

Original Article

DUSP5 regulates ferroptosis in pancreatic cancer by downregulating SCD1 expression via ERK1/2

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) remains a highly lethal malignancy with limited therapeutic options. The Ras/Raf/MEK/ERK signaling pathway is critically involved in PDAC progression, yet targeting its downstream effectors remains challenging. DUSP5, a nuclear phosphatase that dephosphorylates ERK1/2, has emerged as a potential tumor suppressor, but its role in PDAC and underlying mechanisms are poorly understood. In this study, we find that DUSP5 is significantly downregulated in PDAC tissues compared to adjacent normal tissues (ANTs) and is correlated with a favorable prognosis. Overexpression of DUSP5 inhibits PDAC cell proliferation *in vitro* and *in vivo*. Mechanistically, DUSP5 physically interacts with ERK1/2 in the nucleus, leading to ERK1/2 dephosphorylation and inactivation, which downregulates SCD1 expression, a key enzyme converting saturated fatty acids to mono-unsaturated fatty acids (MUFAs). Reduced SCD1 levels decrease MUFA synthesis (C16:1 and C18:1), increase lipid peroxidation, and induce mitochondrial dysfunction characteristic of ferroptosis. Rescue experiments confirm that supplementation with MUFAs or ferroptosis inhibitors reverses DUSP5-mediated growth inhibition. In conclusion, this study identifies DUSP5 as a novel suppressor of PDAC progression that induces ferroptosis via the ERK1/2/SCD1 axis. Targeting the DUSP5/ERK1/2/SCD1 pathway may represent a promising therapeutic strategy for PDAC.

Key words pancreatic cancer, DUSP5, SCD1, ferroptosis, proliferation

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly malignant digestive system tumor, with approximately 66,440 new cases and 51,750 deaths in the United States every year, ranking as the fourth leading cause of cancer-related death [1]. To date, radical surgical resection is the only potentially curative treatment for early PDAC [2]. However, the disease is commonly diagnosed at advanced stages, and thus, the majority of PDAC patients lose the opportunity for surgical resection. Moreover, pancreatic cancer is generally less

sensitive to cytotoxic chemotherapy, targeted therapy and immunotherapy than other malignancies. Although considerable progress has been made in pancreatic cancer research in recent years [3,4], the 5-year overall survival rate of PDAC remains poor, at approximately 13% [1]. Therefore, it is urgent to elucidate the molecular mechanisms underlying PDAC progression to identify novel therapeutic targets and improve clinical outcomes.

The gene *DUSP5* (dual specificity phosphatase 5), a member of the *DUSP* family, is located on chromosome 10 (10q25) and was

initially identified by Martell *et al.* [5]. It is also known as *VH3* (*VH1*-like phosphatase-3). *VH1* phosphatase, encoded by the vaccinia virus genome, is a dual-specificity protein-tyrosine phosphatase (PTPase) that catalyzes the hydrolysis of substrates phosphorylated at tyrosine and serine/threonine residues. It can negatively regulate the mitogen-activated protein kinase (MAPK) signaling pathway, specifically dephosphorylating nuclear extracellular signal-regulated kinases 1 and 2 (ERK1/2), thereby modulating cell proliferation, differentiation, and apoptosis. These biological functions are closely associated with tumor initiation and progression. Previous studies have demonstrated that DUSP5 acts as a tumor suppressor and is downregulated in multiple malignancies, including gastric cancer, colorectal cancer, and hilar cholangiocarcinoma [6–8]. Accumulating evidence indicates that DUSP5 expression is modulated by multiple upstream regulators, which further alters ERK1/2 phosphorylation status and thereby controls tumor proliferation and progression [9–11]. However, the biological function of DUSP5 in PDAC remains largely unexplored, and its underlying molecular mechanisms remain unclear.

ERK1/2 is a serine/threonine protein kinase that plays a crucial role in the MAPK family, participating in both normal and pathological cellular activities by phosphorylating various substrates within the cytoplasm and nucleus [12]. As a pivotal component of the Ras/Raf/MEK/ERK signaling pathway, ERK1/2 phosphorylation is implicated in the initiation and progression of numerous tumors, including breast cancer, liver cancer, and intestinal tumors [13–15]. Aberrant activation of the Ras/Raf/MEK/ERK pathway is a hallmark feature of pancreatic cancer [16]. Consequently, inhibiting ERK pathway activation can effectively impede tumor advancement [17]. Accumulating studies have confirmed that blocking ERK1/2 phosphorylation inhibits gastric cancer tumorigenesis [18]. Combining inhibitors targeting the ERK signaling pathway can further enhance pancreatic cancer treatment outcomes [19]. Inhibiting the activity of ERK can block the metabolic processes driven by *KRAS* mutation, which in turn further enhances the dependency of pancreatic cancer on autophagy, thereby increasing sensitivity to treatment with autophagy inhibitors [20]. Therefore, we hypothesize that in pancreatic cancer, DUSP5 suppresses tumor growth by inhibiting the activity of ERK through dephosphorylation. However, the precise underlying mechanism remains elusive.

Ferroptosis is a distinct form of cell death characterized by morphological, biochemical, and genetic features that set it apart from previously recognized mechanisms such as apoptosis, various forms of necrosis, and autophagy [21]. Its main characteristics include the accumulation of iron ions, lipid peroxidation, and reactive oxygen species. The process of ferroptosis is metabolic cell death, which is related to both polyunsaturated fatty acid metabolism and monounsaturated fatty acid (MUFA) metabolism [22,23]. Research has found that ferroptosis is related to the activation of ERK1/2 in tumor cells [21]. Activation of the RAS/RAF/MEK/ERK pathway is involved in the regulation of ferroptosis [24]. Therefore, we speculate that DUSP5 may regulate ferroptosis by modulating the activity of ERK1/2. To date, no relevant study has reported this regulatory axis in pancreatic cancer.

In this study, we investigated the impact of DUSP5 on ferroptosis in pancreatic cancer. We found that DUSP5 promotes ferroptosis in PDAC cells. Mechanistically, DUSP5 could downregulate the expression of stearoyl-CoA desaturase 1 (SCD1) by inactivating p-

ERK1/2 through dephosphorylation, which could promote ferroptosis. Our findings identify the role of DUSP5 in pancreatic cancer, suggesting that the DUSP5/ERK1/2/SCD1 regulatory axis may be a potential therapeutic target for PDAC.

Materials and Methods

Tissue samples

The pancreatic cancer and adjacent normal tissue samples used in this study were obtained from patients who underwent surgery and were pathologically diagnosed at Fudan University Shanghai Cancer Center (FUSCC). This study obtained the informed consent of the patients and the approval of the Institutional Research Ethics Committee of FUSCC.

Immunohistochemical (IHC) staining

The IHC steps were performed following the standard procedures described in our previous study [25]. The primary antibodies were incubated as follows: anti-DUSP5 (1:100, D163746; Sangon Biotech, Shanghai, China), anti-p-ERK1/2 (1:200, 4370S; Cell Signaling Technology, Beverly, USA), anti-SCD1 (1:100, D162163; Sangon Biotech) and anti-Ki67 (1:200, ab16667; Abcam, Cambridge, UK). HRP-conjugated affinity-purified goat anti-mouse/rabbit IgG (1:250, GK500710; Gene Tech, Shanghai, China) was used as a secondary antibody. Under the microscope, three different fields of view were randomly selected from each slide to score the protein expression level. The immunoreactive score (IRS) was used as the scoring criterion, with a range of 0–12 as a product of multiplication between the positive cell proportion score (0, not positive; 1, < 10%; 2, 10%–50%; 3, 51%–80%; and 4, > 80%) and the staining intensity score (0, negative; 1, mild reaction; 2, moderate reaction; and 3, intense reaction). An overall IHC score > 6 is considered high expression, while a score ≤ 6 is defined as low expression.

Cell lines and cell culture

Two human PDAC cell lines (PANC-1 and MIA PaCa-2) were purchased from the American Type Culture Collection (ATCC; Manassas, USA). The cells were cultured following the standard protocols provided by ATCC. HEK 293T cells were purchased from Invitrogen (Carlsbad, USA). PANC-1 cells and 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone, Carlsbad, USA), which contained 10% fetal bovine serum (FBS; Wisent, Saint-Jean-Baptiste, Canada) with 100 U/mL penicillin (Yuanpei, Shanghai, China) and 100 µg/mL streptomycin (Yuanpei); MIA PaCa-2 cells were grown in DMEM supplemented with an additional 2.5% horse serum (Gibco, Carlsbad, USA) in addition to 10% FBS. The cells described above were cultured in a 37°C incubator with 5% CO₂.

Chemicals

The ferroptosis inhibitor ferrostatin-1 (Fer-1) was purchased from Selleck (Houston, USA). The mitogen-activated protein kinase (MAPK) kinase inhibitor U0126 was purchased from MedChemExpress (Monmouth Junction, USA). Oleic acid and palmitoleic acid were purchased from CSNpharm (Chicago, USA).

Plasmids

To generate DUSP5 overexpression plasmids, the coding sequences of human *DUSP5* and the relevant C263S mutant *DUSP5* (catalytically inactive) were cloned and inserted into the lentiviral vector

pCDH-CMV-MCS-EF1-puro (SBI, Palo Alto, USA). Empty vector (EV) was used as the control. Lentiviruses were produced from HEK293T cells by co-transfection of plasmids with psPAX2 and pMD2.G vectors (Addgene, Watertown, USA). PANC-1 and MIA PaCa-2 cells were infected with lentiviral particles, and puromycin was used to select stably transfected cells.

Western blot analysis

The specific steps for protein extraction and western blotting were performed as described in our previous study [25]. The primary antibodies were as follows: anti-DUSP5 (1:1000, ab200708; Abcam), anti-p-ERK1/2 (1:1000), anti-ERK1/2 (1:1000, 4695S; Cell Signaling Technology), anti-SCD1 (1:1000), and anti- β -actin (1:1000, 4970S; Cell Signaling Technology). The secondary antibody was HRP-conjugated goat anti-rabbit IgG (H+L) (1:5000, AS014; ABclonal, Wuhan, China). Finally, the protein bands were visualized with an automatic chemiluminescence image analysis system after adding the ultrasensitive chemiluminescence detection reagent (Beyotime, Shanghai, China).

RNA extraction and quantitative real-time PCR

First, total RNA was extracted using the EZ-press RNA Purification Kit (B0004D; EZBioscience, Beijing, China). Next, cDNA was obtained by reverse transcription using the PrimeScript RT reagent Kit (RR036A; TaKaRa, Tokyo, Japan). Finally, quantitative real-time PCR was conducted using a QuantStudio™ 6 Flex Real-Time PCR system (Thermo Fisher Scientific, Waltham, USA). All data were normalized to β -actin and analyzed using the $2^{-\Delta\Delta C_t}$ method. The primers used were as follows: human *DUSP5*: 5'-GCCAGCTTAT-GACCAGGGTG-3' (forward), 5'-GTCCGTCGGGAGACATTCAG-3' (reverse); human *SCD1*: 5'-CCTGGTTTCACTTGGAGCTGTG-3' (forward), 5'-TGTGGTGAAGTTGATGTGCCAGC-3' (reverse); and human *β -actin*: 5'-CATGTACGTTGCTATCCAGGC-3' (forward), 5'-CTCCTTAATGTCACGCACGAT-3' (forward).

Cell proliferation assay

Cell Counting Kit-8 reagents (CCK-8; Yeasen Biotechnology, Shanghai, China) were used to assess the viability of PDAC cells. In brief, cells from each group were seeded in 96-well plates (3×10^3 cells/well) and then cultured for different days (0, 1, 2, 3, 4, and 5 days) at 37°C with 5% CO₂. Subsequently, CCK-8 reagent (10 μ L) was added to each well, followed by incubation for 1.5 h. Finally, the absorbance of each well was measured at 450 nm using a microplate reader (Molecular Devices, San Jose, USA).

Colony formation assay

Cells from different groups were seeded into 6-well plates at a density of 1000 cells per well and cultured for 10 days. Subsequently, the cells were fixed with 4% paraformaldehyde for 15 min and stained with 0.1% crystal violet for another 15 min. Finally, the number of clones formed in each group was counted using the analytical software ImageJ (National Institutes of Health, Bethesda, USA).

RNA-sequencing (RNA-seq) and data analysis

Total RNA was extracted from vector control and DUSP5-over-expressing PANC-1 cells with TRIzol/chloroform. RNA integrity was detected using an Agilent Bioanalyzer 2100 system (Agilent, Santa Clara, USA). For library construction, 3 μ g RNA per sample was

processed using the NEB Next® Ultra™ RNA Library Prep Kit (NEB, Ipswich, USA) for Illumina®. After cDNA synthesis, end repair, adapter ligation and fragment purification, PCR-amplified libraries were quality-checked and sequenced on the Illumina NovaSeq platform to generate 150 bp paired-end reads. Raw reads were filtered by fastp to obtain clean reads, which were mapped to the reference genome using Hisat2 v2.0.5. FeatureCounts v1.5.0-p3 was used to calculate gene expression (FPKM). Differential expression analysis between two groups was performed with DESeq2 R package (v1.16.1). Genes with $|\log_2FC| > 1$ and $P < 0.05$ were defined as DEGs, and volcano plots and heatmaps were drawn via ggplot2. GO and KEGG enrichment analyses of DEGs were conducted using cluster Profiler. Gene Set Enrichment Analysis (GSEA) was performed based on Hallmark gene sets from MSigDB.

Fatty acid metabolomic profiling via LC-MS/MS

Fatty acid metabolomic profiling was conducted on logarithmically growing PANC-1 and MIA PaCa-2 cells stably expressing empty vector or DUSP5 overexpression plasmid. Cell pellets were harvested, rinsed with pre-cooled PBS, and subjected to total intracellular fatty acid extraction using methanol-chloroform solvent. Untargeted lipid detection was performed by Shanghai Baipu Biotechnology Co., Ltd. (Shanghai, China) via UHPLC-Q-TOF LC-MS/MS; pooled quality control (QC) samples were inserted every 10 injections to assess instrumental stability. Raw spectral data were processed for peak alignment and normalization, with metabolite annotation accomplished against Bioprofile in-house BPDB lipid database as well as public HMDB and Lipid Maps databases. Relative levels of representative MUFAs (palmitic acid C16:0, stearic acid C18:0) were quantified, and intergroup differences were assessed by two-tailed unpaired Student's *t*-test, where $P < 0.05$ denoted statistical significance.

BODIPY 581/591 C11 staining

For confocal fluorescence microscopy measurement, cells were seeded in a confocal culture dish (2×10^5 cells/well). After incubation for 24 h, BODIPY-C11 probe (10 μ M; Thermo Fisher Scientific) was added and incubated for another 30 min. After washing with PBS, a confocal fluorescence microscope (Leica, Heidelberg, Germany) was used to take photos. For flow cytometry measurement, cells from each group were seeded in a 6-well cell plate at 5×10^5 cells/well. After 24 h of incubation, the cells were digested, resuspended, washed with PBS, and then stained with 10 μ M BODIPY-C11 probe for 30 min. The cells were then subjected to analysis with a flow cytometer (Beckman, Pasadena, USA).

Reactive oxygen species (ROS) assay

Cells were seeded in 6-well plates ($3-4 \times 10^5$ cells/well) and cultured for 24 h. The culture medium was then removed, and 1 mL of serum-free culture medium containing an ROS fluorescent probe (10 μ M; Beyotime) was added. Cells were incubated at 37°C for 20 min. After washing the cells three times with serum-free cell culture medium, the cells were digested and resuspended in serum-free medium for flow cytometry detection.

MDA detection

The MDA content in cells was detected using a Lipid Oxidation (MDA) Detection Kit (Beyotime). Briefly, after cells were collected,

they were lysed, and the protein concentration of the cells was detected using the BCA kit (Epizyme Biomedical, Shanghai, China). According to the instructions, the processed samples to be tested were added to a 96-well plate, and then the absorbance was measured at 532 nm using a microplate reader (Molecular Devices). The MDA content was calculated based on the standard curve. Finally, the MDA content per unit weight of protein was calculated.

GSH/GSSG assay

According to the instructions, GSH and GSSG Assay Kits (Beyotime) were used to measure the content of GSH and GSSG in each group of cells, and then the GSH/GSSG ratio was calculated.

Transmission electron microscopy (TEM) imaging

PANC-1 cells were cultured to confluence, and then the culture medium was removed. The cells were collected after trypsin digestion and centrifuged at 92 g for 2 min, and the supernatant was discarded. Subsequently, the cells were fixed with 2.5% glutaraldehyde fixation solution at room temperature for 30 min in a dark environment. TEM imaging was conducted by Servicebio (Wuhan, China).

Co-immunoprecipitation (Co-IP)

The FLAG-tag Protein IP Assay Kit with Magnetic Beads was purchased from Beyotime. The cell lysates were mixed with anti-Flag magnetic beads or IgG magnetic beads in a rotational incubator at 4°C overnight, and the protein-protein complexes obtained after magnetic bead elution were subjected to western blot analysis. IgG (Beyotime) was used as a negative control.

Immunofluorescence (IF) staining

For immunofluorescence staining, the adherent cells were fixed with 4% paraformaldehyde for 20 min and then incubated with 0.5% Triton X-100 for 15 min to permeabilize the cell membrane. Subsequently, the cells were blocked with 5% BSA for 30 min and incubated with the primary antibody overnight at 4°C. The fluorescent secondary antibody was added and incubated for 1 h. Next, anti-fade mounting medium with DAPI was added for nuclear staining. Images were collected using a confocal laser scanning microscope (Leica) to observe the co-localization of the two target molecules. The primary antibodies used were as follows: anti-Flag (1:100, GNI4110-FG; GNI, Tokyo, Japan) and anti-ERK1/2 (1:400; 4695S; Cell Signaling Technology). The secondary antibodies used were as follows: CoraLite 488 goat anti-mouse IgG (H+L) (1:100, SA00013-1; Proteintech, Wuhan, China) and CoraLite 594 goat anti-rabbit IgG (H+L) (1:100, SA00013-4; Proteintech).

Animal studies

The research protocol was approved by the Ethics Committee for the Use of Live Animals in Teaching and Research at Fudan University and implemented according to relevant guiding principles. In this study, cells with stable overexpression of DUSP5 and control PANC-1 cells (1×10^7) were cultured and injected subcutaneously into the right axillary region of nude mice (GemPharmatech, Nanjing, China) to observe their proliferative capacity. After 4 weeks, the nude mice were euthanized using carbon dioxide, and the tumors were excised for histological analysis. Tumor volumes were calculated using the ellipsoid volume formula ($1/2 \times L \times W \times H$).

Statistical analysis

Each experiment was repeated three times. All data were analyzed using GraphPad Prism 9 (GraphPad Software, San Diego, USA). Two-tailed unpaired Student's *t* tests were used to compare the differences between any two groups. Multiple groups were compared using one-way analysis of variance (ANOVA). Logistic regression analysis was employed to determine the correlation between DUSP5 and SCD1 expression levels. Survival curves were plotted by the Kaplan-Meier method and compared by the log-rank test. Statistical significance was set at a *P* value less than 0.05.

Results

DUSP5 expression is positively correlated with the PDAC

First, we conducted IHC analysis to assess the expression levels of DUSP5 in pancreatic cancer tissues ($n = 60$) and adjacent non-tumor tissues (ANTs) ($n = 60$), sourced from Fudan University Shanghai Cancer Center (FUSCC). Our findings revealed that DUSP5 expression was significantly downregulated in cancer tissues compared to their ANTs (Figure 1A,C). Furthermore, we analyzed the correlation between DUSP5 expression levels and prognosis in pancreatic cancer tissues, revealing that patients with low DUSP5 expression exhibited poorer prognosis (Figure 1B). Finally, Figure 1D presents representative immunohistochemical staining images of DUSP5 from the tissue microarray.

DUSP5 overexpression suppresses PDAC proliferation *in vitro* and *in vivo*

To further investigate the function of DUSP5 in pancreatic cancer, we overexpressed DUSP5 in PANC-1 and MIA PaCa-2 cell lines using lentiviral transfection techniques. We then verified the efficiency of DUSP5 overexpression through qPCR and western blot analysis (Figure 2A,B). First, we conducted a series of functional experiments *in vitro* and found that, compared to control cells, the proliferation capacity (Figure 2C,D) and colony formation ability (Figure 2E,F) of cells overexpressing DUSP5 were significantly reduced. Next, to further validate the impact of DUSP5 on cellular proliferation, we investigated the effect of DUSP5 on tumor formation capacity by using a xenograft mouse model. PANC-1 cells stably overexpressing DUSP5 and their corresponding control cells were cultured and subcutaneously injected into the right axilla of nude mice to observe their proliferative capacity. After 4 weeks, the mice were euthanized, and the tumors were removed for photography and histological analysis. *In vivo*, overexpression of DUSP5 significantly inhibited the tumorigenic ability and growth capacity of PANC-1 cells (Figure 2G-I). After HE staining was performed on the xenograft tumors, we further compared the proliferation index (Ki-67) in tumors from different groups using IHC methods. The results showed that the level of Ki-67 was significantly reduced in tumors formed by PANC-1 cells overexpressing DUSP5 compared with the control group (Figure 2J).

RNA-seq data analysis

To further explore the potential molecular mechanisms by which DUSP5 regulates the proliferation of pancreatic cancer, RNA-seq was conducted in DUSP5-overexpressing PANC-1 cells and their control groups. The RNA sequencing results revealed that DUSP5 was markedly upregulated, confirming the successful establishment of our stably transfected cell line, and among the downregulated genes, SCD1 was the most prominent. SCD1 is a crucial gene

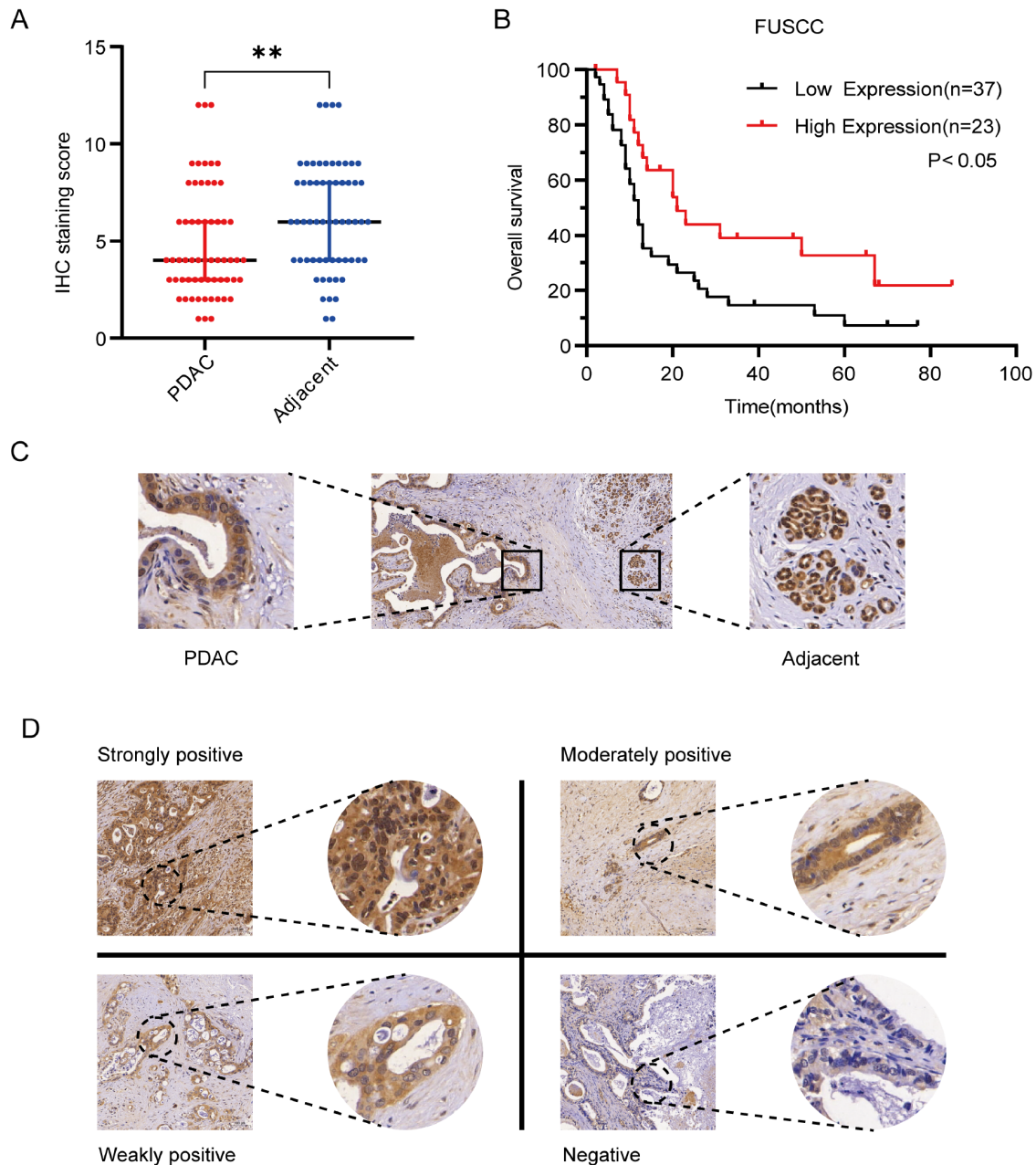


Figure 1. The expression of DUSP5 is positively correlated with the prognosis of PDAC (A) DUSP5 expression is significantly downregulated in PDAC samples compared with normal tissues ($n = 60$, $**P < 0.01$). (B) The low DUSP5 expression group exhibited poorer prognosis than the high group ($*P < 0.05$). (C) Representative images of IHC staining for DUSP5 in PDAC tumor tissues (T) and adjacent normal tissues (ANT). (D) Representative images of IHC staining for DUSP5 in tissue microarrays. Magnification, 100x and 400x, respectively.

regulating fatty acid metabolism and is associated with cellular proliferation in tumors. A heatmap of the top 50 genes with significant differences between the DUSP5-overexpressing group and the control group was created (Figure 3A). A volcano plot was generated, also showing the significant upregulation of DUSP5 and downregulation of SCD1 (Figure 3B).

DUSP5 downregulates SCD1 expression in pancreatic cancer

Next, qPCR and western blot analysis were conducted to further validate the downregulation of SCD1 in the two cell lines PANC-1

and MIA PaCa-2. The results indicated that SCD1 was significantly downregulated in the DUSP5 overexpression group compared to the control group (Figure 3C,D). Subsequently, we detected the expression of DUSP5 and its correlation with SCD1 expression in 35 PDAC specimens. We found that the expression levels of the two molecules were negatively correlated in PDAC specimens (Figure 3E,F; $r = 0.359$, $P < 0.05$, $n = 35$). Similarly, in the tissues of xenograft tumors, DUSP5 and SCD1 exhibited a negative correlation (Figure 3G). Therefore, the above experimental results suggested that DUSP5 may regulate the proliferation of pancreatic cancer by downregulating the expression of SCD1.

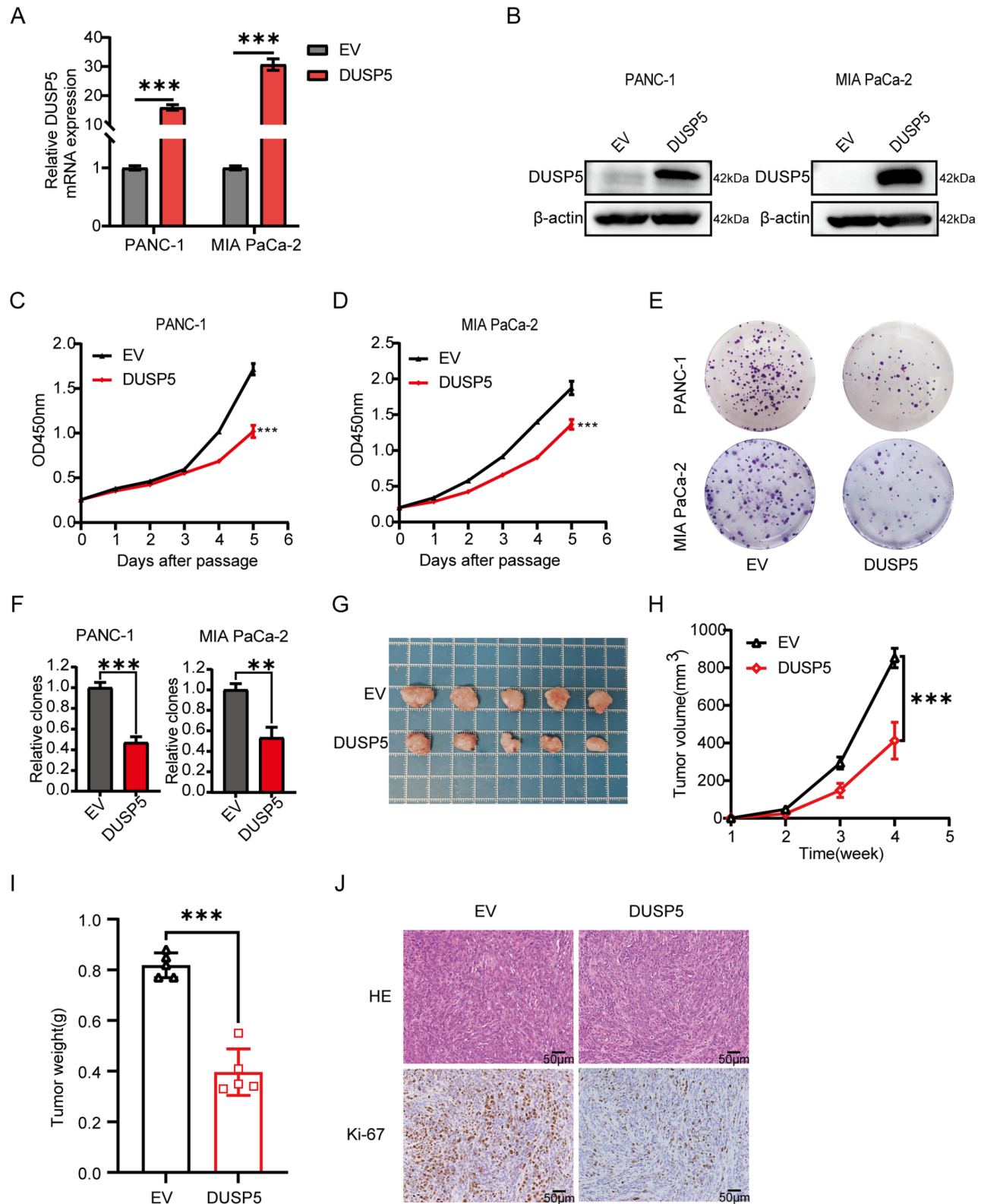


Figure 2. DUSP5 overexpression suppress PDAC proliferation *in vitro* and *in vivo* (A) The overexpression effect of DUSP5 was analyzed by qRT-PCR in PANC-1 and MIA PaCa-2 cells. (B) Western blot analysis of the effect of DUSP5 overexpression. (C–E) CCK-8 assays (C,D) and colony formation assays (E,F) were used to test the proliferation of PDAC cells. (G–I) *In vivo*, overexpression of DUSP5 significantly inhibited the tumorigenic ability and growth capacity of PANC-1 cells. (J) HE staining and IHC of the proliferation index (Ki-67) from different groups. The level of Ki-67 was significantly reduced in tumors formed by PANC-1 cells overexpressing DUSP5. Scale bar: 50 μm. Data are presented as the mean ± SD from at least three independent experiments. Statistical significance was determined by Student's *t*-test. ***P* < 0.01 and ****P* < 0.001.

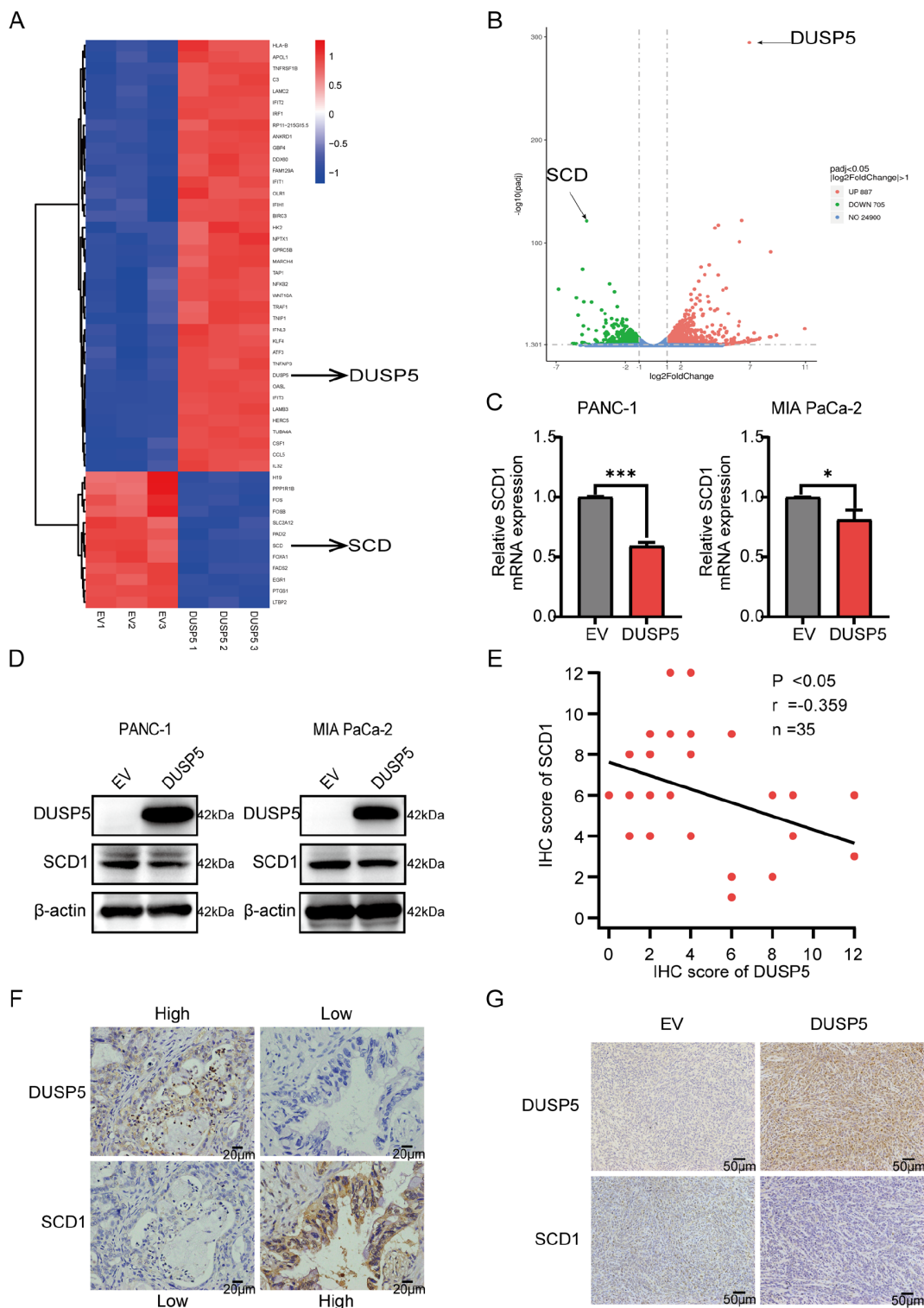


Figure 3. DUSP5 downregulates SCD1 expression in pancreatic cancer (A,B) The heatmap (A) and the volcano plot (B) of gene alterations for EV and overexpressing DUSP5 in PANC-1 cells. (C) The downregulated SCD1 was analyzed by qRT-PCR in PANC-1 ($n = 3$, $***P < 0.001$) and MIA PaCa-2 ($n = 3$, $*P < 0.05$) cells of DUSP5 overexpression. (D) Western blot analysis of the downregulated SCD1. (E,F) Correlation analysis of DUSP5 expression and SCD1 expression in PDAC tissues, as determined by the IHC score ($*P < 0.05$). Scale bar: 20 μm . (G) DUSP5 and SCD1 exhibited a negative correlation in the tissues of xenograft tumors. Scale bar: 50 μm .

DUSP5 promotes ferroptosis in pancreatic cancer cells
The above experiments showed that DUSP5 could downregulate the expression of SCD1. SCD1, as an enzyme that catalyzes the rate-

limiting step in the conversion of saturated fatty acids (SFAs, C16:0 and C18:0) to MUFAs (C16:1 and C18:1), can regulate ferroptosis [26], in which lipid peroxidation is the most significant character-

istic. Subsequently, oxidative lipids were detected using the BODIPY 581/591C11 probe, and subsequent analysis was performed via flow cytometry and confocal microscopy. When lipid peroxidation occurs, the fluorescence shifts from red to green. Confocal microscopy results revealed that DUSP5 increased lipid peroxidation levels (Figure 4A). Subsequent electron microscopy analysis of cellular mitochondria revealed mitochondrial shrinkage, increased membrane density, decreased cristae density, and disrupted mitochondrial membrane integrity (Figure 4B). Similarly, flow cytometry analysis confirmed this finding (Figure 4C,D). Furthermore, lipid peroxidation could result in the accumulation of intracellular ROS, increased levels of MDA, and decreased GSH levels, leading to mitochondrial shrinkage and mitochondrial membrane rupture. The aforementioned experimental assays were conducted in PANC-1 and MIA PaCa-2 stable cell lines overexpressing empty vector or DUSP5 to validate this hypothesis. Consistent with our predictions, DUSP5 was observed to induce elevation of ROS and MDA levels (Figure 4E–G), accompanied by a reduction in the GSH/GSSG ratio (Figure 4H). Subsequently, pancreatic cancer cells overexpressing empty vector or DUSP5 were subjected to mass spectrometry for fatty acid profiling. The results revealed a statistically significant reduction in MUFAs (palmitoleic acid C16:1 and oleic acid C18:1), while no statistically significant differences were detected in saturated fatty acids (palmitic acid C16:0 and stearic acid C18:0) between the experimental groups (Figure 4I). Collectively, these findings suggest that DUSP5 promotes ferroptosis.

Oleic acid and palmitoleic acid reverse DUSP5-induced ferroptosis and cell proliferation inhibition in pancreatic cancer cells

To further confirm that DUSP5 overexpression induces ferroptosis and subsequent proliferation inhibition through downregulation of MUFA biosynthesis, the following experiments were conducted. Confocal microscopy and ROS assays revealed that the addition of oleic acid (50 μ M) and palmitoleic acid (50 μ M) to cells reversed the lipid peroxidation and ROS accumulation induced by DUSP5 overexpression, respectively (Figure 5A,B). Fer-1, one of the primary inhibitors of ferroptosis, similarly reversed lipid peroxidation and ROS accumulation induced by DUSP5 overexpression (Figure 5A,B). Subsequently, CCK-8 and colony formation assays demonstrated that OA, POA, and Fer-1 reversed the inhibition of cell proliferation induced by DUSP5 overexpression (Figure 5C–F). These experimental results indicated that DUSP5 induces ferroptosis, thereby leading to cell proliferation inhibition in pancreatic cancer cells. As shown in Figure 5B, treatment with OA, POA, or Fer-1 reduced ROS levels in control cells. However, these treatments did not significantly affect cell proliferation or colony formation in control cells (Figure 5C–F). These data reveal an apparent uncoupling between ROS levels and proliferative/clonogenic phenotypes under basal, non-stressed conditions.

DUSP5 interacts with ERK1/2 and inhibits its activation, thereby suppressing SCD1 expression

To elucidate the regulatory mechanism of DUSP5-mediated downregulation of SCD1 expression in pancreatic cancer, Co-IP and immunofluorescence assays were conducted. As previously mentioned, DUSP5 negatively regulates ERK1/2 in the MAPK pathway. To further validate the interaction between DUSP5 and ERK1/2 *in*

vivo, total protein extracts were prepared from Flag-tagged DUSP5-overexpressing PANC-1 and MIA PaCa-2 cells, and Co-IP assays were performed following the manufacturer's protocol for the Flag-tag Protein IP Assay Kit with Magnetic Beads. The results demonstrated that DUSP5 co-immunoprecipitated with ERK1/2, indicating their physical interaction in pancreatic cancer cells (Figure 6A). Additionally, IF staining confirmed the colocalization of DUSP5 and ERK1/2 within the nucleus, thereby validating their endogenous interaction (Figure 6B). To further investigate whether DUSP5 exerts its function through modulating ERK1/2 signaling pathway phosphorylation, we examined the expression levels of ERK1/2 and its phosphorylated form (p-ERK1/2). The results revealed that overexpression of wild-type DUSP5 (DUSP5^{WT}) significantly reduced the level of p-ERK1/2, whereas the DUSP5^{C263S} mutant did not exhibit such an effect (Figure 6C). Similarly, a negative correlation was observed between the expression levels of DUSP5 and p-ERK1/2 in human PDAC tissues and xenograft tumors in nude mice (Figure 6D,E). To investigate the regulatory relationship between ERK1/2 inactivation and SCD1 expression in pancreatic cancer, western blot and qPCR assays were performed. The pancreatic cancer cell lines PANC-1 and MIA PaCa-2 were treated with varying concentrations of U0126 (0, 10, or 20 μ M), a selective MAPK/ERK kinase (MEK) inhibitor. The results demonstrated that 20 μ M U0126 significantly induced dephosphorylation of p-ERK1/2 in both cell lines. Concurrently, SCD1 expression at both the mRNA and protein levels was markedly downregulated in a dose-dependent manner following ERK1/2 inhibition (Figure 6F,G). Furthermore, IHC staining in human PDAC tissues and xenograft tumors established in nude mice revealed a significant positive correlation between p-ERK1/2 and SCD1 expression (Figure 6H,I). These findings suggest that ERK1/2 signaling may transcriptionally or post-transcriptionally regulate SCD1 expression in pancreatic cancer.

In summary, DUSP5 inactivates ERK1/2 through direct interaction, thereby downregulating SCD1 expression (Figure 7). This cascade subsequently reduces MUFAs, promotes ferroptosis, and ultimately suppresses pancreatic cancer cell proliferation.

Discussion

PDAC represents a major global health challenge characterized by low early diagnosis rates and high mortality. Despite its relatively low incidence (3% of all cancer diagnoses), PDAC accounts for 7% of global cancer deaths, underscoring its lethal nature [27]. Although systemic therapies have advanced, pancreatic cancer remains characterized by low resectability rates (< 20% at diagnosis), with chemotherapy remaining the cornerstone of treatment for both resectable and metastatic disease. Current standard-of-care chemotherapy regimens include gemcitabine monotherapy, the FOLFIRINOX combination (5-fluorouracil, leucovorin, irinotecan, and oxaliplatin), and nab-paclitaxel plus gemcitabine. While these regimens have extended median overall survival to 11–28 months in advanced PDAC, objective response rates remain disappointingly low (< 10%) in metastatic settings, highlighting critical limitations in therapeutic efficacy. There is an urgent need to identify novel therapeutic targets.

Oncogenic *KRAS* mutations are observed in over 90% of PDAC cases, driving constitutive activation of the Ras/Raf/MEK/ERK signaling cascade and facilitating tumor progression [28]. Despite three decades of intensive research, direct *RAS* inhibition remains

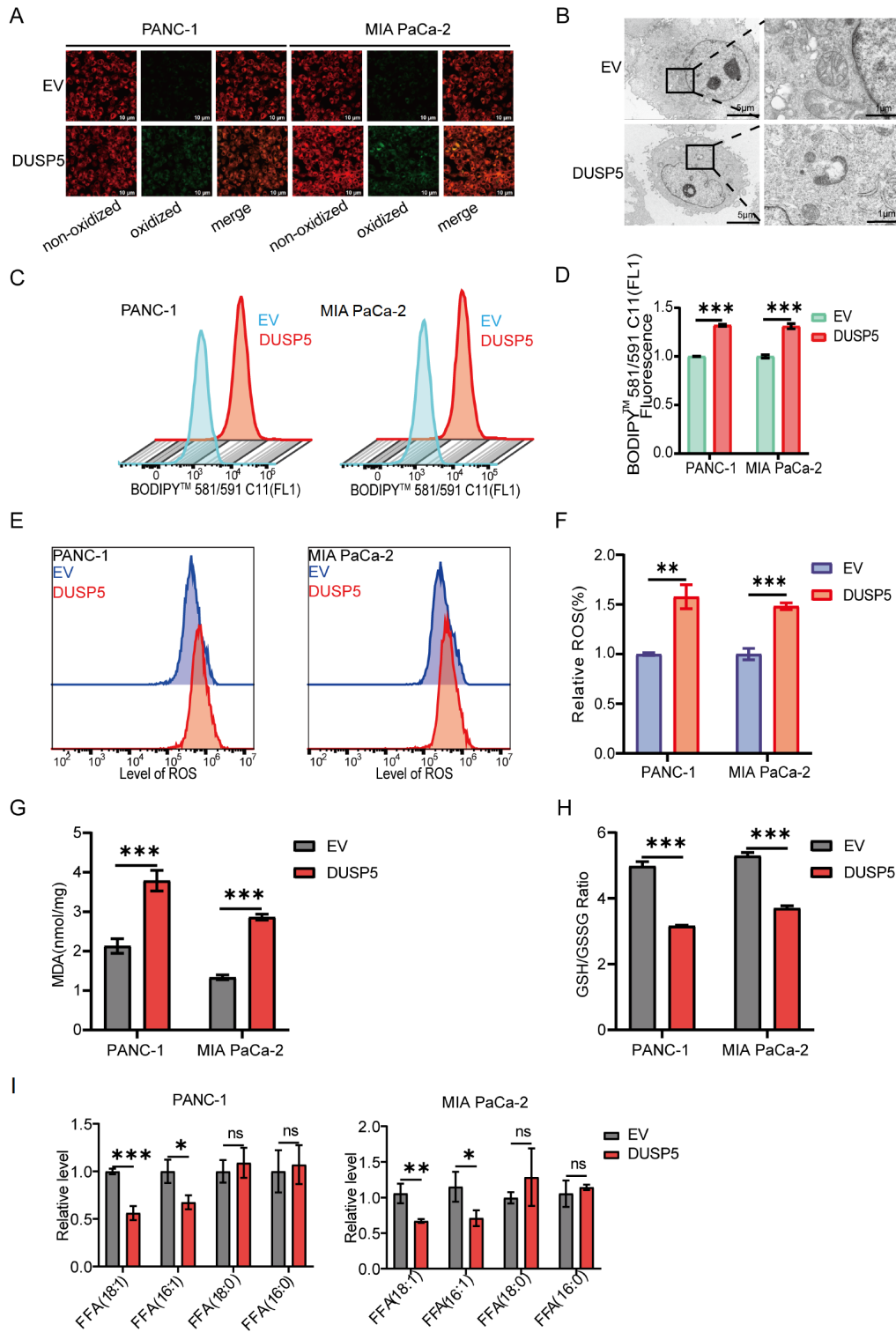


Figure 4. DUSP5 promotes ferroptosis in pancreatic cancer cells (A) Confocal imaging revealed the effect of DUSP5 on lipid peroxidation. Scale bar: 10 μ m. (B) TEM imaging was conducted in DUSP5-overexpressing PANC-1 cells. The result showed the shrunken mitochondria, increased membrane density, and disrupted mitochondrial membrane integrity. Scale bars: 5 μ m (left); 1 μ m (right). (C,D) BODIPY 581/591C11 was detected in the DUSP5-overexpressing PANC-1 and MIA PaCa-2 cells. (E,F) DUSP5 resulted in the accumulation of intracellular ROS. (G) Concentrations of MDA were detected in DUSP5-overexpressing PANC-1 and MIA PaCa-2 cell lines. (H) The GSH/GSSG ratio were detected in DUSP5-overexpressing PANC-1 and MIA PaCa-2 cell lines. (I) Fatty acid profiling were detected in PANC-1 and MIA PaCa-2 stable cell lines ectopically expressing empty vector or DUSP5 by mass spectrometry. Data are presented as the mean $\pm$ SD from at least three independent experiments. Multiple groups were compared using oneway analysis of variance (ANOVA). Statistical significance was determined by Student's *t*-test. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. ns, not significant.

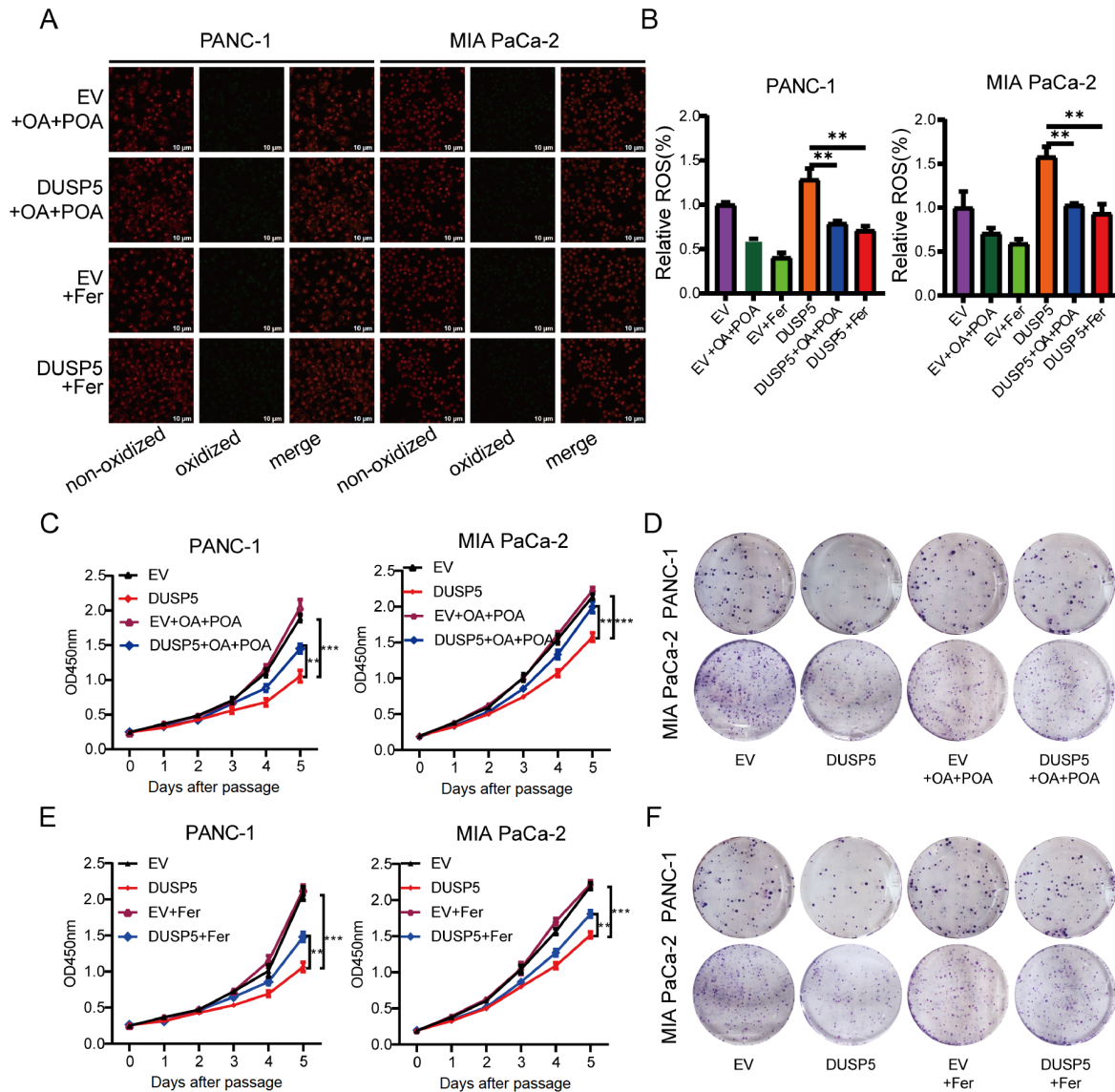


Figure 5. Oleic acid and palmitoleic acid reverse DUSP5-induced ferroptosis and cell proliferation inhibition in pancreatic cancer cells (A) Confocal imaging detected the lipid peroxidation by addition of oleic acid and palmitoleic acid, or Fer-1. Scale bar: 10 μ m. (B) ROS accumulation induced by DUSP5 overexpression was reversed by addition of oleic acid and palmitoleic acid, or Fer-1. (C,D) CCK-8 assays and colony formation assays were used to test the proliferation of PDAC cells by addition of oleic acid and palmitoleic acid. (E,F) CCK-8 assays and colony formation assays were used to test the proliferation of PDAC cells by addition of Fer-1. Concentrations were: oleic acid (50 μ M), palmitoleic acid (50 μ M), and Fer-1 (2 μ M). Data are presented as the mean $\pm$ SD from at least three independent experiments. Multiple groups were compared using oneway analysis of variance (ANOVA). Statistical significance was determined by Student's *t*-test. ***P* < 0.01 and ****P* < 0.001.

an unmet clinical challenge. This challenge stems from the protein's picomolar GTP/GDP affinity, the lack of druggable binding pockets, and complex resistance mechanisms mediated by parallel signaling networks. Accumulating evidence has highlighted the critical role of ERK1/2, the terminal effectors of this pathway, in regulating tumor initiation, proliferation, metastasis, and therapeutic resistance, which has been increasingly underscored by emerging evidence [16]. Our previous studies have demonstrated that the expression of p-ERK1/2 exhibits significant variability among pancreatic cancer patients and is correlated with prognosis [25]. Therefore, identifying therapeutic targets to inhibit p-ERK1/2 activation holds paramount importance in improving clinical outcomes.

DUSP5 can negatively regulate the MAPK signaling pathway,

specifically acting on ERK1/2 in the nucleus to dephosphorylate it [29]. Similarly, our study demonstrated that DUSP5 mediated the dephosphorylation of p-ERK1/2 in pancreatic cancer while exhibiting no significant effect on the expression levels of total ERK1/2. In contrast, DUSP5^{C263S}, a catalytically inactive mutant of DUSP5 in which the active-site cysteine residue was mutated to serine (C263S), failed to mediate the dephosphorylation of p-ERK1/2, and ERK activation was maintained. This observation was consistent with previously reported findings [30]. The dephosphorylation and inactivation of p-ERK1/2 resulted in the suppression of tumor proliferation.

Accumulating studies have shown that DUSP5 inhibits tumor proliferation in gastric cancer, colorectal cancer, and cholangiocar-

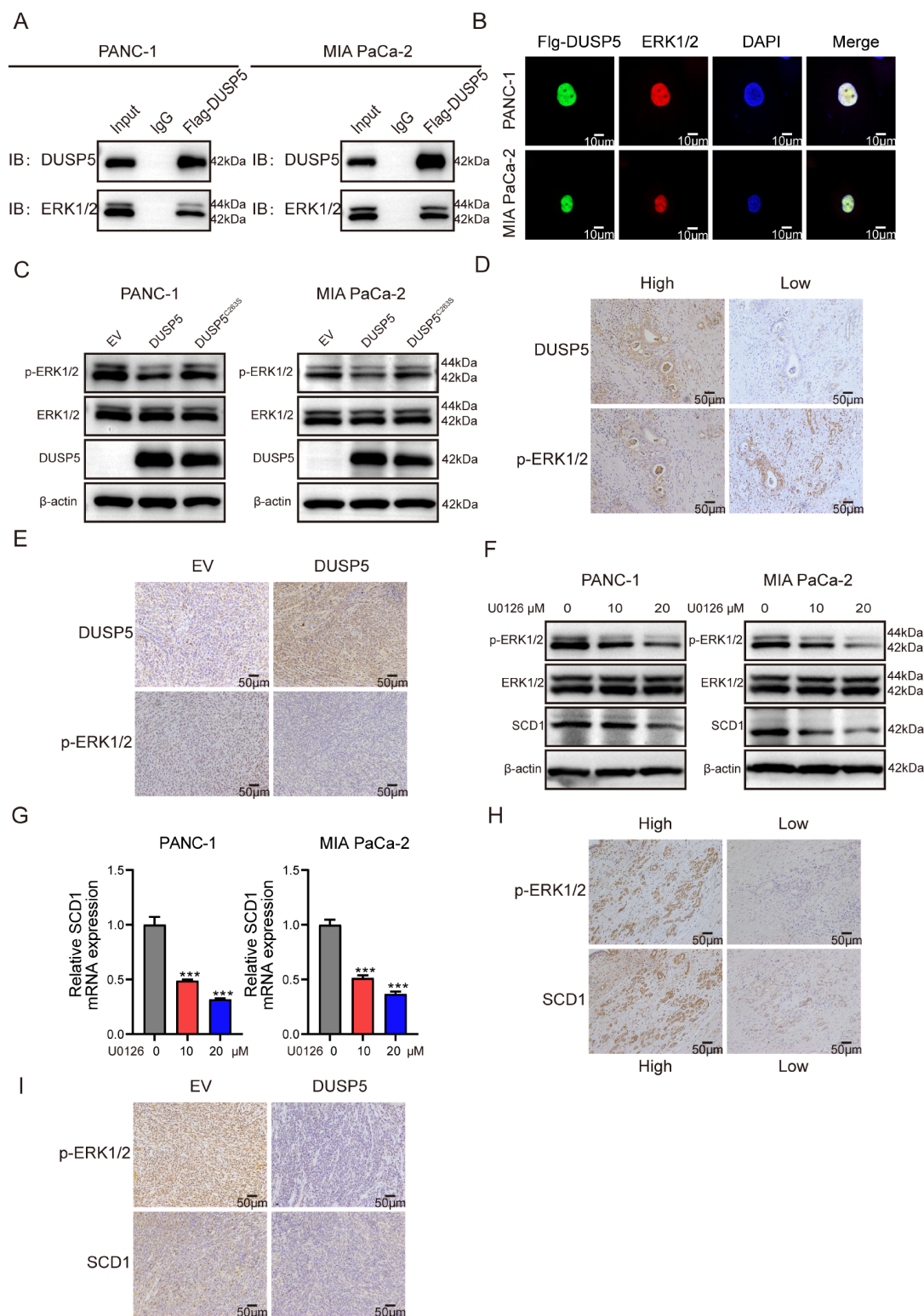


Figure 6. DUSP5 interacts with ERK1/2 and inhibits its activation, thereby suppressing SCD1 expression (A) Co-IP validated DUSP5 interaction with ERK1/2 in PANC-1 and MIA PaCa-2 cells. (B) Colocalization of DUSP5 and ERK1/2 in PANC-1 and MIA PaCa-2 cells as detected by confocal microscopy. Scale bar: 10 μ m. (C) Overexpression of DUSP5 but not DUSP5^{C263S} led to the significant decrease of p-ERK1/2. (D,E) Representative images of IHC staining of DUSP5 and p-ERK1/2 in PDAC tumors and xenograft tumors. Scale bar: 50 μ m. (F,G) Western blot and qRT-PCR analyses detected the downregulated SCD1 in PANC-1 and MIA PaCa-2 cells in the presence of U0126 (10 or 20 μ M). (H,I) Representative images of IHC staining of p-ERK1/2 and SCD1 in PDAC tumors and xenograft tumors. Scale bar: 50 μ m. Data are presented as the mean $\pm$ SD from at least three independent experiments. Multiple groups were compared using oneway analysis of variance (ANOVA). Statistical significance was determined by Student's *t*-test. *** P < 0.001.

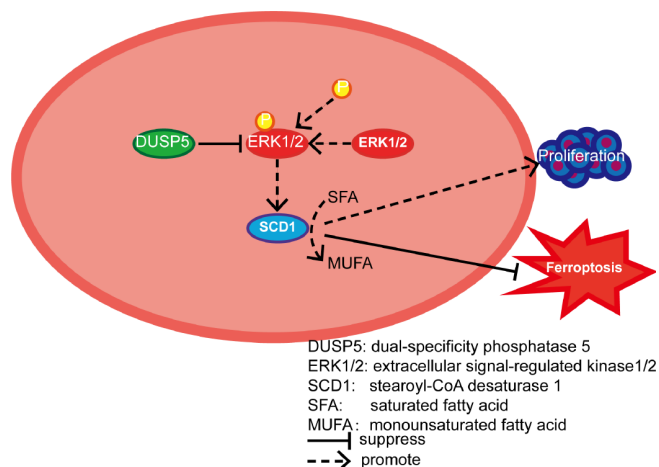


Figure 7. DUSP5 suppresses pancreatic cancer cell proliferation via ERK1/2-SCD1-mediated ferroptosis A graphical summary showing that DUSP5 downregulates SCD1 expression by inactivating ERK1/2, which reduces MUFAs, thereby promoting ferroptosis, and ultimately suppressing pancreatic cancer cell proliferation.

cinoma [6–8]. However, limited research has been conducted on the role of DUSP5 in pancreatic cancer. Our study demonstrated that *DUSP5* inhibited the proliferation of pancreatic cancer in both *in vitro* and *in vivo* experiments. Furthermore, DUSP5 was downregulated in tumor tissues compared with adjacent normal tissues, and the expression of DUSP5 was correlated with the prognosis of pancreatic cancer patients.

To further explore the molecular mechanisms underlying DUSP5-mediated suppression of pancreatic cancer proliferation, RNA sequencing was performed, which revealed a significant downregulation of SCD1. SCD1 is a lipogenic enzyme responsible for converting saturated fatty acids into monounsaturated fatty acids (MUFAs) and acts as a critical regulator of fatty acid metabolism. SCD1 expression has been implicated in poor prognosis in lung adenocarcinoma, gastric cancer, and colorectal cancer [31–33]. Our findings from western blot and qPCR analyses further confirmed that overexpression of DUSP5 downregulated SCD1 expression in pancreatic cancer. Furthermore, a negative correlation between SCD1 and DUSP5 expression was observed in human pancreatic cancer tissues and xenograft tumors. These results were consistent with findings reported in other tumor types. Notably, SCD1 functions as a critical mediator of ferroptosis—an iron-dependent form of regulated cell death—while its upregulation confers cytoprotective effects against ferroptotic stress in cancer cells. Our previous studies demonstrated that both overexpression of FBW7 and knockdown of *NCOA6* downregulated SCD1 expression, thereby promoting ferroptosis in pancreatic cancer or enhancing its sensitivity to ferroptosis-inducing agents [26,34].

A series of experiments were conducted to investigate ferroptosis, including confocal microscopy, flow cytometry, ROS measurement, MDA detection, and GSH quantification. These experiments collectively confirmed that DUSP5 promotes ferroptosis in pancreatic cancer cells. Subsequent electron microscopy analysis of cellular mitochondria revealed mitochondrial shrinkage, increased membrane density, reduced cristae abundance, and compromised mitochondrial membrane integrity. Since SCD1 facilitates the conversion of saturated fatty acids to monounsaturated fatty acids, our findings demonstrated that DUSP5 downregulated SCD1,

leading to reduced production of monounsaturated fatty acids (C16:1 and C18:1). Subsequent rescue experiments were conducted, where supplementation with C16:1 and C18:1 in pancreatic cancer cells inhibited ferroptosis, consistent with the effects observed following the addition of ferroptosis inhibitors. The discrepancy that these agents reduced ROS without altering proliferation or colony formation in control cells can be explained by the low basal ROS level under physiological conditions. Further lowering ROS in unstressed cells provides no additional growth advantage and therefore does not change proliferative or clonogenic activity. In contrast, OA, POA, and Fer-1 mainly function under stress conditions characterized by excessive ROS accumulation or ferroptosis activation. These results suggest that the tested compounds modulate redox homeostasis in a context-dependent manner without interfering with normal cell physiology. Importantly, the inhibition of ferroptosis in pancreatic cancer cells reversed the suppression of cell proliferation caused by DUSP5 overexpression. These results indicate that the inhibitory effect of DUSP5 on the proliferation of pancreatic cancer cells is associated with its induction of ferroptosis.

Our findings demonstrated that regulatory relationships existed not only between DUSP5 and ERK1/2 and between DUSP5 and SCD1 but also between ERK1/2 and the expression of *SCD1*. U0126, a MEK inhibitor, dephosphorylates and inactivates p-ERK1/2. Our findings revealed that treatment with 20 μ M U0126 significantly reduced the expression levels of both SCD1 mRNA and protein. Previous studies demonstrated that reduced expression of SCD1 is correlated with ERK1/2 inactivation in breast cancer [35]. Similarly, a positive correlation between p-ERK1/2 and SCD1 expression was observed in human pancreatic cancer tissues and xenografts. However, the regulatory mechanism by which ERK1/2 modulates SCD1 expression remains poorly defined to date. Conversely, in metabolic disorders, leptin has been reported to suppress *SCD1* transcription via activation of ERK1/2 [36]. It has been documented that the activation of ERK1/2 can activate the transcription factor SREBP-2 (sterol regulatory element-binding protein 2) in hepatocellular carcinoma [37]. In breast cancer, the inhibition of ERK1/2 activity suppressed epithelial-mesenchymal transition (EMT) through the downregulation of *SREBP1* transcription [38]. SREBP1, which transcriptionally regulates SCD1 as a key target, plays a crucial role in the regulation of tumor ferroptosis [39]. We therefore speculate that in pancreatic cancer, ERK1/2 may mediate the regulation of *SCD1* expression through the transcription factor SREBP1. Further investigations are warranted to elucidate this interaction in depth.

DUSP5 downregulation in PDAC is regulated by epigenetic and transcriptional mechanisms. Epigenetic silencing via DNA methylation (e.g., *DUSP5* promoter hypermethylation observed in CIMP-high colorectal cancer [7]) may also extend to PDAC, requiring further validation via bisulfite sequencing across subtypes. Transcriptionally, wild-type p53 directly activates DUSP5 expression by binding to its promoter [40], but *TP53* mutations (approximately 70% in PDAC) disrupt this pathway. Additionally, natural compounds, such as quercetin, induce DUSP5 through serum response factor (SRF), suggesting that SRF or PDAC-dysregulated transcription factors (e.g., SREBPs, NF- κ B) may repress *DUSP5* transcription [41]. Future ChIP assays should map transcription factor-binding sites and histone modifications to clarify these regulatory networks, advancing therapeutic target identification.

Several limitations of the present study should be acknowledged.

First, the correlation between DUSP5 expression and distinct molecular subtypes or mutational backgrounds (e.g., KRAS status) of PDAC remains unclear and requires further investigation. In addition, several potential challenges still exist for the clinical translation of our findings, which need to be overcome in future studies.

In summary, our study identified DUSP5 as a tumor suppressor that inhibits pancreatic cancer proliferation both *in vitro* and *in vivo*. Mechanistically, DUSP5 interacts with ERK1/2, inducing its dephosphorylation and inactivation, which subsequently down-regulates SCD1 expression. The reduction in SCD1 leads to decreased biosynthesis of MUFAs, thereby promoting ferroptosis in pancreatic cancer cells. Collectively, these findings elucidate a novel regulatory pathway (the DUSP5-ERK1/2-SCD1-MUFA-ferroptosis axis) in pancreatic cancer pathogenesis and highlight DUSP5 as a potential prognostic biomarker and therapeutic target for clinical intervention.

Data Availability

The raw sequence data reported in this paper have been deposited in the Gene Expression Omnibus under accession number GSE336205. All other data supporting the findings of this study, including raw data and original images, are available from the corresponding author upon reasonable request.

Author Contributions

Zhilei Zhang, Yuming Jia, and Chao Wang: data editing curation; formal analysis; investigation; validation; visualization; writing—original draft. Guixiong Fan, Borui Li and Desheng Jing: investigation; methodology; supervision. Xiang Liu: investigation; resources. Wuhan Yang: investigation; software. Chong Zhang: investigation; formal analysis. Xiaowu Xu and Yi Qin: conceptualization; methodology; project administration; supervision; writing—review and editing. Li Peng: conceptualization; funding acquisition; project administration; supervision; writing—original draft, review, and editing.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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