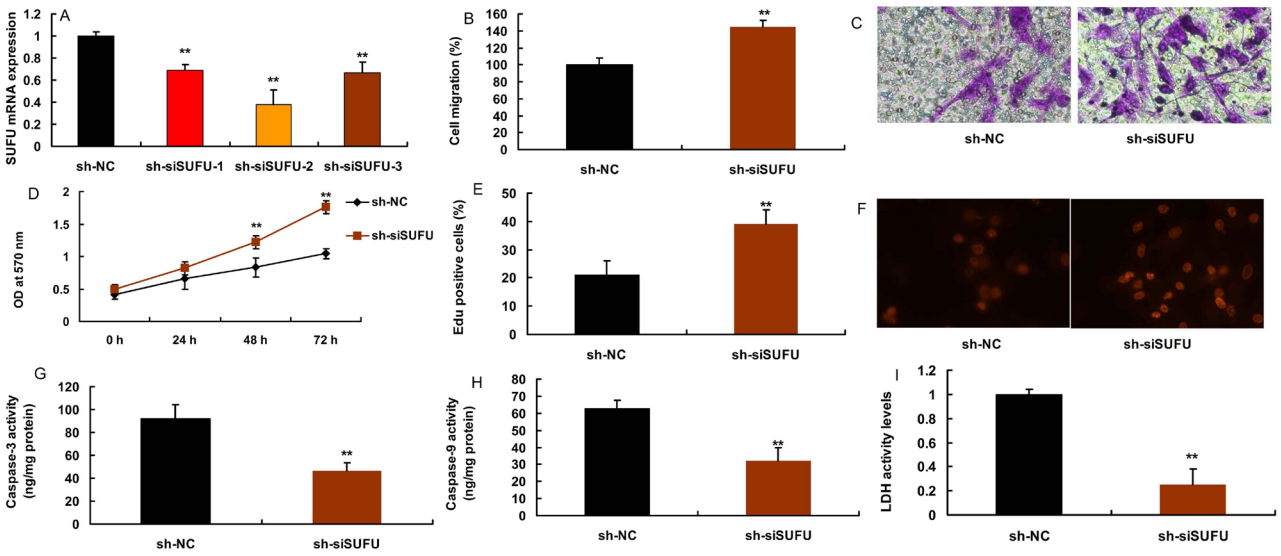

Figure 3. The inhibition of SUFU promoted pancreatic cancer cell growth. SUFU mRNA expression (A), cell migration (B, C), cell growth (D), Edu positive cells (E, F), caspase-3/9 activity and LDH activity levels (G-I). Negative, negative mimic group; siSUFU, down-regulation of SUFU group. ##p < 0.01 compared with negative mimic group.

of SUFU suppressed SUFU protein expression and β-catenin nucleus protein expression, and induced catenin cytoplasm protein expression in vitro model (Figure 4F, I).

The regulation of Wnt/β-catenin signaling pathway controlled the function of SUFU in pancreatic cancer cell lines

To this end, we investigated the relevance of SUFU in regulating Wnt/β-catenin signaling. β-catenin plasmid induced β-catenin nucleus protein expression, suppressed β-catenin cytoplasm protein expression, reduced cell growth, cell migration and Edu positive cells, and increased caspase-3/9 activity and LDH activity levels in vitro model (Figure 5). Siβ-catenin plasmid reduced β-catenin nucleus protein expression, induced β-catenin cytoplasm protein expression, promoted cell growth, cell migration and Edu positive cells, and reduced caspase-3/9 activity and LDH activity levels in vitro model (Figure 6).

4 Discussion

The incidence of pancreatic cancer is still increasing rapidly (Saito et al., 2021). Smoking, aging, diabetes, pancreatitis, family genetic history, gene mutations, etc. are all risk factors of pancreatic cancer (Saleh et al., 2020; Rafiq et al., 2020). Epidemiological data in 2016 showed that there were 53,070 new cases of pancreatic cancer in the United States, ranking the fourth place in the mortality of malignant tumors. In addition, the incidence rate of pancreatic cancer is increasing year by year (Sancho-Albero et al., 2020; Jiang et al., 2021). Pancreatic cancer is highly of malignant, with extremely poor prognosis (5-year survival rate less than 5%) (Goral, 2015; Heinrich & Lang, 2017). In the present study, we have explored that SUFU mRNA and protein expressions in patients with pancreatic cancer were reduced. SUFU reduced pancreatic cancer cell growth. Yang et al. indicated that SUFU promoted cancer growth and metastasis of gastric cancer (Yang et al., 2018), suggesting a novel mechanism underlying the potential significance of SUFU in pancreatic cancer.

A large number of studies have confirmed that the Wnt/β-catenin signaling pathway is closely associated with tumor cell cycle, tumor cell apoptosis, tumor stem cells and tumor angiogenesis (Ren et al., 2019). However, the present understanding of the relationship between Wnt/β-catenin pathway and pancreatic cancer is incomplete, which requires further investigation (Wu et al., 2019). According to present studies, the relevant components in the Wnt/β-catenin signaling pathway and the connection with other signaling pathways can be used as therapeutic targets for anti-pancreatic cancer drugs (Xue et al., 2018). We have found that SUFU suppressed Wnt/β-catenin signaling pathway in pancreatic cancer cell lines. Peng et al. showed that SUFU promoted Wnt/β-catenin signaling pathway in human gastric cancer (Peng et al., 2020). Together, SUFU was identified to be a target of β-catenin signaling pathway.

The loss-of-function of SUFU has been confirmed to be related to the pathogenesis and development of multiple malignancies in human, such as medulloblastoma, Gorlin syndrome, meningioma and prostate cancer (Raducu et al., 2016). Studies have confirmed that abnormal SUFU signal transduction is vitally involved in the pathogenesis and progression of various human malignancies (including ovarian cancer) (Gormley et al., 2015; Sandhu et al., 2019). Therefore, the inhibition of SUFU signaling pathway activity has been tried in multiple types of human malignancies. In addition, we found that the regulation of Wnt/β-catenin signaling pathway controlled the function of SUFU in pancreatic cancer cell lines. Peng et al. showed that miRNA-194 activated Wnt/β-catenin signaling pathway by targeting SUFU in gastric cancer (Peng et al., 2017).

The present study revealed that SUFU reduced cell proliferative and cell migration through activating β-catenin signaling pathway. Further work will be required to understand if SUFU has diagnostic and prognostic value for pancreatic cancer intervention.

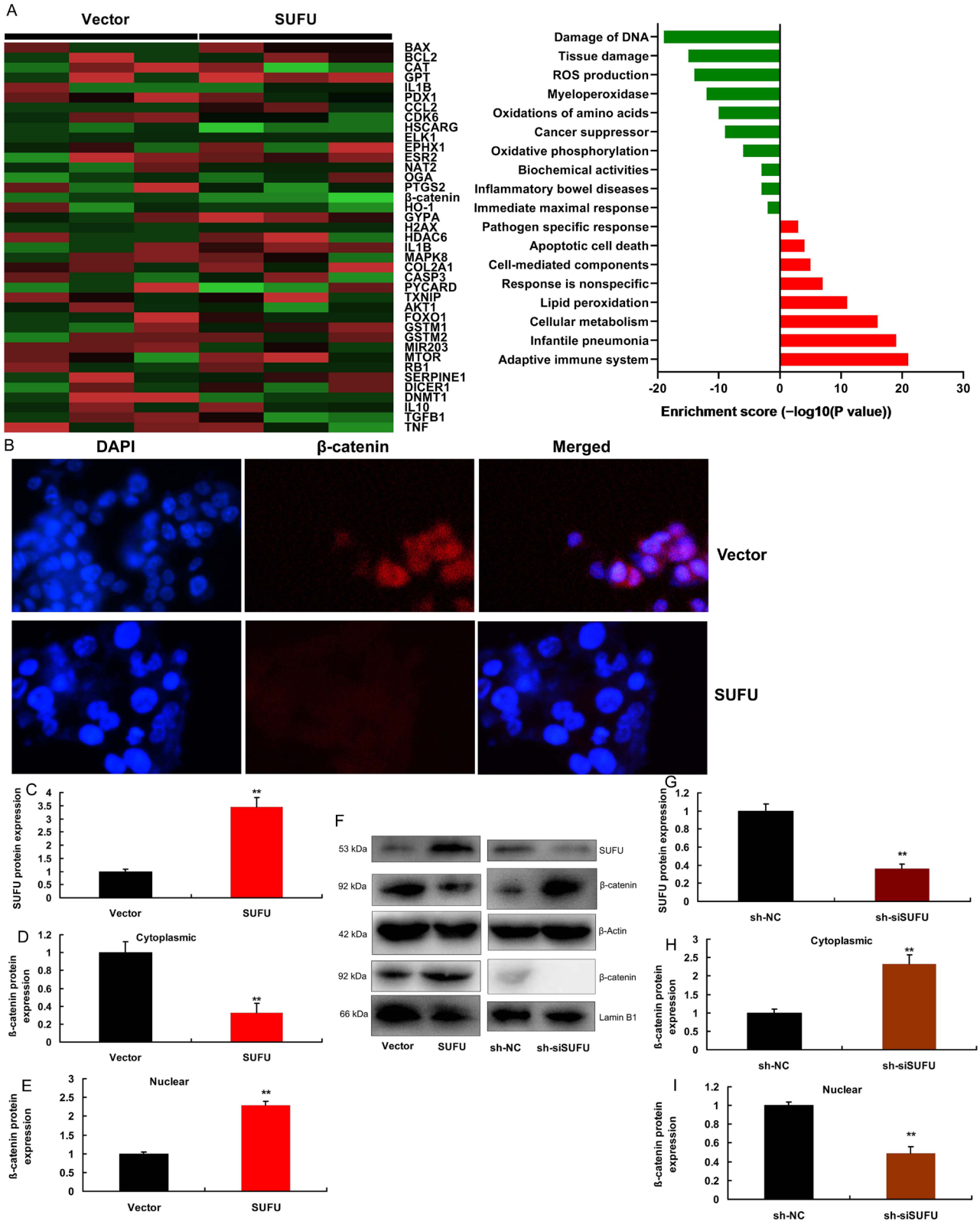

Figure 4. SUFU suppressed Wnt/β-catenin signaling pathway in pancreatic cancer cell lines. Heat map (A), β-catenin expression (Immunofluorescence, B), SUFU and β-catenin protein expression (C-F) by over-expression of SUFU; SUFU and β-catenin protein expression (F-I) by down-regulation of SUFU. Negative, negative mimic group; SUFU, over-expression of SUFU group; siSUFU, down-regulation of SUFU group. ##p < 0.01 compared with negative mimic group.

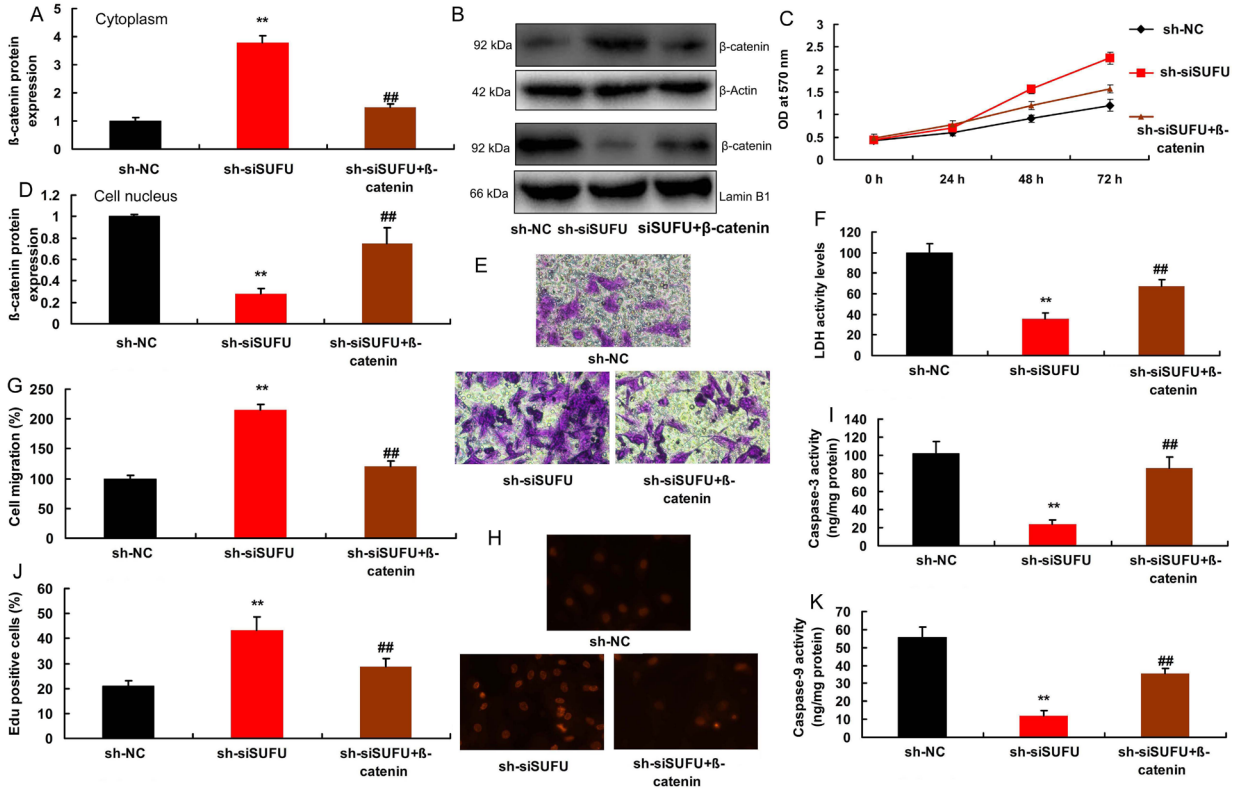

Figure 5. The activation of Wnt/β-catenin signaling pathway controlled the function of SUFU in pancreatic cancer cell lines. β-catenin protein expression (A, B, D), cell growth (C), cell migration (E, G), LDH activity levels (F), caspase-3/9 activity (I, K), Edu positive cells (J, H). Negative, negative mimic group; SUFU, over-expression of SUFU group; SUFU+ siβ-catenin, over-expression of SUFU and down-regulation of β-catenin group. ##p < 0.01 compared with negative mimic group; **p < 0.01 compared with over-expression of SUFU group.

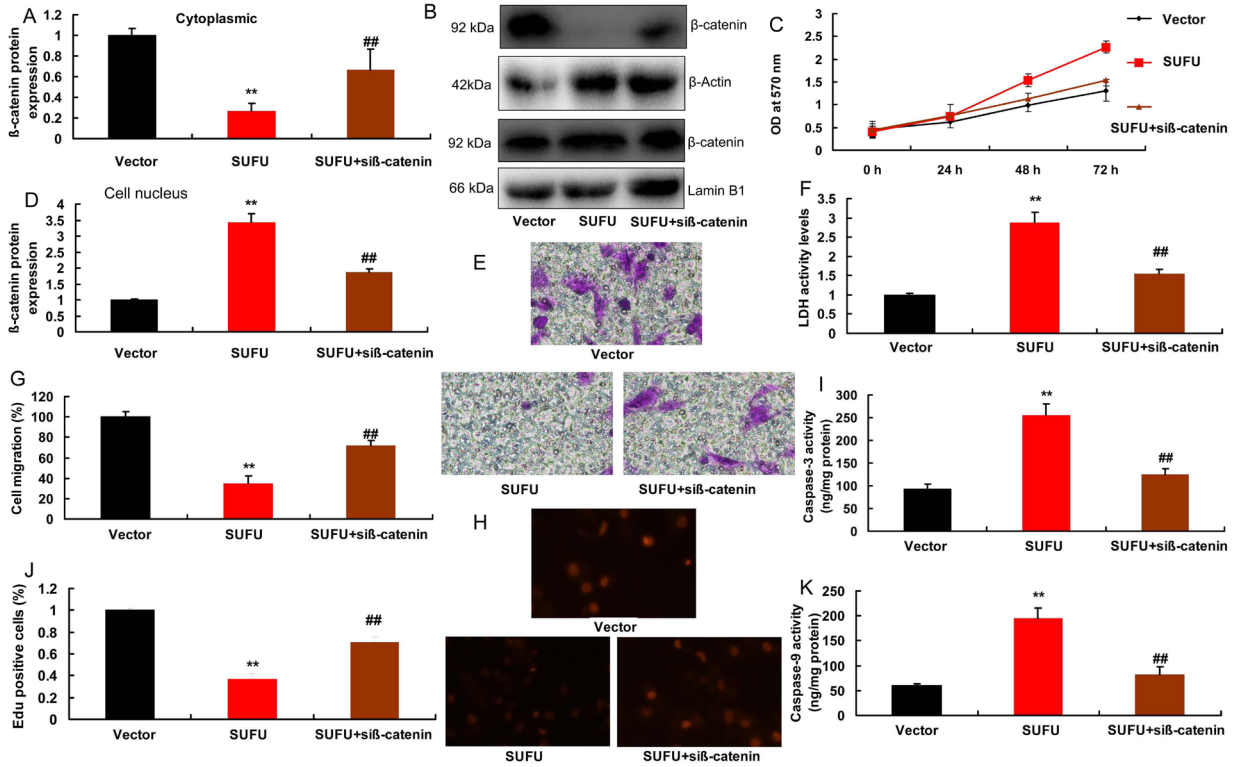

Figure 6. The inhibition of Wnt/β-catenin signaling pathway controlled the function of SUFU in pancreatic cancer cell lines. β-catenin protein expression (A, B, D), cell growth (C), cell migration (E, G), LDH activity levels (F), caspase-3/9 activity (I, K), Edu positive cells (J, H). Negative, negative mimic group; siSUFU, down-regulation of SUFU group; SUFU+ siβ-catenin, down-regulation of SUFU and over-expression of β-catenin group. ##p < 0.01 compared with negative mimic group; **p < 0.01 compared with down-regulation of SUFU group.

Ethical approval

This study was approved by the Ethics Committee of the The Second Affiliated Hospital of Soochow University and performed in accordance with the Declaration of Helsinki.

Conflict of interest

The author declare that they have no conflict of interest.

Availability of data and material

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

Author contributions

Limin Liu was the guarantor of integrity of the entire study, study concepts and design and analyzed the data. Duanmin Hu participated in literature research, experimental studies and helped to draft the manuscript. All authors read and approved the final manuscript.

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