

Targeted inhibition of OTUD7B enhances therapeutic sensitivity in KRAS- mutant pancreatic ductal adenocarcinoma by promoting ubiquitination of DHODH

Lirui Tang

Clinical Oncology School of Fujian Medical University

Zhirui Zeng

The Affiliated Hospital of Guizhou Medical University

Shan Lei

Guizhou Medical University

Fei Huang

The Affiliated Hospital of Guizhou Medical University

Yating Sun

Guizhou Medical University

Haohao Zeng

Guizhou Medical University

Huading Tai

Guizhou Medical University

Hangyi Li

The Affiliated Hospital of Guizhou Medical University

Huahai Kuang

Guizhou Medical University

Wanyuan Ruan

Guizhou Medical University

Wenpeng Cao

Guizhou Medical University

Yunhuan Zhen

The Affiliated Hospital of Guizhou Medical University

Yiran Chen

chengyiran@bjmu.edu.cn

Clinical Oncology School of Fujian Medical University

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Abstract

Background

Pancreatic ductal adenocarcinoma (PDAC) is frequently driven by oncogenic KRAS and remains highly refractory to therapy. Ferroptosis represents a therapeutically exploitable vulnerability in KRAS-mutant PDAC, yet the molecular mechanisms that sustain ferroptosis resistance remain incompletely understood.

Methods

An in vivo CRISPR/Cas9 screen was performed in KRAS-mutant and KRAS-wild-type PDAC models under erastin treatment to identify genotype-selective ferroptosis regulators. The function and mechanism of OTUD7B were investigated using PDAC cell lines, patient tissues, biochemical and ubiquitination assays, and spontaneous PDAC mouse models. An OTUD7B-targeting antisense oligonucleotide (ASO) was further evaluated alone or in combination with erastin or anti-PD-1 therapy.

Results

OTUD7B was identified as a selective determinant of erastin resistance in KRAS-mutant PDAC. OTUD7B was preferentially upregulated in KRAS-mutant PDAC, and oncogenic KRAS enhanced OTUD7B transcription through STAT3 activation. Mechanistically, OTUD7B directly interacted with dihydroorotate dehydrogenase (DHODH) and stabilized DHODH by removing K48-linked ubiquitin chains at K169 and K254, thereby reinforcing ferroptosis defense. OTUD7B depletion increased reactive oxygen species, iron accumulation and lipid peroxidation, reduced glutathione levels, and markedly potentiated erastin-induced ferroptosis in vitro and in spontaneous PDAC models. Therapeutic OTUD7B-ASO recapitulated these effects and enhanced the antitumor efficacy of anti-PD-1 therapy, accompanied by increased intratumoral CD8⁺ T-cell infiltration.

Conclusions

These findings establish the KRAS–STAT3–OTUD7B–DHODH axis as a key ferroptosis-protective pathway in KRAS-mutant PDAC and identify OTUD7B as a potential therapeutic target for sensitizing tumors to ferroptosis induction and immunotherapy.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) persists as one of the most fatal malignancies globally, currently positioned as the seventh leading cause of cancer-related mortality, with projections indicating it will ascend to the second leading cause by 2030 [1]. Despite extensive research efforts spanning

several decades, the 5-year overall survival rate for this condition remains below 15% [2]. This dismal prognosis is primarily due to late-stage diagnosis, aggressive tumor biology, and significant resistance to chemotherapy and radiotherapy [3]. The presence of a dense desmoplastic stroma, inadequate vascularization, and a highly immunosuppressive tumor microenvironment (TME) presents formidable barriers [4]. These characteristics reduce the efficacy of existing treatments, often resulting in therapeutic failure in advanced stages. Consequently, there is an urgent clinical imperative to elucidate the unique molecular vulnerabilities of PDAC.

Genetically, oncogenic mutations in the KRAS gene represent the principal driving force behind PDAC, occurring in over 90% of cases [5]. The KRAS^{G12D} variant is detected in approximately 45% of patients [6]. As a key regulator of cellular signaling, mutant KRAS operates as a constitutively active GTPase, persistently activating downstream pathways such as MAPK and PI3K-AKT [7, 8]. These pathways facilitate uncontrolled cellular proliferation, migration, and metabolic reprogramming. In addition to its traditional mitogenic roles, mutant KRAS reconfigures cellular metabolism by enhancing glycolysis, modifying lipid metabolism, and inducing mitochondrial dysfunction and increased oxidative stress [9, 10]. These genotype-driven changes support cellular survival and contribute to the formation of an immune-excluded TME through cytokine signaling and metabolic interactions [11]. The unique molecular and metabolic characteristics of KRAS-mutant tumors make them reliant on specific adaptive mechanisms, thereby providing a rationale for targeting these vulnerabilities. This approach enables the development of genotype-selective therapeutic strategies for KRAS-mutant PDAC.

Ferroptosis is a recently characterized form of regulated cell death, distinguished by the iron-dependent accumulation of lipid peroxides, which ultimately results in membrane rupture [12]. Tumor cells have developed multiple sophisticated anti-ferroptotic defense mechanisms to counteract this lethal process [13]. These defenses include the canonical System xc⁻/GSH/GPX4 axis and the mitochondrial dihydroorotate dehydrogenase (DHODH)-CoQH2 pathway, the latter serving as a critical non-GPX4-dependent defense in the regulation of ferroptosis [14–16]. A notable relationship exists between KRAS-mutant tumors and ferroptosis, wherein oncogenic KRAS imparts certain vulnerabilities to ferroptosis inducers, such as erastin, by modulating iron metabolism [17, 18]. However, PDAC cells paradoxically demonstrate significant intrinsic resistance to ferroptosis, achieved through specific molecular adaptations that suppress this form of cell death [19]. Consequently, therapeutically targeting and overcoming these ferroptosis defense barriers holds significant promise for suppressing the growth of KRAS-mutant PDAC and overcoming chemoresistance.

In this study, we aimed to identify genotype-specific regulators of ferroptosis resistance in KRAS-mutant PDAC. Utilizing *in vivo* CRISPR/Cas9 screening, we identified the deubiquitinase OTUD7B as a crucial determinant of resistance to erastin specifically within KRAS-mutant contexts. Oncogenic KRAS induces the upregulation of OTUD7B through the activation of STAT3. Mechanistically, OTUD7B enhances the stability of DHODH by removing K48-linked ubiquitin chains, thereby strengthening mitochondrial antioxidant defenses. Furthermore, we validated that targeting OTUD7B with a specific antisense oligonucleotide (ASO) increases the sensitivity of PDAC to ferroptosis induction and exhibits synergy

with anti-PD-1 immunotherapy by promoting intratumoral infiltration of CD8⁺ T-cells. Collectively, our results establish the KRAS–STAT3–OTUD7B–DHODH axis as a critical ferroptosis-protective pathway and underscore OTUD7B as a promising therapeutic target for enhancing the sensitivity of KRAS-mutant PDAC to combination therapies.

2. Methods and materials

2.1 Human ethics and PDAC sample collection

Human pancreatic ductal adenocarcinoma (PDAC) and matched adjacent non-tumor tissue specimens were collected from patients who underwent surgical resection at Guizhou Medical University Affiliated Hospital. The diagnosis of all cancer tissue specimens was confirmed through postoperative pathological examination. The study protocol was approved by the Human Research Ethics Review Committee of Guizhou Medical University (Approve number: 202140), and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to sample collection.

2.2 Spontaneous pancreatic ductal adenocarcinoma mouse model

All animal experiments involved in this study were conducted under the supervision and with the approval of the Animal Ethics Committee of Guizhou Medical University (Approve number: 2100017). The spontaneous PDAC mouse model used in this study harbored the LSL-Kras^{G12D/+}; LSL-Trp53^{R172H/+}; Pdx1-Cre genetic background. All mouse strains were obtained from the Shanghai Model Organisms Center, Inc. (Shanghai, China). Mice were housed in a specific pathogen-free barrier facility within individually ventilated cages, with environmental conditions strictly maintained at 22 ± 2°C and 50 ± 10% relative humidity under a 12-hour light/12-hour dark cycle. All animals had ad libitum access to irradiated chow and autoclaved water, and all animal care and experimental procedures were performed in strict accordance with institutional guidelines and animal ethics regulations.

2.3 Cell culture and lentiviral transduction

HPDE and PDAC cells were cultured in DMEM or RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and incubated at 37°C with 5% CO₂. HEK293T cells were maintained under similar conditions. All cell lines underwent short tandem repeat (STR) authentication and were confirmed to be free of mycoplasma contamination. For stable gene knockdown, short hairpin RNAs (shRNAs) cloned into the pLKO.1 vector were packaged in HEK293T cells using the psPAX2 and pMD2.G plasmids. Viral supernatants were collected after 48 hours and used to infect target cells in the presence of polybrene, followed by selection with puromycin. For gene overexpression, Flag-tagged wild-type OTUD7B, catalytically inactive OTUD7B^{C194S}, HA-tagged DHODH, and their mutant variants were transiently transfected using Lipofectamine 3000. To establish PDAC cell lines stably expressing, cells were treated with 1.0 µg/mL puromycin for 14 days post-infection.

2.4 In vivo CRISPR/Cas9 screening

A genome-wide CRISPR/Cas9 library screen was conducted in KRAS-mutant PANC-1 and wild-type BXPC-3 cells, which had been orthotopically implanted in mice. Upon tumor establishment, erastin was administered, and tumors were subsequently harvested for genomic DNA extraction and single-guide RNA (sgRNA) sequencing. The analysis of sgRNA alterations was performed using Model-based Analysis of Genome-wide CRISPR-Cas9 Knockout (MAGeCK).

2.5 CCK-8 assay

The in vitro proliferation ability of PDAC cells was detected using cell count kit-8 (CCK-8) assay. PDAC cells were seeded into 96-well plates at a density of 3,000 cells per well and allowed to adhere overnight. The following day, the culture medium was replaced with fresh medium containing the specified treatments, including OTUD7B-ASO or escalating concentrations of erastin, and the cells were further incubated for an additional 48 h. At the indicated time points, 10 μ L of CCK-8 reagent was added to each well and the plates were incubated at 37°C in the dark. Following a 2-hour incubation period, absorbance was measured at 450 nm using an iMark microplate reader.

2.6 LDH release assay

PANC-1 and AsPC-1 cells, exhibiting logarithmic growth, were seeded into 96-well plates and subsequently subjected to OTUD7B-ASO transfection followed by erastin treatment. At specified time points, culture supernatants were harvested and centrifuged to eliminate cellular debris. Equivalent volumes of the supernatants were then combined with the LDH reaction solution and incubated in the dark. Absorbance was measured at 490 nm following the addition of the stop solution. The release of LDH was quantified as the percentage calculated. Each experiment was conducted in triplicate and repeated on three separate occasions.

2.7 Measurement of intracellular reactive oxygen species

PANC-1 and AsPC-1 cells were seeded into 6-well plates at a density of 2×10^5 cells per well and allowed to adhere overnight. Subsequently, the cells were subjected to treatment with erastin, OTUD7B-ASO, or a combination of both. the culture medium was aspirated, and the cells were incubated with 10 μ M DCFH-DA diluted in serum-free DMEM for 30 minutes at 37°C in the absence of light. Following incubation, the cells were washed twice with 1 $\times$ PBS, harvested, and resuspended in 500 μ L of PBS. Fluorescence intensity was quantified using a flow cytometer with an excitation wavelength of 488 nm and an emission wavelength of 525 nm. Data were analyzed utilizing FlowJo software, and the mean fluorescence intensity was employed to determine relative reactive oxygen species (ROS) levels.

2.8 Ferroptosis detection

Cellular and tissue levels of ferroptosis markers, iron content, malondialdehyde (MDA), and glutathione (GSH), were assessed utilizing specific detection kits in accordance with the manufacturer's protocols. Lipid peroxidation was evaluated through C11-BODIPY 581/591 staining, with quantification performed

using flow cytometry or fluorescence microscopy. *In vivo*, the levels of iron and lipid peroxides in tumor tissues were quantified through biochemical assays, while the expression of 4-hydroxynonenal (4-HNE) was determined using immunofluorescence staining.

2.9 Immunohistochemistry (IHC)

Following deparaffinization and rehydration, tissue sections were subjected to antigen retrieval using a citrate buffer at pH 6.0 by pressure cooker antigen retrieval. The sections were then treated with 3% hydrogen peroxide to inhibit endogenous peroxidase activity and subsequently blocked with 10% goat serum. Primary antibodies (**Supplemental Table S1**) were applied, and the sections were incubated overnight at 4°C. After thorough washing, HRP-conjugated secondary antibodies were introduced, and immunoreactivity was visualized using a DAB substrate, with hematoxylin serving as the counterstain. The sections were then dehydrated, sealed with a mounting medium, and examined under a microscope. IHC scoring was determined by integrating the proportion of positive cells with the intensity of staining.

2.10 quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from PDAC cell lines and tissue specimens utilizing the TRIzol reagent, following the protocol provided by the manufacturer. after which reverse transcription reagents were used to convert the extracted RNA into complementary DNA (cDNA). qRT-PCR was then performed to measure the expression levels of messenger RNA (mRNA), with GAPDH employed as the endogenous internal control to standardize gene expression levels. The relative mRNA expression levels were determined using the comparative $2^{-\Delta\Delta C_t}$ method. Each sample was analyzed in technical triplicates, and the findings are expressed as fold changes relative to the control group. Primers used in study was showed in **Supplemental Table S2**.

2.11 Western blotting

Cells were lysed using RIPA buffer supplemented with protease and phosphatase inhibitors, and protein concentrations were quantified employing a BCA protein assay kit. Equivalent amounts of total protein were resolved via SDS-PAGE and subsequently transferred onto PVDF membranes. The membranes were blocked with 5% skim milk and incubated overnight at 4°C with primary antibodies (**Supplemental Table S1**). After three washes with TBST, the membranes were incubated with HRP-conjugated secondary antibodies for 1 hour at room temperature, and protein bands were visualized using an enhanced chemiluminescence detection system. GAPDH was utilized as the internal loading control. Band intensities were quantified using ImageJ software.

2.12 Untargeted metabolomic analysis

For untargeted metabolomic profiling, cells were harvested and subsequently washed with ice-cold PBS. Metabolites were extracted using a chilled methanol, and the samples were stored at -80°C until further analysis. Post-centrifugation, the supernatants were analyzed via ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS) in both positive and negative ionization modes, employing gradient elution. Quality control samples were interspersed throughout the analytical run to

ensure the stability of the analysis. The raw data underwent processing for peak detection, alignment, and normalization. Metabolic pathway enrichment analysis (MPWEA) was performed to systematically assess the altered metabolic pathways. Differentially abundant metabolites were visualized using hierarchical clustering and presented as heatmaps, with Z-score normalization applied.

2.13 Immunofluorescence staining

Cells were fixed using paraformaldehyde for 30 minutes and subsequently incubated with 0.5% Triton X-100. To minimize non-specific binding, the background was blocked with bovine serum albumin (BSA). The cells were then incubated with a primary antibody including anti-OTUD7B (1:100; ab238678, Abcam, USA), anti-DHODH (1:100; 14877-1-AP, Proteintech, Wuhan, China), and anti-CD8 (1:400; CL488-65113, Proteintech, Wuhan, China) at 4°C for 12 hours, followed by a 2-hour incubation with a fluorescently labeled secondary antibody at room temperature. After counterstaining with DAPI, the sections were examined using a confocal laser scanning microscope.

2.14 Chromatin immunoprecipitation (ChIP)

The cells were cross-linked using 1% formaldehyde and subsequently lysed. Chromatin was fragmented into 200–500 base pair segments via sonication. Immunoprecipitation was carried out employing an anti-STAT3 antibody, with normal rabbit IgG utilized as a negative control. Following a series of washing steps, the protein-DNA complexes were eluted and subjected to reverse cross-linking at 65°C overnight. The purified DNA was subsequently analyzed through quantitative real-time PCR (qPCR) utilizing site-specific primers that flank the predicted STAT3 binding sites within the OTUD7B promoter region. Relative enrichment was determined using the $2^{-\Delta\Delta Ct}$ method, with normalization to the input DNA.

2.15 Dual-luciferase reporter assay

A dual-luciferase reporter assay was performed to confirm the binding of OTUD7B to the STAT3 or the KRAS^{G12D} promoter. Cells were seeded in a 24-well plate and incubated overnight. Firefly luciferase reporter constructs, containing either the wild-type or site-mutated OTUD7B promoter, were co-transfected with specified expression plasmids encoding STAT3. A renilla luciferase plasmid was included as an internal control to assess transfection efficiency. Luciferase activity was measured 24 h post-transfection to evaluate promoter activity.

2.16 Immunoprecipitation coupled with mass spectrometry (IP-MS)

Lysates of PANC-1 cells underwent immunoprecipitation using an anti-OTUD7B antibody or a normal IgG control, followed by capture with Protein A/G magnetic beads. After thorough washing, the immunocomplexes were eluted and subjected to in-solution trypsin digestion. The resulting peptides were analyzed using liquid chromatography-tandem mass spectrometry.

2.17 GST pull-down assay

GST-tagged OTUD7B fusion proteins were expressed in *Escherichia coli* and subsequently immobilized on glutathione-Sepharose beads. These beads were then incubated with cell lysates derived from 293T cells that overexpressed either Flag-tagged DHODH or its structural domain deletion mutants, under conditions of gentle agitation overnight at 4°C. Following this incubation, the beads underwent three washes with elution buffer to remove non-specific interactions. The proteins bound to the beads were then denatured and eluted by boiling in 2 x SDS loading buffer for 5 minutes. The eluates, along with whole-cell lysates serving as input, were analyzed via Western blotting using specific antibodies.

2.18 Protein–protein molecular docking

The three-dimensional structures of OTUD7B and DHODH were retrieved from the Protein Data Bank. Initial protein preparation was conducted in PyMOL 2.5, where all bound ligands, water molecules, and other heteroatoms were removed to optimize the docking surfaces. The cleaned structures were subsequently imported into AutoDock Tools for further processing. Molecular docking simulations were subsequently performed to generate the binding conformations. The resultant binding modes were visualized and analyzed using PyMOL 2.5.

2.19 Ubiquitination assay

The cells were transfected with the specified plasmids and subsequently exposed to MG132 for a duration of 6 hours prior to harvesting. Following this, the cells were lysed using RIPA buffer supplemented with 1% SDS, and sonication was performed on ice. The lysates underwent pre-clearance with Protein A/G Plus-Agarose beads for 1 hour at 4°C. Thereafter, the samples were incubated with the specified primary antibodies overnight at 4°C. The immune complexes were collected by centrifugation at 10,000 ×g for 5 minutes at 4°C and were washed four times with the lysis buffer. Ubiquitination was subsequently assessed by western blotting.

2.20 Subcutaneous tumor model

NC and OTUD7B-knockout KPC1 cells were subjected to trypsinization, quantified, and subsequently resuspended in PBS. A total of 4×10^6 cells were then administered via injection into the right anterior axillary region of 6-week-old C57BL/6 mice. Upon successful tumor establishment, the mice were administered intraperitoneal injections of either an IgG control or an anti-PD-1 antibody every three days. Tumor dimensions, specifically length and width, were assessed every three days utilizing a digital caliper. Tumor volume was calculated using the formula $V = \pi \times \text{Length} \times \text{Width}^2/6$. At the study's conclusion, when the tumor diameter reached 15 mm, mice were euthanized, and tumor tissues were collected for further analysis.

2.21 Statistical analysis

All captured cellular images were analyzed using ImageJ software. Statistical analyses were conducted employing SPSS version 22.0 and GraphPad Prism version 9.0. Quantitative data are presented as the mean ± standard deviation (SD), based on a minimum of three independent experiments. The statistical significance of differences between two independent groups was assessed using an unpaired two-tailed

Student's t-test. For comparisons involving paired samples, a paired two-tailed Student's t-test was employed. A two-tailed *P*-value of less than 0.05 was considered indicative of statistical significance.

3. Results

3.1 Knockdown of OTUD7B specifically potentiates erastin-induced ferroptosis in KRAS-mutant PDAC

KPC mice were first employed to establish a spontaneous PDAC model. Concurrently, at week 10, these mice were administered 20 mg/kg erastin or vehicle control (DMSO) via tail vein injection every one week for a total of 10 treatment cycles (Fig. 1A). Following the treatment regimen, we observed that erastin-treated mice bearing spontaneous PDAC exhibited markedly reduced tumor volumes (Fig. 1B-C) and prolonged overall survival to a certain extent compared to the control group (Fig. 1D).

On this basis, we aim to develop relevant targets to further enhance the inhibitory effect of erastin on KRAS-mutant PDAC. To this end, we selected KRAS-mutant PDAC cells (PANC-1) and KRAS wild-type PDAC cells (BXPC-3), and performed a CRISPR/Cas9 library screen by establishing an orthotopic pancreatic tumor model (Fig. 1E). The CRISPR/Cas9 library screen results showed that, under erastin treatment, the sgRNA abundance of 57 genes was significantly reduced in BXPC-3 cells (β -score ≤ -2 and $P < 0.05$), whereas in PANC-1 cells, the sgRNA abundance of 276 genes was significantly reduced (Fig. 1F). Through intersection analysis, we found that only sgRNA of 270 genes were specifically depleted in KRAS-mutant cells following erastin treatment (Fig. 1G). Among them, the genes with the greatest sgRNA depletion abundance in KRAS-mutant cells after erastin treatment were OTUD7B, TRIM45, SSU72, OTUD5, THBS1, RPL3, WDR43, DHX15, and RNGTT (Fig. 1G). To verify whether these genes are associated with the sensitivity of KRAS-mutant cells to erastin, we overexpressed the aforementioned genes in PANC-1 and BXPC-3 cells, respectively (**Fig. S1A**). The results showed that overexpression of OTUD7B, TRIM45, SSU72, THBS1, WDR43, DHX15, and RNGTT in PANC-1 cells significantly enhanced cell resistance to erastin (Fig. 1H). However, overexpression of these genes in BXPC-3 cells had no such effect (Fig. 1H). Among these, overexpression of OTUD7B mediated the strongest resistance to erastin in PANC-1 cells; therefore, we focused our subsequent investigation on OTUD7B.

Three shRNAs targeting OTUD7B were used to construct OTUD7B-knockdown PDAC cells in PANC-1 (KRAS-mutant) and AsPC-1 (KRAS-mutant). The results showed that the first shRNA exhibited the highest knockdown efficiency for OTUD7B (**Fig. S1B**). Therefore, we used the first shRNA to generate OTUD7B-knockdown PANC-1 and AsPC-1 cells. CCK-8 assays revealed that knockdown of OTUD7B significantly enhanced the sensitivity of PANC-1 (Fig. 1I) and AsPC-1 cells (**Fig. S1C**) to erastin. Flow cytometry further demonstrated that OTUD7B knockdown significantly potentiated erastin-mediated increases in ROS levels in both PANC-1 (Fig. 1J) and AsPC-1 cells (**Fig. S1D**). Additionally, kit-based assays showed that OTUD7B knockdown significantly enhanced erastin-mediated increases in iron ion (Fig. 1K; Fig.S1E) and MDA levels (Fig.S1G), along with a decrease in GSH levels (Fig.S1F). Similarly,

observation of fluorescence microscopy indicated that lipid peroxide deposition was increased in PANC-1 cells with OTUD7B-knockdown after erastin treatment (Fig. 1L).

Moreover, to further validate the role of OTUD7B in erastin-induced cell death, we performed rescue experiments on OTUD7B-knockdown cells treated with erastin, using the ferroptosis inhibitors DFO and Fer-1, the apoptosis inhibitor Z-VAD, and the necroptosis inhibitor Nec-1. The results demonstrated that the DFO and Fer-1 exerted significant rescue effects, whereas the Z-VAD and the Nec-1 failed to rescue (Fig. 1M; **Fig. S1H**). Furthermore, metabolomic analysis further revealed that among the metabolites with altered abundance in erastin-treated OTUD7B-knockdown cells compared to Erastin-treated NC cells, 13.6% were classified as lipids and lipid-related metabolites (Fig. 1N). Collectively, these findings indicate that knockdown of OTUD7B enhances erastin-induced ferroptosis in KRAS-mutant PDAC cells.

3.2 OTUD7B exhibits elevated expression levels in PDAC harboring KRAS mutations

We next investigated OTUD7B expression in PDAC tissues and cells, particularly in the context of KRAS mutation status. IHC showed that OTUD7B protein levels were not significantly different between KRAS-WT PDAC tissues and their corresponding adjacent non-tumor tissues (Fig. 2A). However, when comparing KRAS-mutant PDAC tissues with their corresponding adjacent tissues, OTUD7B protein expression was markedly upregulated (Fig. 2B). In addition, qRT-PCR further revealed that OTUD7B mRNA levels were specifically elevated in KRAS-mutant PDAC tissues in comparison to adjacent tissues and KRAS-WT PDAC (Fig. 2C-E). Furthermore, we analyzed OTUD7B expression in PDAC tissues using the TIMER cohort, and found that OTUD7B mRNA levels were significantly upregulated in KRAS-mutant PDAC (Fig. 2F). Similarly, through immunofluorescence staining, we observed that OTUD7B protein expression was significantly higher in KRAS-mutant PDAC tissues than in PanIN lesions (Fig. 2G).

Moreover, we detected the mRNA and protein levels of OTUD7B in PDAC cell lines and normal pancreatic ductal epithelial cell HPDE. qRT-PCR (Fig. 2H) and western blotting assays (Fig. 2I) indicated that, compared with its expression in HPDE, OTUD7B mRNA and protein levels were significantly up-regulated in KRAS-mutant PDAC cell lines; however, only a modest upregulation of both mRNA and protein levels was observed in the KRAS-wild-type PDAC cell line BxPC-3.

3.3 KRAS upregulates OTUD7B transcription through the activation of STAT3

To investigate why OTUD7B is highly expressed in KRAS-mutant PDAC, we examined whether KRAS regulates OTUD7B expression. To this end, PANC-1 and AsPC-1 cells were treated with KRpep-2d (KR), a KRAS^{G12D} inhibitory peptide. The results showed that KR treatment significantly reduced both the mRNA (Fig. 3A) and protein levels (Fig. 3B) of OTUD7B in PANC-1 and AsPC-1 cells. Conversely, ectopic expression of KRAS^{G12D} by plasmid transfection markedly increased OTUD7B expression at both the mRNA (Fig. 3C) and protein levels (Fig. 3D) in these cells. Collectively, these findings indicate that oncogenic KRAS positively regulates OTUD7B expression in PDAC cells.

Previous studies have shown that KRAS can promote the transcription of target genes by inducing the phosphorylation and activation of multiple transcription factors. We therefore sought to identify the transcription factors through which KRAS enhances OTUD7B expression. Using the PROMO (<http://alggen.lsi.upc.es/>), ChIPBase (<https://rnasysu.com/chipbase3/>), and HumanTFDB databases (<http://bioinfo.life.hust.edu.cn/HumanTFDB/>), we predicted potential transcription factors that may regulate OTUD7B transcription. This analysis identified SPI1, ELK1, STAT3, and YY1 as candidate transcription factors targeting OTUD7B (Fig. 3E). We next performed ChIP-PCR assays in PANC-1 and AsPC-1 cells transfected with a KRAS^{G12D}-expressing plasmid. Among the four candidate transcription factors, only STAT3 exhibited significantly increased binding to the OTUD7B promoter following KRAS^{G12D} overexpression in PANC-1 (Fig. 3F) and AsPC-1 cell (**Fig. S2A**).

We next investigated how STAT3 promotes OTUD7B transcription. Using the JASPAR database (<https://jaspar.elixir.no/>), we obtained STAT3 motif (Fig. 3G) and predicted potential STAT3-binding sites within the OTUD7B promoter. Four putative STAT3-binding sites were identified at positions – 2868 to – 2860 (site 1), – 2004 to – 1996 (site 2), – 1597 to – 1589 (site 3), and – 913 to – 905 (site 4) relative to the transcription start site (Fig. 3H). ChIP-PCR assays using site-specific primers (**Fig. S2B**) revealed that STAT3 selectively bound to site 3 within the predicted OTUD7B promoter region (Fig. 3I). Furthermore, dual-luciferase reporter constructs harboring mutations at each predicted binding site were generated (Fig. 3J) and transfected into PANC-1 and AsPC-1 cells. Notably, only mutation of site 3 significantly attenuated the STAT3 overexpression-induced increase in OTUD7B promoter activity (Fig. 3K). Collectively, we indicated that STAT3 transcriptionally activates OTUD7B primarily through direct binding to site 3 within the OTUD7B promoter.

Furthermore, analysis of PDAC samples from TCGA revealed a positive correlation between STAT3 and OTUD7B expression in PDAC (Fig. 3L). Consistent with this finding, in our cohort of KRAS-mutant PDAC specimens, tissues with high OTUD7B expression more frequently exhibited elevated levels of KRAS and p-STAT3 (Fig. 3M; **Fig. S2C**). Furthermore, western blotting showed that treatment with a STAT3 inhibitor reversed the KRAS^{G12D} overexpression-induced increase in OTUD7B protein levels in PANC-1 (Fig. 3N) and AsPC-1 cells (**Fig. S2D**). These findings suggest that oncogenic KRAS promotes STAT3 phosphorylation and activation, thereby enhancing STAT3-mediated transcription of OTUD7B (**Fig. S2E**).

3.4 OTUD7B binds with DHODH and increased its protein stability in KRAS mutated PDAC

OTUD7B is a cysteine protease-type deubiquitinase whose biological functions are primarily mediated through the deubiquitination of its substrate proteins [20]. To investigate how OTUD7B regulates ferroptosis in KRAS-mutant PDAC cells, we performed IP-MS using an anti-OTUD7B antibody. The analysis identified XXX potential OTUD7B-interacting proteins in PANC-1 cells (**Table.S2**). Notably, DHODH, a key regulator of ferroptosis, was among the most abundant proteins identified in the OTUD7B immunoprecipitates (**Table.S3**). Based on this finding, we subsequently focused on characterizing the interaction between OTUD7B and DHODH.

Through endogenous Co-IP assays, we demonstrated an interaction between OTUD7B and DHODH in PANC-1 and AsPC-1 cells (Fig. 4A). To further validate this association, 293T cells were co-transfected with Flag-tagged OTUD7B and HA-tagged DHODH expression constructs, followed by exogenous Co-IP analysis. Consistently, OTUD7B was found to physically interact with DHODH in 293T cells (Fig. 4B–E). Moreover, GST pull-down assays confirmed a direct interaction between OTUD7B and DHODH in 293T, whereas no binding was detected with GST alone (Fig. 4F). To analyze the binding model between OTUD7B and DHODH, molecule docking was performed. We found that the interaction between OTUD7B and DHODH is primarily mediated by hydrogen bonds formed between Glu339 and Gly224 within the OTU domain of OTUD7B and Lys169 and Lys254 within the DHOD domain of DHODH (Fig. 4G). Consistent with the molecular docking results, exogenous co-immunoprecipitation assays in 293T cells showed that deletion of either the OTU domain of OTUD7B (Fig. 4H) or the DHOD domain of DHODH (Fig. 4I) markedly impaired the interaction between the two proteins. These results suggest that OTUD7B interacts with DHODH through its OTU domain, which binds to the DHOD domain of DHODH.

Moreover, transfection of an OTUD7B construct with deletion of the catalytic sites failed to increase DHODH expression in PANC-1 and AsPC-1 cells (Fig. 4J). Moreover, OTUD7B knockdown reduced DHODH protein levels in both PANC-1 and AsPC-1 cells, whereas this effect was attenuated by treatment with the proteasome inhibitor MG132 (Fig. 4K). Consistently, CHX chase assays showed that overexpression of wild-type OTUD7B markedly enhanced the protein stability of DHODH in PANC-1 and AsPC-1 cells (Fig. 4L). Taken together, OTUD7B binds with DHODH and increased its protein stability in KRAS mutated PDAC.

3.5 OTUD7B suppresses K48-linked ubiquitination of DHODH at K169 and K254 site

To investigate the mechanism by which OTUD7B regulates DHODH protein stability, ubiquitination assays were performed. We found that knockdown of OTUD7B markedly increased the ubiquitination level of DHODH in both PANC-1 (Fig. 5A) and AsPC-1 cells (Fig. 5B). In contrast, overexpression of wild-type OTUD7B significantly reduced DHODH ubiquitination in PANC-1 and AsPC-1 cells, whereas the catalytically inactive OTUD7B mutant failed to exert a similar effect (Fig. 5C–D). Furthermore, *in vitro* ubiquitination assays yielded consistent results, demonstrating that wild-type OTUD7B directly decreased DHODH ubiquitination, while the enzymatically inactive OTUD7B mutant lost this regulatory function (Fig. 5E). Collectively, these results indicate that OTUD7B stabilizes DHODH by suppressing its ubiquitination.

We next sought to determine the ubiquitin linkage type through which OTUD7B regulates DHODH ubiquitination. HEK293T cells were transfected with wild-type ubiquitin or ubiquitin mutants retaining only lysine 48 (K48) or lysine 63 (K63), followed by ubiquitination assays. Overexpression of wild-type OTUD7B markedly reduced DHODH ubiquitination in cells expressing either wild-type ubiquitin or the K48-only ubiquitin mutant, whereas the catalytically inactive OTUD7B mutant failed to exert this effect (Fig. 5F). In contrast, no comparable reduction in DHODH ubiquitination was observed in cells expressing

the K63-only ubiquitin mutant (Fig. 5F). Meanwhile, HEK293T cells were transfected with wild-type ubiquitin, the K48R ubiquitin mutant, or the K63R ubiquitin mutant. The results showed that OTUD7B retained its ability to reduce DHODH ubiquitination in cells expressing either wild-type ubiquitin or the K63R mutant (Fig. 5G). In contrast, this effect was markedly attenuated in cells expressing the K48R ubiquitin mutant (Fig. 5G). These findings suggest that OTUD7B preferentially removes K48-linked ubiquitin chains from DHODH.

Moreover, potential ubiquitination residues in DHODH were first predicted using the GPS online platform (<https://gps.biocuckoo.cn/online.php>), followed by experimental validation of the candidate sites using ubiquitination assays. To determine which residues were involved in K48-linked ubiquitination, HEK293T cells were co-transfected with HA-tagged DHODH mutants together with Myc-tagged K48 ubiquitin. The ubiquitination analysis revealed that K169 and K254 were the major residues responsible for K48-linked ubiquitination of DHODH (Fig. 5H; **Fig. S3**). To further determine whether these residues were targeted by OTUD7B-mediated deubiquitination, DHODH mutants carrying substitutions at K169 or K254 were generated. HEK293T cells were subsequently co-transfected with HA-tagged DHODH mutants, Myc-tagged K48 ubiquitin, and Flag-tagged OTUD7B. The results demonstrated that OTUD7B markedly reduced K48-linked ubiquitination of DHODH associated with the K169 and K254 residues (Fig. 5I). Taken together, these evidences indicated that OTUD7B suppresses K48-linked ubiquitination of DHODH at K169 and K254 site.

3.6 Knockdown of OTUD7B increased the sensitivity of erastin in spontaneous PDAC mouse model

Given the ability of OTUD7B to reduce DHODH ubiquitination, we hypothesized that OTUD7B-depletion might decrease DHODH expression, thereby weakening the ferroptosis defense capacity of PDAC cells and increasing their sensitivity to erastin. To test this hypothesis *in vivo*, we used an AAV-mediated approach to knock down OTUD7B expression in a spontaneous PDAC mouse model and evaluated the response of PDAC tumors to erastin treatment (Fig. 6A). We found that AAV-mediated OTUD7B knockdown alone had no significant effect on spontaneous PDAC tumor growth; however, it markedly enhanced the tumor-suppressive effect of erastin (Fig. 6A-B). Moreover, mice receiving the combination of AAV-mediated OTUD7B knockdown and erastin exhibited significantly prolonged overall survival compared with those treated with erastin alone (Fig. 6C).

IHC analysis of PDAC tissues from each treatment group showed that OTUD7B depletion alone markedly reduced DHODH expression, while having no significant effect on CK19 expression (Fig. 6D-E). However, when combined with erastin treatment, OTUD7B depletion led to a significant reduction in CK19 expression in PDAC tissues (Fig. 6D-E). Additionally, OTUD7B depletion slightly increased the levels of iron and lipid peroxides in PDAC tissues (Fig. 6F-G). Notably, OTUD7B depletion markedly potentiated the erastin-induced accumulation of both iron and lipid peroxides (Fig. 6F-G). Consistently, immunofluorescence staining demonstrated that combined OTUD7B depletion and erastin treatment

markedly elevated intratumoral 4-HNE levels, a recognized marker of lipid peroxidation, in PDAC tissues (Fig. 6H).

3.7 OTUD7B-ASO potentiates erastin-mediated ferroptosis in KRAS-mutation PDAC

Recently, more and more attention has been paid to the development of ASO, which offer several advantages over traditional drugs, such as enhanced targeting specificity and prolonged efficacy [21, 22]. We developed three kinds of OTUD7B-ASO with 2'-MOE terminal five bases and phosphorothioate backbone modification (Fig. 7A), which all significantly suppressed the OTUD7B level of both at the mRNA and protein levels (Fig. 7B-C). Among the three ASOs, OTUD7B-ASO2 exhibited the strongest ability to inhibit OTUD7B (Fig. 7C). Therefore, this ASO was selected for subsequent studies.

Ubiquitination assays demonstrated that treatment with OTUD7B-ASO significantly increased the ubiquitination levels of DHODH protein in PANC-1 and AsPC-1 cells (Fig. 7D). CCK-8 (Fig. 7E) and LDH release assay (Fig. 7F) further showed that OTUD7B-ASO enhanced the ability of erastin to inhibit the proliferation of PANC-1 and AsPC-1 cells, as well as inducing cell death. Flow cytometry analysis for ROS indicated that OTUD7B-ASO enhanced the ability of erastin to increase ROS level in PANC-1 and AsPC-1 cells (Fig. 7G). Furthermore, analysis of ferroptosis-associated markers indicated that the combined treatment further increased iron content (Fig. 7H), oxidized lipids (Fig. 7I), and MDA levels (Fig. 7J), while causing a more pronounced depletion of GSH (Fig. 7K). Taken together, these evidences indicated that OTUD7B-ASO potentiates erastin-mediated ferroptosis in KRAS-mutation PDAC *in vitro*.

3.8 OTUD7B-ASO synergizes with anti-PD-1 therapy to suppress the progression of KRAS-mutant PDAC

Previous studies have demonstrated that inducing ferroptosis in tumor cells represents a promising strategy to enhance the responsiveness to immunotherapies, including anti-PD-1 treatment [23]. Given the role of OTUD7B in regulating DHODH stability and ferroptosis, we investigated whether OTUD7B inhibition could synergize with anti-PD-1 therapy. NC and OTUD7B-knockout KPC1 cells were subcutaneously implanted into mice. After tumor establishment, the mice were treated with either IgG control or an anti-PD-1 antibody (Fig. 8A). It was demonstrated that knockdown of OTUD7B slightly reduced the proliferation of KPC1 proliferation *in vivo*, but significantly increased the inhibitory effects of PD-1 antibody (Fig. 8A). Similarly, we found that tumor tissues derived from OTUD7B-knockdown after PD-1 antibody treatment exhibited the smallest size (Fig. 8B-C). No significant differences in body weight were observed among mice bearing different tumor types or receiving different treatment regimens (Fig. 8D). Moreover, IHC for tumor tissues indicated that tumor tissues derived from OTUD7B-knockdown after PD-1 antibody treatment exhibited the highest CD8 expression and lowest KI67 expression (Fig. 8E-F).

Furthermore, KPC tumor-bearing mice were treated with OTUD7B-ASO, an anti-PD-1 antibody, or the combination of both (Fig. 8G). Compared with either OTUD7B-ASO or anti-PD-1 monotherapy, the

combination treatment significantly prolonged overall survival (Fig. 8H) and markedly reduced tumor burden (Fig. 8I). Consistently, multiplex immunofluorescence analysis revealed that combined OTUD7B-ASO and anti-PD-1 treatment significantly increased CD8 expression within tumor tissues (Fig. 8J-K), indicating enhanced infiltration of cytotoxic T cells into the tumor microenvironment. Taken together, these results suggested that OTUD7B-ASO synergizes with anti-PD-1 therapy to suppress the progression of KRAS-mutant PDAC.

4. Discussion

PDAC persists as one of the most lethal human malignancies, distinguished by its significant resistance to conventional therapies and a highly immunosuppressive TME[24]. Oncogenic KRAS signaling primarily drives this immunosuppressive environment. It is characterized by marked T cell dysfunction [25, 26]. Substantial infiltration of immunosuppressive cells and dense stromal fibrosis are also prominent. Collectively, these features constitute a prototypical “cold tumor” phenotype [27]. The KRAS-driven TME impedes cytotoxic T cell infiltration and promotes immune evasion, contributing to the immunosuppressive and refractory nature of PDAC.

Recent evidence has identified ferroptosis as a therapeutically exploitable vulnerability in tumors with KRAS mutations [28]. Ferroptosis is an iron-dependent form of regulated cell death driven by lipid peroxidation [29]. Nonetheless, PDAC cells paradoxically display intrinsic resistance to the induction of ferroptosis, indicating the presence of complex molecular defense mechanisms that support tumor survival under oxidative stress conditions [30, 31]. Our study identifies the deubiquitinase OTUD7B as a crucial factor in conferring resistance to ferroptosis in KRAS-mutant PDAC and elucidates the KRAS–STAT3–OTUD7B–DHODH axis as a key ferroptosis-protective pathway. These findings provide a mechanistic foundation for genotype-selective therapeutic strategies and highlight the potential of targeting OTUD7B to enhance the sensitivity of PDAC to ferroptosis induction and immunotherapy.

OTUD7B is a member of the ovarian tumor (OTU) domain-containing deubiquitinase family, serving as a critical regulator of the ubiquitin-proteasome system and playing a significant role in the pathogenesis of various human diseases [20]. Importantly, OTUD7B demonstrates context-dependent dual functionality in both neoplastic and non-neoplastic environments. It is overexpressed in several solid tumors, such as non-small cell lung cancer and pancreatic cancer, where it facilitates tumor progression by stabilizing oncoproteins like YAP1 and β -catenin [32, 33]. In contrast, in hepatocellular carcinoma and lymphoma, OTUD7B may act as a tumor suppressor by stabilizing p53 or TRAF3, thereby inhibiting NF- κ B signaling [34, 35]. This context-specific behavior highlights the necessity of comprehending OTUD7B's regulatory network within the distinct molecular landscape of KRAS-mutant PDAC. Our research elucidates that oncogenic KRAS induces upregulation of OTUD7B through the activation of STAT3, thereby establishing a direct connection between the most common driver mutation in PDAC and the maintenance of resistance to ferroptosis. At the mechanistic level, OTUD7B enhances the stability of DHODH by cleaving K48-linked ubiquitin chains at lysine residues K169 and K254, thereby strengthening the mitochondrial antioxidant defense system. DHODH has recently been identified as a crucial suppressor of ferroptosis

independent of GPX4, and our findings uncover a previously unrecognized regulatory layer of this pathway through deubiquitination [36, 37]. Significantly, a recent study has shown that OTUD7B can protect cardiomyocytes from ferroptosis by deubiquitinating HNF4 α [38], indicating that OTUD7B-mediated regulation of ferroptosis may represent a conserved mechanism across various pathophysiological contexts.

The therapeutic potential of targeting OTUD7B encompasses more than merely modulating ferroptosis sensitivity [39, 40]. Our research demonstrates that pharmacological inhibition of OTUD7B via ASOs not only amplifies erastin-induced ferroptosis but also acts synergistically with anti-PD-1 immunotherapy, as evidenced by increased intratumoral infiltration of CD8⁺ T-cells. This finding is consistent with recent studies in PDAC, which indicate that the induction of ferroptosis can effectively transform the immunosuppressive tumor microenvironment from a “cold” to a “hot” state, thereby enhancing the efficacy of checkpoint blockade therapies. Moreover, research has shown that targeting ferroptosis regulators, such as METTL3 and TRIM21, can augment antitumor immunity in pancreatic cancer [41]. This study integrates ferroptosis induction with immune checkpoint blockade, offering a promising therapeutic strategy for PDAC. Our findings identify OTUD7B as a pivotal component in this approach. Notably, the application of ASOs presents several advantages over small-molecule inhibitors, including increased target specificity, extended efficacy, and the potential for tissue-selective delivery [42–44]. Collectively, these results advocate for the clinical translation of therapies targeting OTUD7B in KRAS-mutant PDAC.

Several limitations of this study merit careful consideration. Although our findings elucidate the KRAS–STAT3–OTUD7B–DHODH axis in KRAS-mutant PDAC, the functional significance of this regulatory network in other cancer types remains to be investigated. Given the conserved role of OTUD7B in mediating deubiquitination and the universal function of DHODH in lipid metabolism modulation, future research is necessary to determine whether this axis contributes to ferroptosis resistance in other lipid-dependent cancers. Furthermore, while our studies provide compelling preclinical evidence supporting the efficacy of OTUD7B ASOs, the optimization of delivery strategies and combination regimens in clinical settings remains an area for further exploration. Nonetheless, our findings reveal a previously unrecognized mechanism by which oncogenic KRAS maintains ferroptosis defense and identify OTUD7B as a therapeutically viable target for overcoming treatment resistance in PDAC.

5. Conclusion

In conclusion, our research identifies the KRAS–STAT3–OTUD7B–DHODH axis as a pivotal pathway conferring protection against ferroptosis in KRAS-mutant PDAC. Importantly, the targeted inhibition of OTUD7B enhances tumor sensitivity to ferroptosis induction and augments the efficacy of anti-PD-1 immunotherapy. This positions OTUD7B as a promising therapeutic target for overcoming treatment resistance and improving clinical outcomes in this highly lethal malignancy.

Declarations

Ethics approval and consent to participate:

Human pancreatic ductal adenocarcinoma (PDAC) and matched adjacent non-tumor tissue specimens were collected from patients who underwent surgical resection at the Affiliated Hospital of Guizhou Medical University. The study protocol was approved by the Human Research Ethics Review Committee of Guizhou Medical University (Approval No. 202140) and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to sample collection. All animal experiments were approved by the Animal Ethics Committee of Guizhou Medical University (Approval No. 2100017) and were performed in accordance with institutional guidelines and relevant animal ethics regulations.

Competing interests:

The authors declare that they have no competing interests.

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Author Contribution

L.T., Z.Z., H.Z., H.T., H.L., and H.K. contributed to investigation. L.T., Z.Z., S.L., F.H., Y.S., H.Z., H.T., H.L., and H.K. contributed to methodology. L.T., S.L., and F.H. contributed to data curation. L.T. contributed to formal analysis. Z.Z. contributed to validation and visualization. S.L., F.H., and Y.S. contributed to software. L.T. and Z.Z. contributed to writing the original draft. W.R., W.C., Y.Z., and Y.C. contributed to conceptualization and project administration. W.R., W.C., and Y.Z. contributed to supervision. Y.Z. and Y.C. contributed to resources. L.T., Z.Z., Y.Z., and Y.C. contributed to funding acquisition. All authors had read and approved to submit the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*. 2021; 71(3): 209–249.
2. Nagaraju GP, Nellipudi H, Ganji C, Kuppala V, Ganji SP, Kumari S, Farran B, Srilatha M, Guenter R, El-Rayes BF. Pancreatic ductal adenocarcinoma: integrating molecular insights for targeted interventions. *Signal Transduct Target Therapy*. 2026;11(1):165.
3. Shah A, Ganguly K, Rauth S, Sheree SS, Khan I, Ganti AK, Ponnusamy MP, Kumar S, Jain M, Batra SK. Unveiling the resistance to therapies in pancreatic ductal adenocarcinoma. *Drug Resist Updates: Reviews Commentaries Antimicrob Anticancer Chemother*. 2024;77:101146.
4. Joseph AM, Al Aiyan A, Al-Ramadi B, Singh SK, Kishore U. Innate and adaptive immune-directed tumour microenvironment in pancreatic ductal adenocarcinoma. *Front Immunol*. 2024;15:1323198.
5. Gurreri E, Genovese G, Perelli L, Agostini A, Piro G, Carbone C, Tortora G. KRAS-Dependency in Pancreatic Ductal Adenocarcinoma: Mechanisms of Escaping in Resistance to KRAS Inhibitors and Perspectives of Therapy. *Int J Mol Sci*. 2023;24(11):9313.
6. Stickler S, Rath B, Hamilton G. Targeting KRAS in pancreatic cancer. *Oncol Res*. 2024;32(5):799–805.
7. Lee JE, Woo MG, Jung KH, Kang YW, Shin SM, Son MK, Fang Z, Yan HH, Park JH, Yoon YC, Kim YS, Hong SS. Combination Therapy of the Active KRAS-Targeting Antibody inRas37 and a PI3K Inhibitor in Pancreatic Cancer. Volume 30. *Biomolecules & Therapeutics*; 2022. pp. 274–83. 3.
8. Tan YQ, Sun B, Zhang X, Zhang S, Guo H, Basappa B, Zhu T, Sethi G, Lobie PE, Pandey V. Concurrent inhibition of pBADS99 synergistically improves MEK inhibitor efficacy in KRASG12D-mutant pancreatic ductal adenocarcinoma. *Cell Death Dis*. 2024;15(2):173.
9. Wang F, Qi XM, Wertz R, Mortensen M, Hagen C, Evans J, Sheinin Y, James M, Liu P, Tsai S, Thomas J, Mackinnon A, Dwinell M, Myers CR, Bartrons Bach R, Fu L, Chen G. p38 γ MAPK Is Essential for Aerobic Glycolysis and Pancreatic Tumorigenesis. *Cancer Res*. 2020;80(16):3251–64.
10. Hafner P, Keller SJ, Chen X, Alrawashdeh A, Jumaa H, Nollmann FI, Besson S, Kemming J, Gorka O, Das T, Appiah B, Lehmann A, Li M, Apostolova P, Bengsch B, Zeiser R, Tholen S, Schilling O, Gross O, Vlachos A, Wittel UA, Von Elverfeldt D, Reichardt W, Boerries M, Andrieux G, Heynen GJ, Fichtner-Feigl S, Hannibal L, Ruess DA. Vertical RAS pathway inhibition in pancreatic cancer drives therapeutically exploitable mitochondrial alterations. *Signal Transduct Target Therapy*. 2026;11(1):33.
11. Huang C, Mai G, Li Y, Liu H, Huang W. KRAS-driven cytokine–metabolic crosstalk shapes immune exclusion in pancreatic ductal adenocarcinoma. *Front Genet*. 2026;17:1899014.

12. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, Morrison B, Stockwell BR. Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. *Cell*. 2012;149(5):1060–72.
13. Yan B, Ai Y, Sun Q, Ma Y, Cao Y, Wang J, Zhang Z, Wang X. Membrane Damage during Ferroptosis Is Caused by Oxidation of Phospholipids Catalyzed by the Oxidoreductases POR and CYB5R1. *Mol Cell*. 2021;81(2):355–e36910.
14. Yang WS, Sriramaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB, Brown LM, Girotti AW, Cornish VW, Schreiber SL, Stockwell BR. Regulation of Ferroptotic Cancer Cell Death by GPX4. *Cell*. 2014;156(1–2):317–31.
15. Bersuker K, Hendricks JM, Li Z, Magtanong L, Ford B, Tang PH, Roberts MA, Tong B, Maimone TJ, Zoncu R, Bassik MC, Nomura DK, Dixon SJ, Olzmann JA. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature*. 2019;575(7784):688–92.
16. Xia T, Liu C, Lin Q, Jiang M, Jin Q, Guo D, Zhu X, Liu N. Co-disruption of GPX4 and DHODH Ferroptosis Defense Systems via Excipient-Free Self-Assembled Nanoassemblies for Enhanced Ferroptosis Therapy in Triple-Negative Breast Cancer. *ACS Appl Mater Interfaces*. 2026;18(6):9471–82.
17. Chen Y, Yan Q, Ruan S, Cui J, Li Z, Zhang Z, Yang J, Fang J, Liu S, Huang S, Hou B, Zhang C. GCLM lactylation mediated by ACAT2 promotes ferroptosis resistance in KRASG12D-mutant cancer. *Cell Rep*. 2025;44(6):115774.
18. ZHAO H, HUANG Q, Ao LIUY. Oncogenic KRAS Promotes Ferroptosis in Pancreatic Cancer Through Regulation of the Fosl1-Tfr Axis. *Pancreas*. 2025;54(3):e235–45.
19. Celesia A, Piccoli F, Wang T, Hu Y, Danzi F, Aparo A, Cisterna B, Pacchiana R, Poles M, Scupoli MT, Luchini C, Ugel S, Donadelli M, Fiore A. Metabolic plasticity underlies ferroptosis resistance driven by mutant p53 in pancreatic ductal adenocarcinoma. *Molecular Cancer*, 2026[2026-09-02].
<https://link.springer.com/10.1186/s12943-026-02746-y>
20. Wang Q, Xu S, Wen L, Meng J, Wang X, Luo T, Luo R, Li Z, Lin D, Yin J. Research progress of OTUD7B: from structural function and disease mechanisms to clinical translation. *Front Immunol*. 2026;17:1850848.
21. Xu L, Zhang H, Jiang B, Jiang Y, Lu H. Antisense Oligonucleotides: Technological Advances, Clinical Progress, and Expanding Therapeutic Frontiers. *Pharmaceutics*. 2026;18(4):446.
22. Çakan E, Lara OD, Szymanowska A, Bayraktar E, Chavez-Reyes A, Lopez-Berestein G, Amero P, Rodriguez-Aguayo C. Therapeutic Antisense Oligonucleotides in Oncology: From Bench to Bedside. *Cancers*. 2024;16(17):2940.
23. Zhang Y, Yu R, Li Q, Song S, Fu Y, Shen X, Xu T, Zhang Y, Liang J, Zhang Z, Xu S, Tang J, Zhao Y, Shen C, Zhuang Y, Zhang J, Wang L, Zou X, Chen D, Lv Y, Zhang S. Epigenetic suppression of Nrf2–Slc40a1 axis induces ferroptosis and enhances immunotherapy in pancreatic cancer. *J Immunother Cancer*. 2025;13(10):e013269.

24. Li M, Wang X, Li P, Gong H, Wu J, Mao Y. Current advances in immunotherapy for KRAS-Mutant pancreatic cancer. *Clin Experimental Med*. 2026;26(1):285.
25. Kern KA. KRAS-driven immune exclusion in pancreatic ductal adenocarcinoma: mechanisms and therapeutic reversal. *Oncologist*. 2026;31(7):oyag160.
26. Wang CA, Hou YC, Hong YK, Tai YJ, Shen C, Hou PC, Fu JL, Wu CL, Cheng SM, Hwang DY, Su YY, Shan YS, Tsai SJ. Intercellular TIMP-1-CD63 signaling directs the evolution of immune escape and metastasis in KRAS-mutated pancreatic cancer cells. *Mol Cancer*. 2025;24(1):25.
27. Kung HC, Zheng KW, Zimmerman JW, Zheng L. The tumour microenvironment in pancreatic cancer - new clinical challenges, but more opportunities. *Nat Rev Clin Oncol*. 2025;22(12):969–95.
28. Zhao L, Deng H, Zhang J, Zamboni N, Yang H, Gao Y, Yang Z, Xu D, Zhong H, Van Geest G, Bruggmann R, Zhou Q, Schmid RA, Marti TM, Dorn P, Peng R W. Lactate dehydrogenase B noncanonically promotes ferroptosis defense in KRAS-driven lung cancer. *Cell Death Differ*. 2025;32(4):632–45.
29. Alarcón-Veleiro C, López-Calvo I, Berjawi L, Lucio-Gallego S, Mato-Basalo R, Quindos-Varela M, Lesta-Mellid R, Santamarina-Caínzos I, Varela-Rodríguez S, Fraga M, Quintela M, Vizoso-Vázquez A, Arufe M, Fafián-Labora J. Ferroptosis: An emerging strategy for managing epithelial ovarian cancer. Volume 187. *Biomedicine & Pharmacotherapy*; 2025. p. 118065.
30. Padhye A, Yu Q, Sindoni N, Zhang H, Dilly J, Feng H, Aguirre A, Mancias J. Abstract C018: Targeting KRAS inhibitor resistance by ferroptosis induction in PDAC. *Cancer Res*. 2024;84(17Supplement2):C018–018.
31. Pereira-Vieira J, Granja S, Celeiro SP, Barbosa-Matos C, Preto A, Queirós O, Ko YH, Casal M, Baltazar F. 3-Bromopyruvate boosts the effect of chemotherapy in acute myeloid leukemia by a pro-oxidant mechanism. *Free Radic Biol Med*. 2025;234:192–202.
32. Wang B, Jie Z, Joo D, Ordureau A, Liu P, Gan W, Guo J, Zhang J, North BJ, Dai X, Cheng X, Bian X, Zhang L, Harper JW, Sun SC, Wei W. TRAF2 and OTUD7B govern a ubiquitin-dependent switch that regulates mTORC2 signalling. *Nature*. 2017;545(7654):365–9.
33. Chen S, Cai K, Zheng D, Liu Y, Li L, He Z, Sun C, Yu C. RHBDL2 promotes the proliferation, migration, and invasion of pancreatic cancer by stabilizing the N1ICD via the OTUD7B and activating the Notch signaling pathway. *Cell Death Dis*. 2022;13(11):945.
34. Ding C, Cao L, Wang R, Wu Q, Li M, Zhang J, Thorne RF, Li J, Ma J, Wu M, Cang S. OTUD7B is a new deubiquitinase targeting p53. *Theranostics*. 2025;15(6):2121–38.
35. Hu H, Brittain GC, Chang JH, Puebla-Osorio N, Jin J, Zal A, Xiao Y, Cheng X, Chang M, Fu YX, Zal T, Zhu C, Sun SC. OTUD7B controls non-canonical NF-κB activation through deubiquitination of TRAF3. *Nature*. 2013;494(7437):371–4.
36. Mao C, Liu X, Zhang Y, Lei G, Yan Y, Lee H, Koppula P, Wu S, Zhuang L, Fang B, Poyurovsky MV, Olszewski K, Gan B. DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. *Nature*. 2021;593(7860):586–90.

37. Yang L, An X, Yang S, Lin X, Chen Z, Xue Q, Chen X, Wang Y, Yan D, Chen S, Fan Y, Tang D, Yu W, Liu J, Chen X. The deubiquitinase USP24 suppresses ferroptosis in triple-negative breast cancer by stabilizing DHODH protein. *Cell Death Dis.* 2025;16(1):564.
38. Zheng R, Song W, Wang C, Du X, Liu C, Sun X, Lu C. Deubiquitinase OTUD7B stabilizes HNF4 α to alleviate pressure overload-induced cardiac hypertrophy by regulating fatty acid oxidation and inhibiting ferroptosis. *Biomark Res.* 2025;13(1):53.
39. Zhang X, Zhang P, Chen X, Liu X, Liu W, Hu X, Sun C, Wang X, Shi J. Deubiquitinase OTUD7B Regulates Cell Proliferation in Breast Cancer. *Clin Breast Cancer.* 2025;25(2):122–e1322.
40. Bonacci T, Emanuele MJ. Dissenting degradation: Deubiquitinases in cell cycle and cancer. *Sem Cancer Biol.* 2020;67:145–58.
41. Mao W, Jiang Q, Feng Y, Peng C, Peng H, Li X, Jiao L, Zhang L, Ma L, Sun T. TRIM21-mediated METTL3 degradation promotes PDAC ferroptosis and enhances the efficacy of Anti-PD-1 immunotherapy. *Cell Death Dis.* 2025;16(1):240.
42. Anand P, Zhang Y, Patil S, Kaur K. Metabolic Stability and Targeted Delivery of Oligonucleotides: Advancing RNA Therapeutics Beyond The Liver. *J Med Chem.* 2025;68(7):6870–96.
43. Tang Y, Zou J, Li Q, Liu Y, Guo X, Bai H, He X, Gan C, Zhao X, Wu W, Xie J, Lee RJ. Small nucleic acid therapeutics: delivery breakthroughs, clinical translation, and future paradigms in precision medicine. *Int J Pharm.* 2025;684:126189.
44. Singh A, Tekade M, Nagaraja S, Bharti A, Tekade RK. Advancements in RNA-based therapies from bench to bedside. *npj Drug Discovery.* 2026;3(1):4.