Propofol mediates pancreatic cancer cell activity through the repression of ADAM8 via SP1

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Abstract. Propofol is a commonly used anesthetic with controversial effects on cancer cells. A growing number of studies have demonstrated that low concentrations of propofol are associated with tumor suppression and when used as an intravenous anesthesia improved recurrence-free survival rates for many cancers, but deeper insights into its underlying mechanism are needed. The study detailed herein focused upon the effect of propofol on pancreatic cancer cells and the mechanism by which propofol reduces A disintegrin and metalloproteinase 8 (ADAM8) expression. The ability of propofol to impact the proliferation, migration and cell cycle of pancreatic cancer cell lines was assessed *in vitro*. This was mechanistically explored following the identification of SP1 binding sites within ADAM8, which enabled the regulatory effects of specificity protein 1 (SP1) on ADAM8 following propofol treatment to be further explored. Ultimately, this study was able to show that propofol significantly inhibited the proliferation, migration and invasion of pancreatic cancer cells and decreased the percentage of cells in S-phase. Propofol treatment was also shown to repress ADAM8 and SP1 expression, but was unable to affect ADAM8 expression following knockdown of SP1. Moreover, a direct physical interaction between SP1 and ADAM8 was verified using co-immunoprecipitation and dual-luciferase reporter assays. Cumulatively, these results suggest that propofol represses pathological biological behaviors associated with pancreatic cancer cells through the suppression of SP1, which in turn results in lower *ADAM8* mRNA expression and protein levels.

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Introduction

In 2018, 432,000 deaths resulting from pancreatic cancer were recorded worldwide (1). It is difficult to detect pancreatic cancer in its early stages; as a result, patients are often already in the advanced stage of the disease at the time of diagnosis, which accounts for the 5-year overall survival rate of <5% (2). Currently, although surgical resection combined with chemotherapy is the predominant method for the treatment of pancreatic cancer, surgical stress can affect the immune and neuroendocrine systems and induce inadvertent seeding of tumor cells during surgery, which are the main causes of tumor recurrence (3). Anesthesia management is an essential part of the perioperative period, and the use of anesthetics can affect different physiological and pathophysiological functions, such as cell proliferation, angiogenesis and apoptosis (4). Recently, a meta-analysis has shown that propofol-based total intravenous anesthesia improved the recurrence-free survival rate (pooled HR, 0.78; 95% CI, 0.65-0.94; P<0.01) and the overall survival rate (pooled HR, 0.76; 95% CI, 0.63-0.92; P<0.01) for various cancer types (5), suggesting that propofol may be involved in tumor suppression. Therefore, the aim of the present study was to examine the mechanisms associated with this phenomenon.

A disintegrin and metalloproteinase 8 (ADAM8) is a type-I transmembrane glycoprotein the expression levels of which in normal tissue are typically low and limited to a few distinct cell types in the lymphatic organs, which are components of the immune system (6), and in the central nervous system (7). However, under pathological stimuli, ADAM8 protein

expression levels are upregulated in several diseases, including osteosarcoma, colorectal cancer, gastric cancer and pancreatic cancer, which suggests that ADAM8 may be pathophysiologically relevant. Once upregulated, ADAM8 is proteolytically active and results in enhanced shedding of cell adhesion molecules, cytokine receptors and extracellular matrix components (8). In our previous study, propofol downregulated ADAM8 expression under hypoxic conditions (9), which partially inhibited its activity; this was not observed with the control drug, batimastat, BB-94) (9, 10). For these reasons, it is possible that other mechanisms participate in the effect of propofol on pancreatic cancer through ADAM8.

Specificity protein 1 (SP1) is a widely studied transcription factor, which regulates target gene expression by binding to GC boxes with the consensus sequence 5'-G/T-GGGCGG-G/A-G/A-C/T-3' or 5'-G/T-G/A-GGCG-G/T-G/A-G/A-C/T-3' within their promoter regions (11). SP1 affects both tumor-suppressor genes and oncogenes, suggesting that it may play an important role in tumor development and metastasis. Recent studies have also demonstrated that SP1 has an impact on tumor invasion and metastasis. Indeed, in oral squamous cell carcinoma, SP1 was found to promote cell invasion and migration by upregulating Annexin A2 transcription (12). Additionally, it has been demonstrated that inhibition of SP1/Syncytin1 axis inhibits the proliferation and metastasis through the AKT and ERK1/2 signaling pathways in non-small cell lung cancer (13). The aim of the present study was to determine whether SP1 mediates the effects of ADAM8 on pancreatic cancer cells following treatment with propofol.

Materials and methods

Cell culture and lentiviral transduction. The human pancreatic cancer cell lines Panc-1 and Bxpc3 were purchased from the American Type Culture Collection. Panc-1 and Bxpc3 cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.), supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere containing 5% CO_2 at 37˚C. Panc-1 is a human pancreatic cancer cell line isolated from a pancreatic carcinoma of ductal cell origin; Bxpc3 is a human pancreatic cancer cell line used in the study of pancreatic adenocarcinomas. We had repeated the experiment using the Bxpc3 cell line. SP1 knockdown cell lines were established by transfecting the cells with short hairpin RNA (shRNA) lentiviral plasmids. The lentiviral shRNA plasmid targeting SP1 (shSP1) was purchased from Vigene Biosciences, and the control sequence (shCtrl) from Sigma-Aldrich (Merck KGaA).

For transduction, a total of $5x10^4$ cells/well were seeded in 6-well plates. The shSP1 or shCtrl lentivirus was added to the cells in the presence of 5 μl polybrene (Sigma-Aldrich; Merck KGaA). After 96 h, the transduced cells were selected with 1 μg/ml puromycin. Subsequently, the selected cells were treated with different concentrations of propofol according to our previous study (10). In order to avoid the possible adverse effects of lipid emulsion, pure propofol was obtained from Sigma-Aldrich (Merck KGaA).

Cell viability assay. Cell viability was assessed using MTT assays. Cells were seeded in 96-well plates ($6x10^3$ cells/well) and treated with propofol for 48 h. MTT solution (Sigma-Aldrich; Merck KGaA) was added for 4 h at 37˚C. The precipitate was then dissolved in 200 μl DMSO. The absorbance was measured at 490 nm using a Multiskan Spectrum plate reader (Thermo Fisher Scientific, Inc.).

Wound healing assay. In a 6-well plate, Panc-1 and Bxpc3 cells ($1x10^6$ cells/well) were incubated in DMEM overnight in order to create a cell monolayer. A scratch was made in the middle of the well using a pipette tip (7 mm) and the debris was washed away prior to the addition of new medium to the wells. Using an optical microscope (magnification, x400), the cells were imaged, and the initial area of the scratch in the field of view was determined as the length multiplied by the width. A total of three fields of view were examined. The plate was incubated at 37˚C for 24 h, after which the same field of view was imaged and the scratch area was measured again using the same methodology. The final area of the scratch wound was divided by the initial area to determine the percentage wound remaining of the initial area covered by migrating cells over the 24-h culture period.

Cell cycle analysis. Cells were incubated with the indicated doses of propofol for 24 h, and then washed with cold PBS. Subsequently, the cells were fixed using cold 75% ethanol overnight at 4˚C, washed with cold PBS and stained with propidium iodide (PI) for 30 min at 37˚C. After staining, the cells were analyzed using flow cytometry (BD FACSDiva™; BD Biosciences).

Total RNA isolation and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from cell lines using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. A NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc.) was used to measure RNA concentration. RT reactions were performed using the PrimeScript RT Reagent kit (Takara Biotechnology Co., Ltd.) and qPCR was performed using FastStart Universal SYBR-Green Master (Roche Diagnostics GmbH) and the Step One Plus Real-time PCR system (Applied Biosystems). According to the manufacturer's protocol, the thermocycling conditions were as follows: i) Initial denaturation, 95˚C for 30 sec; ii) amplification, 95˚C for 5 sec, 60˚C for 20 sec, 40 cycles; and iii) dissociation curve, 95˚C for 60 sec, 55˚C for 30 sec, 95˚C for 30 sec. The $2^{-\Delta\Delta Cq}$ method was used to determine the relative mRNA expression levels, which were normalized to those of β-actin (14). All experiments were performed independently three times and set up in triplicate. The following primer sequences were used: i) ADAM8 forward, 5'-ACAATGCAGAGTTCCAGATGC-3' and reverse, 5'-GGACCACACGGAAGTTGAGTT-3'; ii) SP1 forward, 5'-CGGAATTCATGAGCGACCAAGATCACTCCATG-3' and reverse, 5'-CGGAATTCTTGGACCCATGCTACCTTGCATCC-3'; and iii) GAPDH forward, 5'-GTCAGTGGTGGACCTGACCT-3' and reverse, 5'-TGGTGCTCAGTTTAGCCCAGG-3'.

Western blot analysis. Cells were lysed using RIPA buffer (Beijing Dingguo Changsheng Biotechnology Co., Ltd.) to extract total protein. The extracted protein (50 μg) was separated using SDS-PAGE on 10% gels, and then transferred to PVDF membranes. The membranes were incubated with antibodies against ADAM8 (cat. no. ab255608; dilution 1:1,000; Abcam), SP1 (cat. no. ab231778; dilution 1:1,000; Abcam) and

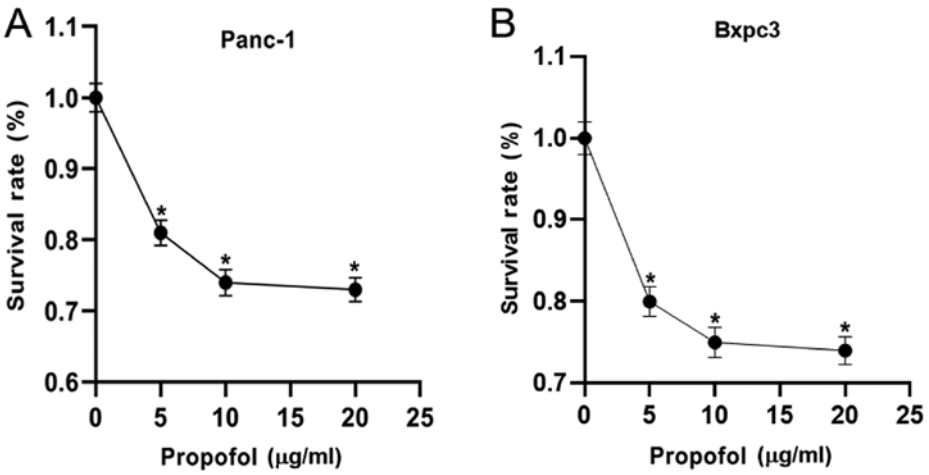

Figure 1. Propofol inhibits the proliferation of pancreatic cancer cells. (A) Panc-1 and (B) Bxpc3 cells were exposed to different concentrations of propofol (0, 5, 10 and 20 μg/ml). The MTT assay was used to assess cell proliferation. The experiments in this figure were performed in triplicate, and the data are expressed as mean ± SD. *P<0.05 compared to the untreated control.

β-actin (cat. no. ab8226; dilution 1:1,000; Abcam) overnight at 4˚C, then with goat anti-rabbit HRP-conjugated antibody (cat. no. ab181662; dilution 1:2,000; Abcam). The protein bands were visualized using the Odyssey system (LI-COR Biosciences).

Lentiviral infection. Lentiviral vectors for SP1 knockdown (shSP1) were purchased from Vigene Biosciences (cat. no. P100029). A total of $50x10^4$ cells/well were seeded in 6-well plates. The shSP1 or shCtrl virus (cat. no. TL506569V) was added to the cells in the presence of 5 μl polybrene (Sigma-Aldrich; Merck KGaA) to increase the efficiency of infection. After 96 h, the transduced cells were selected using 1 μg/ml puromycin for 2 months. The selected cells were then treated with different concentrations of propofol, the transfection efficiencies in all cell lines are over 80%. The following primer sequences were used: i) SP1-shRNA1, 5'-GCAAGTTCTGACAGGACTACCTTCAAGAGAGGTAGTCCTGTCAGAACTTGCTTTTTT-3'; ii) SP1-shRNA2, 5'-GCAACATCATTGCTGCTATGCTTCAAGAGAGCATAGCAGCAATGATGTTGCTTTTTT-3'; iii) SP1-shRNA3, 5'-GCAGACCTTTACAACTCAAGCTTCAAGAGAGCTTGAGTTGTAAAGGTCTGCTTTTTT-3'.

Dual-luciferase reporter assays. Dual luciferase reporter assays were performed as previously described (15). Wild-type or mutant ADAM8 (containing mutations in the putative binding site for SP1 located in 3'-untranslated region), together with a synthesized promoter mimic or vector, were co-transfected for 48 h. The transfected cells were then harvested to determine luciferase activity using a dual luciferase reporter assay system (Promega Corp.).

Co-immunoprecipitation (Co-IP) assay. Panc-1 cells were lysed using RIPA buffer (50 mM Tris-HCl, pH 7.8; 150 mM NaCl; 5 mM EDTA; 0.1% Triton X-100; 0.05% NP-40). Subsequently, the lysates were incubated overnight at 4˚C in an orbital shaker with 2 μg anti-ADAM8 or anti-SP1 antibody alongside a negative control containing 2 μg rabbit IgG antibody. Cell lysate without any antibody (input) was used as a positive control. After incubation, the mixture was incubated with agarose beads at 4˚C for 3 h. The beads were collected and sequentially washed five times with 1 ml RIPA lysis buffer, then analyzed by western blotting using anti-ADAM8 or anti-SP1 antibodies. The intensity of the specific bands was evaluated using ImageJ software, version 1.46 (National Institutes of Health). The assays were repeated at least three times.

Statistical analysis. The data are presented as the mean ± SD of three independent experiments. SSPS 20.0 software (IBM Corp.) was used for statistical analysis. One-way ANOVA followed by Duncan's post hoc test and unpaired Student's t-tests were used to compare the experimental groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Propofol inhibits the viability of pancreatic cancer cells. Firstly, the effect of 0 (negative control), 5, 10 and 20 μg/ml propofol on Panc-1 and Bxpc3 cell viability was evaluated using MTT assays. As shown in Fig. 1, propofol significantly suppressed the viability of Panc-1 and Bxpc3 cells when compared with the negative control. It was also observed that 10 μg/ml propofol resulted in the lowest viability. These findings indicated that propofol treatment could inhibit the viability of pancreatic cancer cells.

Propofol reduces the number of pancreatic cancer cells in the S-phase of the cell cycle. The role of propofol on the cell cycle progression of Panc-1 and Bxpc3 cells was examined. Panc-1 and Bxpc3 cells were treated with 0, 5, 10 and 20 μg/ml propofol, and the distribution of Panc-1 and Bxpc3 cells in different phases of the cell cycle was examined using flow cytometry. The results indicated that propofol affected the cell cycle progression of Panc-1 and Bxpc3 cells, as evidenced by a significant gradual reduction in the number of pancreatic cancer cells in the S-phase with increasing propofol concentration (Fig. 2). While the number of cells in the G1-phase appeared to be increased, this was not statistically significant. This demonstrated that cells were blocked at the S-phase, which may indicate a relative decrease in DNA synthesis and replication.

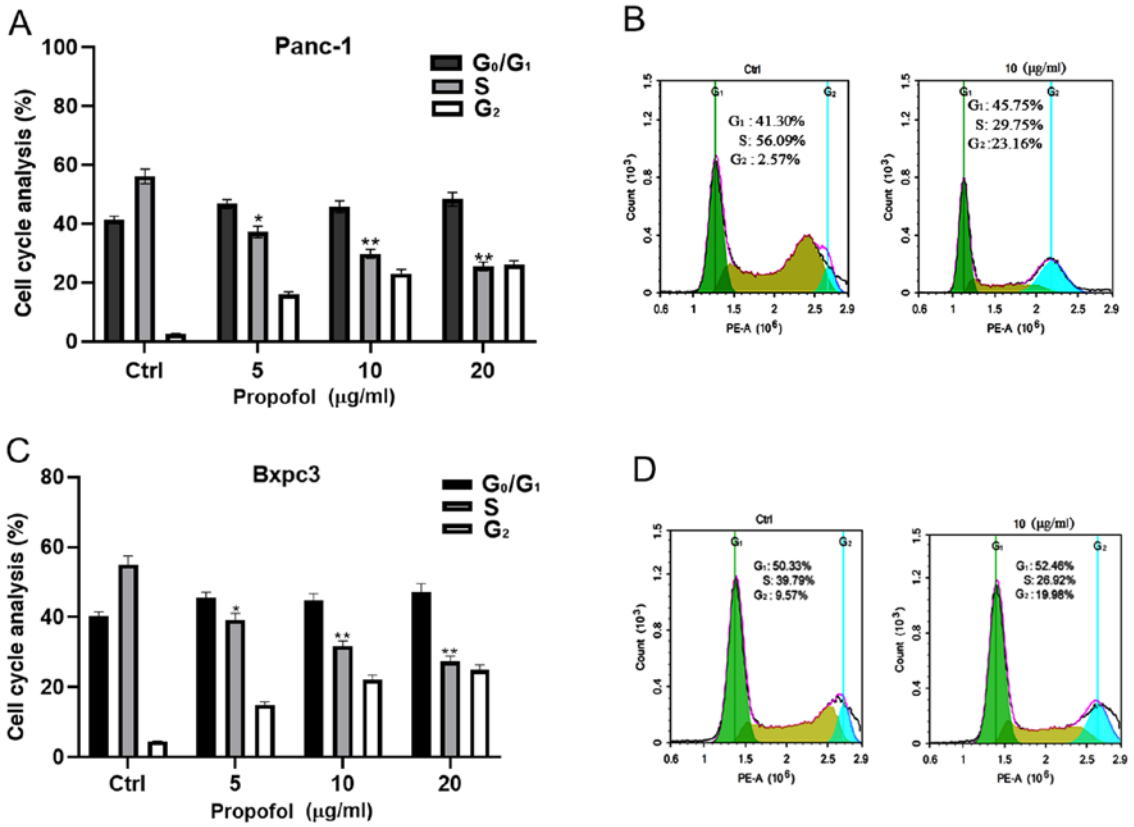

Figure 2. Propofol blocks the cell cycle of pancreatic cancer cells at the S-phase. After Panc-1 and Bxpc3 cells were treated with different concentrations of propofol (0, 5, 10 and 20 μg/ml) for 24 h, (B and D) flow cytometry was used to measure the distribution of the cell cycle and the results were analyzed using FlowJo software. The percentage of (A) Panc-1 and (C) Bxpc3 cells at the S-phase was decreased after propofol treatment. The experiments in this figure were performed in triplicate, and the data are expressed as mean ± SD. *P<0.05; **P<0.005, compared to the untreated control.

Propofol inhibits the migration of pancreatic cancer cells. In addition to its inhibitory effects on cell viability, the potential role of propofol on the malignant behavior of Panc-1 and Bxpc3 cells was also examined. For this purpose, wound healing assays were performed. The results demonstrated that propofol treatment significantly suppressed the migration of Panc-1 and Bxpc3 cells (Fig. 3). Indeed, the wounds healed at a slower rate following treatment with higher concentrations of propofol.

Propofol inhibits the expression of ADAM8. To investigate the effects of propofol on ADAM8, mRNA and protein were extracted from Panc-1 and Bxpc3 cells treated with 0, 5, 10 and 20 μg/ml propofol. It was observed that propofol treatment significantly reduced the ADAM8 mRNA and protein expression levels in a dose-dependent manner (Fig. 4).

Verification of the direct interaction between SP1 and ADAM8. In the University of California Santa Cruz database, the promoter region of ADAM8 was predicted to be located at chr10:133,262,422-133,264,422 (GRCh38). The luciferase reporter vectors containing the indicated genomic fragments of the ADAM8 gene were constructed. To investigate the potential regulators involved in ADAM8 expression, potential transcription factor binding sites in the ADAM8 promoter were identified using three online software packages: PubMed (https://pmlegacy.ncbi.nlm.nih.gov/gene/101), JASPAR (http://jaspar.genereg.net/) and GeneCards (http://genecards.org) (16). Binding sites for the transcription factor SP1 and zinc finger and BTB domain containing 40 were found in the promoter region of ADAM8 (Fig. 5A). Of the two candidate transcription factors, only SP1 mimics markedly enhanced luciferase activity (Fig. 5B). Co-IP experiments were performed in order to confirm whether SP1 binds to the promoter region of ADAM8. The results indicated that SP1 and ADAM8 were detectable in the corresponding precipitated protein complexes, indicating that SP1 interacted directly with ADAM8 (Fig. 5C). This suggested that the SP1 transcription factor may be targeted by propofol.

Propofol potentially targets SP1 to regulate ADAM8. To investigate whether propofol acts through ADAM8 via SP1, Panc-1 and Bxpc3 cells were treated with 0, 5, 10 and 20 μg/ml propofol and used in a dual luciferase reporter assay. The luciferase activity was significantly inhibited, with a concentration of 10 μg/ml propofol resulting in the lowest luciferase activity (Fig. 6A and B). Additionally, Panc-1 shCtrl and three Panc-1 shSP1 cell lines (Panc-1-SP1-shRNA1, 2 and 3) were established, and the shSP1 cell line expressing the lowest mRNA and protein levels of SP1 was used in the subsequent experiments (Fig. 6C-H). Protein and mRNA were extracted from the Panc-1/Bxpc3-shSP1-shRNA and the Panc-1/Bxpc3-NC-shRNA lines to determine whether the expression of ADAM8 was regulated by propofol in the absence of SP1. The expression of ADAM8 was decreased at different concentrations of propofol in the control groups (Figs. 7A-C and 8A-C), but not in the experimental groups (Figs. 7D-F and 8D-F).

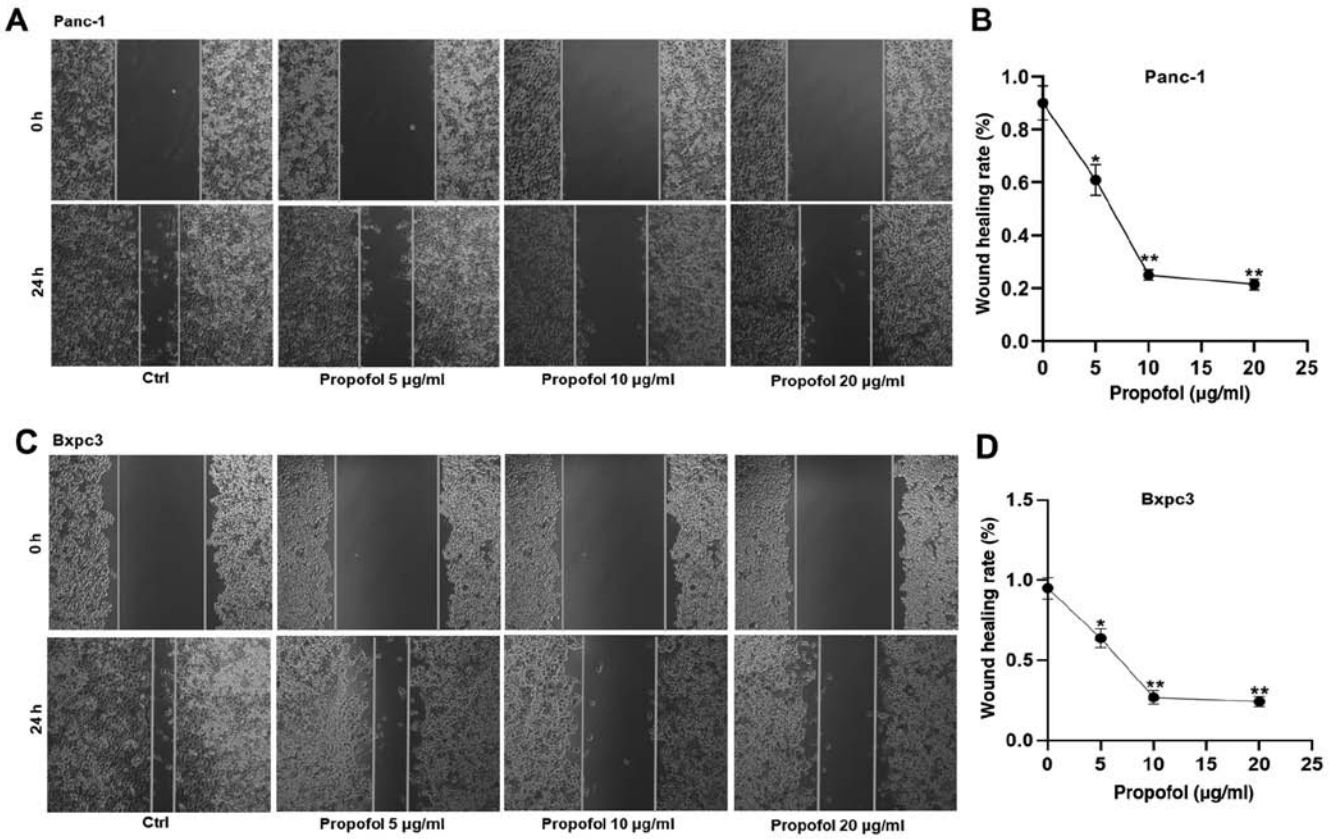

Figure 3. Propofol inhibits the migration of pancreatic cancer cells. Panc-1 and Bxpc3 cells were exposed to 5, 10 and 20 μg/ml propofol and compared to untreated cells for 24 h. (A and C) Cell migration was detected using wound healing assays and the relative migrated surface was analyzed by ImageJ software. (B and D) The relative rate of wound healing was significantly decreased after propofol treatment (i.e. propofol inhibited migration). The experiments in this figure were performed in triplicate, and the data are expressed as mean ± SD. *P<0.05; **P<0.005, compared to the untreated control.

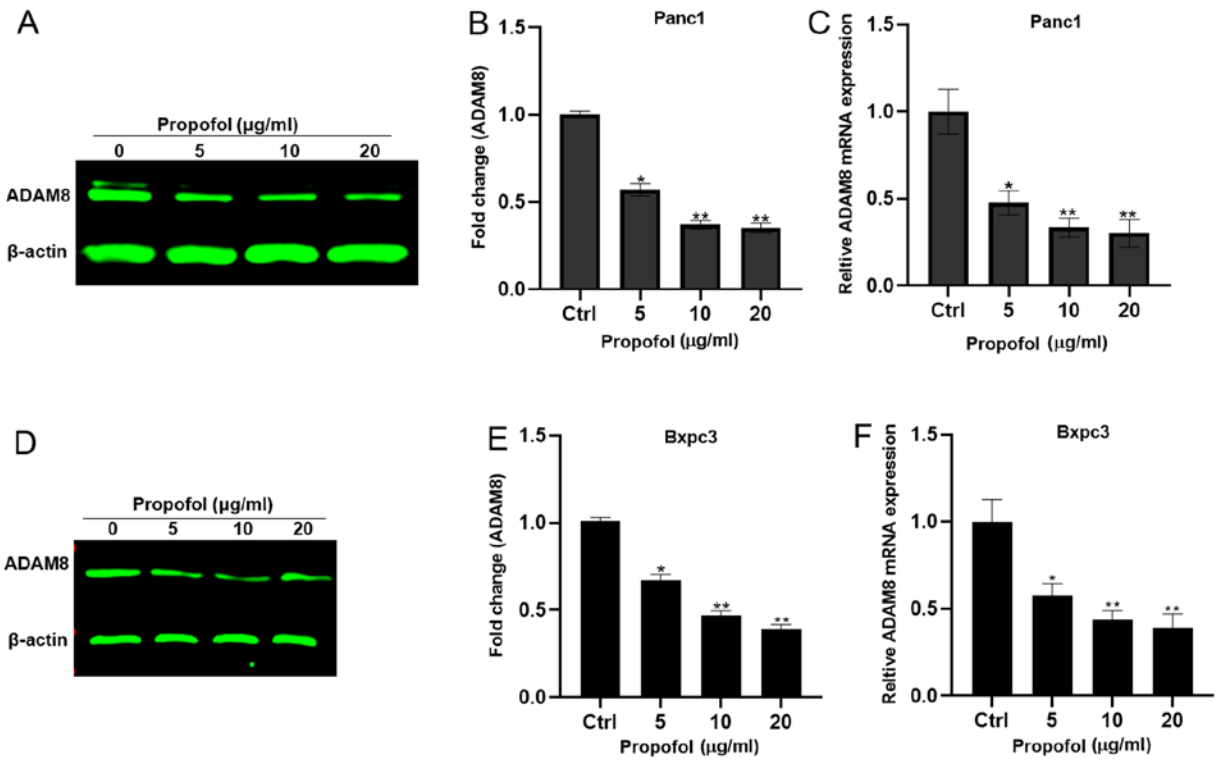

Figure 4. Propofol decreases ADAM8 mRNA expression and protein levels. (A and D) The protein levels of ADAM8 in Panc-1 and Bxpc3 cells following treatment with 0, 5, 10 and 20 μg/ml propofol were detected using western blotting. (B and E) The results were quantified by ImageJ software. (C and F) qPCR was used for the detection of the mRNA level of ADAM8 in Panc-1 cells treated with 0, 5, 10 and 20 μg/ml propofol. All experiments in this figure were performed in triplicate, and the data are expressed as mean ± SD. *P<0.05; **P<0.005, compared to the untreated control (Ctrl). ADAM8, A disintegrin and metalloproteinase 8.

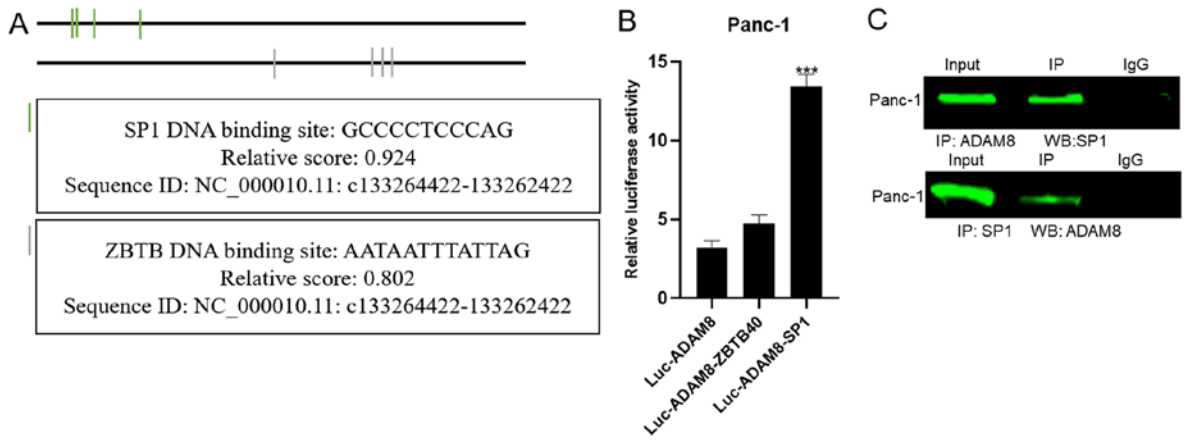

Figure 5. Verification of the direct interaction between SP1 and ADAM8. (A) Prediction of transcription factor binding sites in the ADAM8 promoter region using Pubmed, JASPAR and GeneCards. (B) The influence of transcription factors on ADAM8 promoters were determined by dual luciferase reporter assays and data are expressed as mean ± SD. ***P<0.001, compared to Luc-ADAM8 group. (C) Co-IP assay was performed to detect the interaction between SP1 and ADAM8 in Panc-1 cells. ADAM8, A disintegrin and metalloproteinase 8; SP1, specificity protein 1; Co-IP, Co-immunoprecipitation.

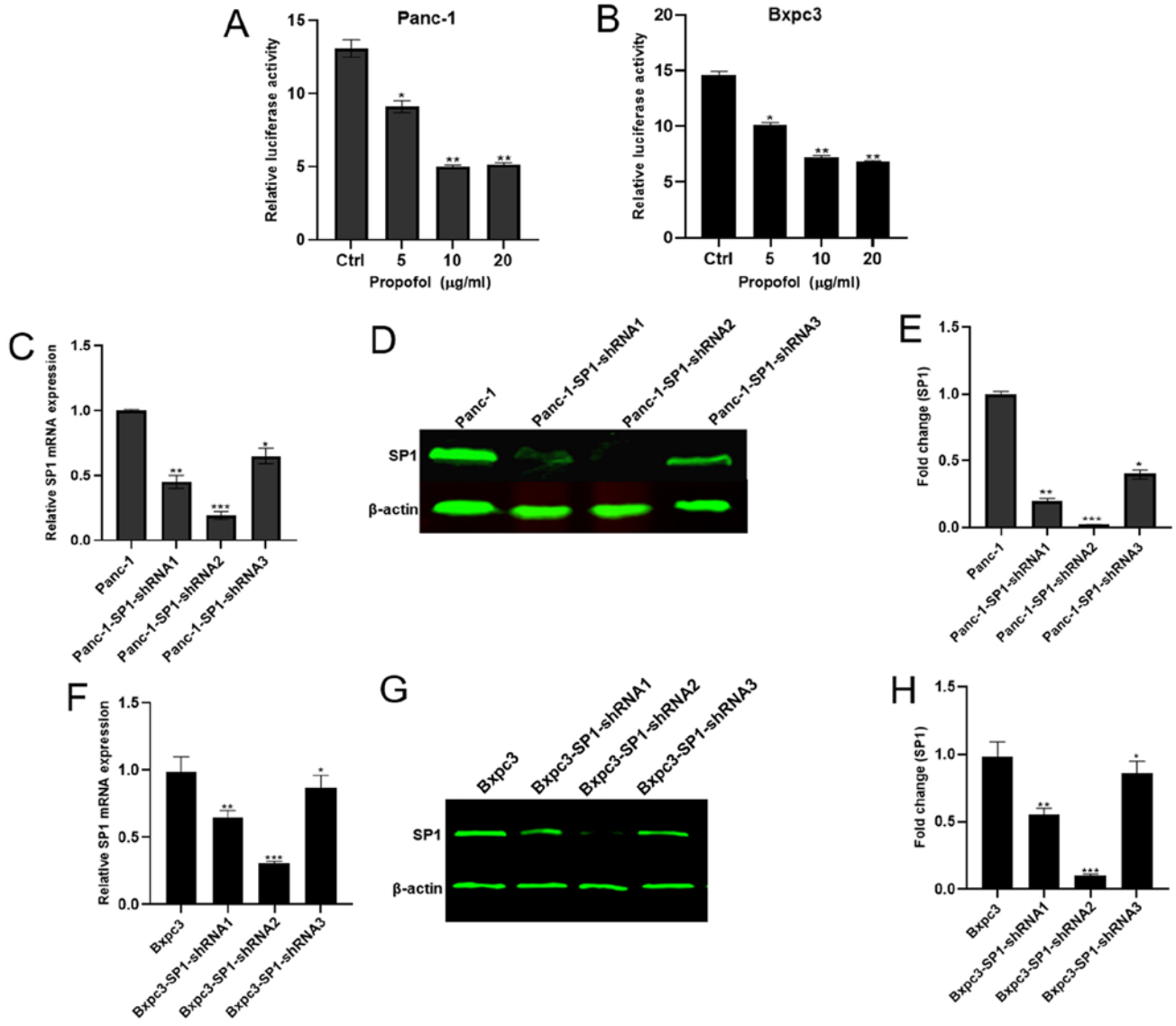

Figure 6. Panc-1 and Bxpc3 cells were treated with 0, 5, 10 and 20 μg/ml propofol. (A and B) Dual-luciferase assay was used for assessing the luciferase activity. *P<0.05; **P<0.005, compared with the Ctrl group. (C-E) The protein and mRNA levels of SP1 in three groups of Panc-1 cells transfected with SP1-knockdown plasmids (Panc-1-SP1-shRNA1, 2 and 3) and in control Panc-1 cells (Panc-1) were detected by western blot analysis and qPCR. (F-H) The protein and mRNA levels of SP1 in three groups of Bxpc3 cells transfected with SP1-knockdown plasmids (Bxpc3-SP1-shRNA1, 2 and 3) and in control Bxpc3 cells (Bxpc3) were detected by western blot analysis and qPCR. *P<0.05; **P<0.005; ***P<0.001, compared with the control Panc-1 or Bxpc3 control cells. SP1, specificity protein 1.

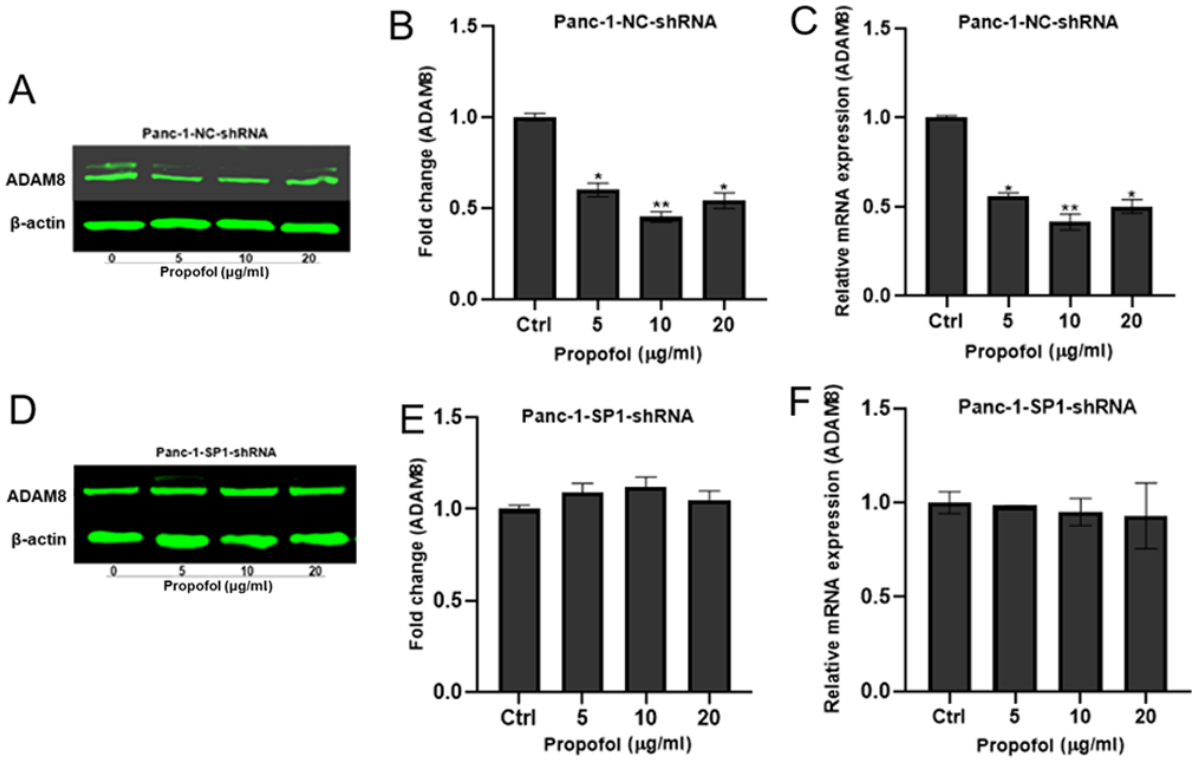

Figure 7. The protein and mRNA levels of ADAM8 in the (A-C) Panc-1-NC-shRNA and (D-F) Panc-1-SP1-shRNA cell line following treatment with 0, 5, 10 and 20 μg/ml propofol. Data are expressed as mean ± SD. *P<0.05; **P<0.005, compared with the Ctrl. ADAM8, A disintegrin and metalloproteinase 8; SP1, specificity protein 1.

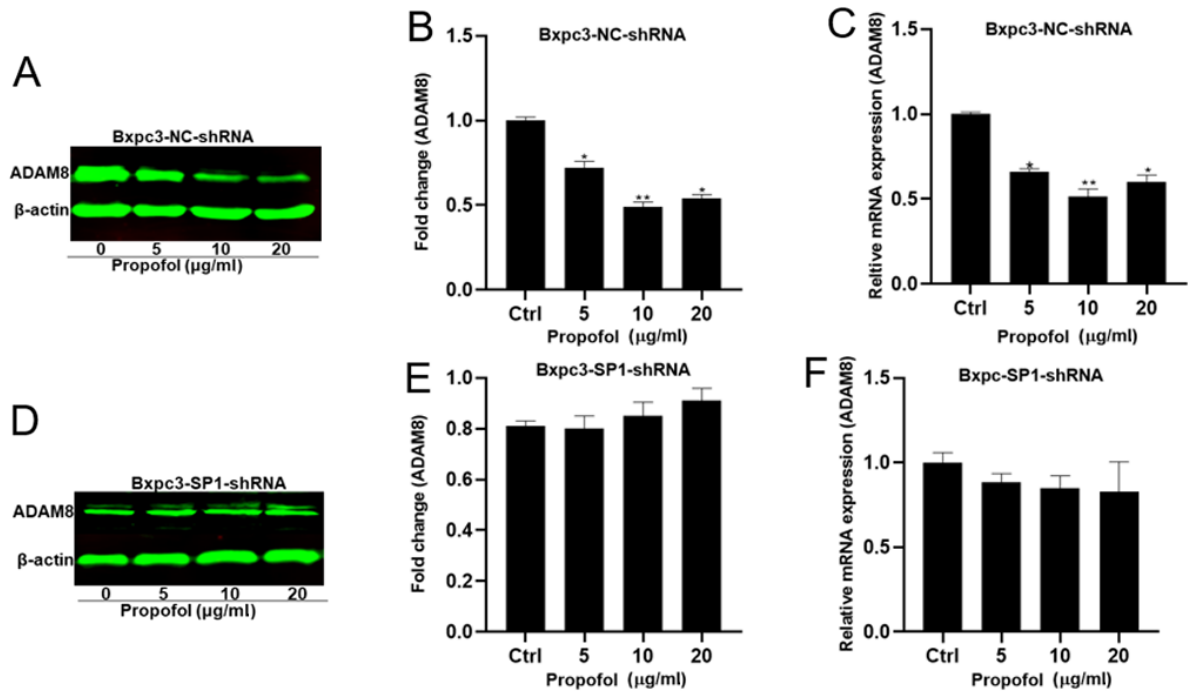

Figure 8. The protein and mRNA levels of ADAM8 in the (A-C) Bxpc3-NC-shRNA and (D-F) Bxpc3-SP1-shRNA cell line following treatment with 0, 5, 10 and 20 μg/ml propofol. Data are expressed as mean ± SD. *P<0.05; **P<0.005, compared with the Ctrl. ADAM8, A disintegrin and metalloproteinase 8; SP1, specificity protein 1.

Discussion

Previous studies have shown that propofol not only affects epigenetic pathways, such as those involving histone acetylation, microRNAs and long non-coding RNAs (17,18), but also modulates signaling pathways, including the SLUG, MAPK, nuclear factor erythroid 2 like 2 and NF-κB pathways (19). The present study demonstrated that propofol could

inhibit the viability, block the cell cycle at the S-phase and suppress the migration of pancreatic cancer cells. To obtain deeper insight into the associated molecular mechanism, several transcription factors for A disintegrin and metalloproteinase 8 (ADAM8) were investigated. Interestingly, specificity protein 1 (SP1) was found to regulate ADAM8 expression, which was affected by propofol treatment in Panc-1 and Bxpc3 cells. Thus, the effect of propofol on pancreatic cancer cells was mediated by ADAM8 via SP1.

Propofol is a commonly used intravenous sedative-hypnotic agent. In addition to its multiple anesthetic advantages, propofol exerts a number of non-anesthetic effects. Indeed, accumulating evidence suggests that it may affect cancer development in direct as well as indirect manners. A number of studies have indicated that propofol suppresses the malignancy of a variety of human cancer types, such as hepatocellular carcinoma (20), breast cancer (21) and lung cancer (22). Moreover, a previous study suggested a possible correlation between propofol and chemotherapy, although the mechanism remains unclear (23). The previous study conducted by our research group demonstrated that propofol inhibits pancreatic tumor growth via ADAM8 (9) and determined that propofol specifically inhibited ADAM8 expression and activation in response to hypoxia in pancreatic cancer (10). The results of the present study are consistent with these previous reports.

ADAM8 is a proteolytically active member of the ADAM protease family. Increased expression of ADAM8 has been observed in breast cancer (24), lung adenocarcinoma (25) and pancreatic cancer (26). ADAM8 has been shown to cleave important extracellular matrix components of the tumor stroma, such as growth factors or cell surface proteins (27).

Epidermal growth factor has been demonstrated to reduce cell attachment, cell-cell interaction and cell spreading, as well as to inhibit the expression levels of cyclin A, D1 and cdk2 (28). Cyclins play an important role in cell proliferation, pluripotency and determination of cell fate. Since DNA synthesis and replication are an important part of the S-phase, the reduced percentage of cells in the S-phase identified in this study could indicate that propofol suppresses pancreatic cancer cell growth through the repression of ADAM8 via SP1, which is a hypothesis that requires further study.

SP1 is involved in basal transcriptional regulation of various genes. SP1 contains three highly homologous C2H2 regions, which directly bind to DNA, thus promoting gene transcription (29). In the present study, SP1 interacted directly with ADAM8, and propofol did not inhibit Panc-1 cell migration and ADAM8 expression in Panc-1 cells following SP1 knockdown by shRNA. Additionally, luciferase activity was reduced with increasing concentrations of propofol in cells transfected with luciferase reporter vectors and SP1 mimics. These results suggest that SP1 directly mediates the expression and the function of ADAM8 following propofol treatment (Figs. S1 and S2).

There were limitations in this study. First, we did not perform *in vivo* experiment, however, our previous study had demonstrated propofol remarkably retarded xenograft tumor progression and inhibited the expression of angiogenesis mediators by ADAM8 *in vivo* (9,10); second, our previous study confirmed that propofol inhibited invasion of pancreatic cancer cell by Transwell assays (10), thus we did not present the results in this manuscript.

In conclusion, the present study findings suggest that propofol plays a critical role in inhibiting the viability and migration of pancreatic cancer cells and also blocks their cell cycle progression at the S-phase by targeting SP1 to regulate ADAM8. These findings may expand the current knowledge in the field of perioperative anesthetics and their effects on tumor cells.

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Availability of data and materials

All data and materials are available without restriction. Researchers can obtain data by contacting the corresponding authors.

Authors' contribution

XY and YBD contributed to the conception and design of the study. YG, CW and YZ contributed to perform the experiments, data acquisition and interpretation. KMS was involved in the bioinformation analysis. YG drafted the manuscript. XY, YBD and KMS reviewed the manuscript critically. All authors contributed to the interpretation of the findings, and reviewed, edited and approved the final manuscript for publication.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2018. CA Cancer J Clin 68: 7-30, 2018.
2. Dudley B and Brand RE: Pancreatic cancer surveillance: Who, when, and how. Curr Treat Options Gastroenterol 17: 681-691, 2019.
3. Horowitz M, Neeman E, Sharon E and Ben-Eliyahu S: Exploiting the critical perioperative period to improve long-term cancer outcomes. Nat Rev Clin Oncol 12: 213-226, 2015.
4. Aguirre JA, Lucchinetti E, Clanachan AS, Plane F and Zaugg M: Unraveling interactions between anesthetics and the endothelium: Update and novel insights. Anesth Analg 122: 330-348, 2016.
5. Yap A, Lopez-Olivo MA, Dubowitz J, Hiller J and Riedel B; Global Onco-Anesthesia Research Collaboration Group: In reply: Comment on 'Anesthetic technique and cancer outcomes: A meta-analysis of total intravenous versus volatile anesthesia'. Can J Anaesth 67: 152-153, 2020.

6. Kelly K, Hutchinson G, Nebenius-Oosthuizen D, Smith AJ, Bartsch JW, Horiuchi K, Rittger A, Manova K, Docherty AJ and Blobel CP: Metalloprotease-disintegrin ADAM8: Expression analysis and targeted deletion in mice. Dev Dyn 232: 221-231, 2005.
7. Schlomann U, Rathke-Hartlieb S, Yamamoto S, Jockusch H and Bartsch JW: Tumor necrosis factor alpha induces a metalloprotease-disintegrin, ADAM8 (CD 156): Implications for neuron-glia interactions during neurodegeneration. J Neurosci 20: 7964-7971, 2000.
8. Schlomann U, Wildeboer D, Webster A, Antropova O, Zeuschner D, Knight CG, Docherty AJ, Lambert M, Skelton L, Jockusch H, *et al*: The metalloprotease disintegrin ADAM8. Processing by autocatalysis is required for proteolytic activity and cell adhesion. J Biol Chem 277: 48210-48219, 2002.
9. Gao Y, Yu X, Zhang F and Dai J: Propofol inhibits pancreatic cancer progress under hypoxia via ADAM8. J Hepatobiliary Pancreat Sci 26: 219-226, 2019.
10. Yu X, Shi J, Wang X and Zhang F: Propofol affects the growth and metastasis of pancreatic cancer via ADAM8. Pharmacol Rep 72: 418-426, 2020.
11. Safe S, Imanirad P, Sreevalsan S, Nair V and Jutooru I: Transcription factor Sp1, also known as specificity protein 1 as a therapeutic target. Expert Opin Ther Targets 18: 759-769, 2014.
12. Kang Y, Nagaraja AS, Armaiz-Pena GN, Dorniak PL, Hu W, Rupaimoole R, Liu T, Gharpure KM, Previs RA, Hansen JM, *et al*: Adrenergic stimulation of DUSP1 impairs chemotherapy response in ovarian cancer. Clin Cancer Res 22: 1713-1724, 2016.
13. Li X, Fu Y, Xia X, Zhang X, Xiao K, Zhuang X and Zhang Y: Knockdown of SP1/Syncytin1 axis inhibits the proliferation and metastasis through the AKT and ERK1/2 signaling pathways in non-small cell lung cancer. Cancer Med 8: 5750-5759, 2019.
14. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
15. Wang S, Zhen L, Liu Z, Ai Q, Ji Y, Du G, Wang Y and Bu Y: Identification and analysis of the promoter region of the human HAS3 gene. Biochem Biophys Res Commun 460: 1008-1014, 2015.
16. Vlieghe D, Sandelin A, De Bleser PJ, Vleminckx K, Wasserman WW, van Roy F and Lenhard B: A new generation of JASPAR, the open-access repository for transcription factor binding site profiles. Nucleic Acids Res 34: D95-D97, 2006.
17. Yu X, Gao Y and Zhang F: Propofol inhibits pancreatic cancer proliferation and metastasis by up-regulating miR-328 and down-regulating ADAM8. Basic Clin Pharmacol Toxicol 125: 271-278, 2019.
18. Xiao Y, Yurievich UA and Yosypovych SV: Long noncoding RNA XIST is a prognostic factor in colorectal cancer and inhibits 5-fluorouracil-induced cell cytotoxicity through promoting thymidylate synthase expression. Oncotarget 8: 83171-83182, 2017.
19. Meng C, Song L, Wang J, Li D, Liu Y and Cui X: Propofol induces proliferation partially via downregulation of p53 protein and promotes migration via activation of the Nrf2 pathway in human breast cancer cell line MDA-MB-231. Oncol Rep 37: 841-848, 2017.
20. Ou W, Lv J, Zou X, Yao Y, Wu J, Yang J, Wang Z and Ma Y: Propofol inhibits hepatocellular carcinoma growth and invasion through the HMGA2-mediated Wnt/β-catenin pathway. Exp Ther Med 13: 2501-2506, 2017.
21. Ecimovic P, Murray D, Doran P and Buggy DJ: Propofol and bupivacaine in breast cancer cell function in vitro - role of the NET1 gene. Anticancer Res 34: 1321-1331, 2014.
22. Cui WY, Liu Y, Zhu YQ, Song T and Wang QS: Propofol induces endoplasmic reticulum (ER) stress and apoptosis in lung cancer cell H460. Tumour Biol 35: 5213-5217, 2014.
23. Li H, Lu Y, Pang Y, Li M, Cheng X and Chen J: Propofol enhances the cisplatin-induced apoptosis on cervical cancer cells via EGFR/JAK2/STAT3 pathway. Biomed Pharmacother 86: 324-333, 2017.
24. Romagnoli M, Mineva ND, Polmear M, Conrad C, Srinivasan S, Loussouarn D, Barillé-Nion S, Georgakoudi I, Dagg Á, McDermott EW, *et al*: ADAM8 expression in invasive breast cancer promotes tumor dissemination and metastasis. EMBO Mol Med 6: 278-294, 2014.
25. Ishikawa N, Daigo Y, Yasui W, Inai K, Nishimura H, Tsuchiya E, Kohno N and Nakamura Y: ADAM8 as a novel serological and histochemical marker for lung cancer. Clin Cancer Res 10: 8363-8370, 2004.
26. Schlomann U, Koller G, Conrad C, Ferdous T, Golfi P, Garcia AM, Höfling S, Parsons M, Costa P, Soper R, *et al*: ADAM8 as a drug target in pancreatic cancer. Nat Commun 6: 6175, 2015.
27. Conrad C, Benzel J, Dorzweiler K, Cook L, Schlomann U, Zarbock A, Slater EP, Nimsky C and Bartsch JW: ADAM8 in invasive cancers: Links to tumor progression, metastasis, and chemoresistance. Clin Sci (Lond) 133: 83-99, 2019.
28. Cao L, Yao Y, Lee V, Kiani C, Spaner D, Lin Z, Zhang Y, Adams ME and Yang BB: Epidermal growth factor induces cell cycle arrest and apoptosis of squamous carcinoma cells through reduction of cell adhesion. J Cell Biochem 77: 569-583, 2000.
29. Nagaoka M, Shiraishi Y and Sugiura Y: Selected base sequence outside the target binding site of zinc finger protein Sp1. Nucleic Acids Res 29: 4920-4929, 2001.