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The role of HSPB1 in modulating ferroptosis in pancreatic cancer via the TP53/SLC7A11/GPX4 axis

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Abstract

Purpose This study investigates the role of HSPB1 in pancreatic cancer, particularly its impact on cell proliferation and migration through ferroptosis regulation and interaction with TP53

Materials and Methods HSPB1 expression was analyzed using BioGPS and GEPIA databases. BxPC-3 cell lines with stable HSPB1 overexpression and knockdown were created via plasmid transfection and siRNA. The study examined HSPB1's effect on TP53 protein levels and its role in ferroptosis using TP53 agonists and inhibitors.

Results HSPB1 mRNA levels were significantly elevated in pancreatic cancer tissues, and both mRNA and protein levels were notably upregulated in cancer cell lines. HSPB1 overexpression promoted BxPC-3 cell proliferation and migration, while silencing HSPB1 reduced these effects. High HSPB11 expression increased the levels of SLC7A11 and GPX4, while HSPB1 knockdown inhibited their expression. Transmission electron microscopy (TEM) showed that HSPB1 overexpression alleviated erastin-induced cellular damage. Although HSPB1 did not significantly affect TP53 mRNA levels, it reduced the degradation of TP53 protein, thereby enhancing the expression of SLC7A11 and GPX4 and reducing ferroptosis. The TP53 agonist significantly attenuated the effects of HSPB1 overexpression on SLC7A11 and GPX4 expression and partially restored TP53 expression. The TP53 inhibitor reversed the decrease in SLC7A11 and GPX4 expression caused by HSPB1 silencing and reduced the elevated levels of ROS and free iron. Moreover, HSPB1 overexpression reduced lipid ROS production.

Conclusion HSPB1 promotes pancreatic cancer progression by suppressing TP53 signaling and increasing SLC7A11 and GPX4 expression, attenuating ferroptosis. These insights suggest HSPB1 as a potential therapeutic target, warranting further development of specific inhibitors.

Graphical abstract

The figure illustrates the mechanism by which HSPB1 promotes pancreatic cancer progression by inhibiting ferroptosis. HSPB1 regulates the TP53 signaling pathway, resulting in downstream effects on key ferroptotic regulators. Specifically, HSPB1 suppresses TP53, which in turn modulates the expression of SLC7A11 and GPX4. These changes in the expression of SLC7A11 and GPX4 inhibit ferroptosis, a form of regulated cell death. The inhibition of ferroptosis ultimately promotes the survival and proliferation of pancreatic cancer cells, contributing to tumor progression.

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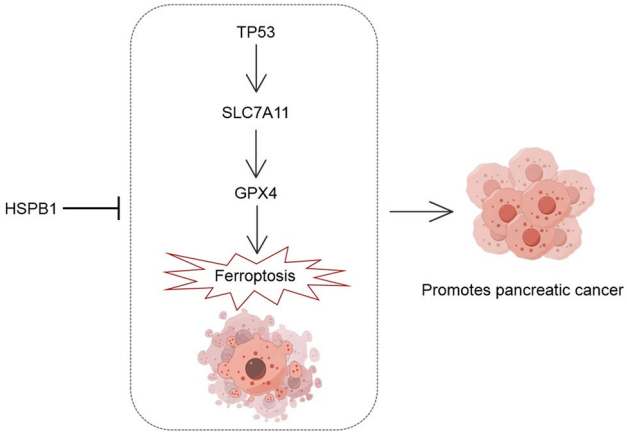


Keywords HSPB1 · Ferroptosis · Pancreatic cancer · TP53 · SLC7A11 · GPX4

1 Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death globally, and the sixth in China, characterized by its exceptionally high malignancy [1]. Despite continuous advancements in treatment, the five-year survival rate for pancreatic cancer patients remains disappointingly low [2]. This is primarily due to the insidious onset of the disease and the poor treatment response, which contribute to its high malignancy. As such, a thorough understanding of the molecular mechanisms driving pancreatic cancer development is crucial for improving patient prognosis.

HSPB1, a key member of the heat shock protein family, plays a critical role in regulating endoplasmic reticulum (ER) stress associated with protein misfolding [3]. Additionally, HSPB1 maintains intracellular glutathione activity, intercellular reactive oxygen species (ROS), and the function of molecular chaperones [4–6]. HSPB1 is highly expressed in osteosarcoma [7], breast cancer [8], esophageal cancer [9], liver cancer [10], and pancreatic cancer [11]. Notably, high expression levels of HSPB1 are associated with poor prognosis, increased tumor invasion, and resistance to chemotherapy in breast cancer [12]. In esophageal squamous cell carcinoma, elevated HSPB1 expression is found in poorly differentiated tissues and is negatively correlated with tissue differentiation [9]. Furthermore, knocking down HSPB1 in gemcitabine-resistant pancreatic cancer cells has been shown to restore their sensitivity to gemcitabine [11].

Ferroptosis is an iron-dependent oxidative cell death caused by lipid peroxide accumulation, elevated intracellular iron levels, and diminished antioxidant defenses [13]. GPX4 regulates ferroptosis by converting lipid hydroperoxides into lipid alcohols, mitigating lipid peroxidation, and impeding ferroptosis progression [14]. Impairment of ferroptosis, such as through the inhibition of GPX4, promotes tumorigenesis and increases cancer cell survival, thus contributing to tumor progression [15]. Recent studies show that the ferroptosis inducer erastin stimulates HSPB1 expression, while HSPB1 knockout enhances erastin-induced ferroptosis. Conversely, HSPB1 overexpression or phosphorylation has been shown to reduce iron-dependent lipid reactive oxygen species, thereby inhibiting ferroptosis and protecting cells from oxidative damage [16]. TP53 is an important biomarker of drug efficacy in pancreatic cancer and a target for individualized treatment strategies, as it plays a critical role in maintaining genomic stability and regulating apoptosis in response to chemotherapy [17]. HSPB1 expression is inversely correlated with nuclear TP53 accumulation [18], suggesting that HSPB1-TP53 protein interactions may be modulated by HSPB1 regulation in pancreatic cancer. However, whether HSPB1 contributes to pancreatic cancer by regulating TP53 remains to be studied.

This study explores the role of HSPB1 in iron-dependent pancreatic cancer cell death and examines the influence of ferroptosis regulators GPX4 and TP53 to clarify the molecular mechanisms of HSPB1 in ferroptosis within pancreatic cancer cells.

2 Materials and methods

2.1 Cell culture

The BxPC-3 cell and PANC-3 cell line were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China. Cells were maintained in RPMI-1640 medium (HyClone) with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco, Rockville, MD, USA) at 37 °C in a 5% CO_2 humidified atmosphere.

2.2 Cell viability assay

HSPB1 vector, empty vector, HSPB1 siRNA, and negative siRNA-transfected BxPC-3 cells were seeded in 96-well plates at 1 × 10^4 cells per well and incubated overnight at 37 °C in a 5% CO_2 incubator. Cells were washed twice with PBS, and 100 µL of RPMI-1640 medium was added. The medium was changed at 0, 24, and 48 h, with 95 µL of medium and 5 µL of CCK-8 added for a 2-h incubation at 37 °C. Optical density (OD) was measured at 450 nm using a microplate reader (Bio-Tech, USA) to detect cell proliferation changes.

2.3 Cell transfection

For the HSPB1 overexpression and silencing experiments, cells were transfected with either HSPB1 vector or HSPB1 siRNA. Specifically, the HSPB1 vector (1 µg/mL, Weizhen Biotechnology, Shandong, China) and HSPB1 siRNA (50 nM, Shanghai Genechem Co., Ltd.) were introduced into the cells plated in a 24-well plate at a density of 1 × 10^5 cells per well. The cells were incubated at 37 °C with 5% CO_2 for 24 h to allow for proper transfection. After 24 h, the medium was aspirated, and the cells were used for subsequent experiments. Transfection was performed using the Lipofectamine 3000 Transfection Reagent (Thermo Fisher Scientific), according to the manufacturer's instructions. Briefly, the HSPB1 vector or HSPB1 siRNA was mixed with the transfection reagent in Opti-MEM medium (Thermo Fisher Scientific) and incubated for 20 min at room temperature before being added to the cells. The transfection efficiency was assessed using a GFP reporter, and the cells were harvested for further analysis after 24 h.

2.4 Wound healing assay

The wound healing assay was conducted to assess the migration ability of pancreatic cancer cells. HSPB1 vector and HSPB1 siRNA were introduced into cells plated in a 24-well plate at a density of 1 × 10^5 cells per well and incubated at 37 °C with 5% CO_2 for 24 h. After the incubation period, the medium was aspirated, and the cell monolayer was wounded using a sterile 10 µL pipette tip to create a scratch in the monolayer. The cells were washed twice with PBS to remove debris, and 1 mL of RPMI-1640 medium was added to each well. Images of the scratch wounds were captured at two time points: 0 h and 24 h. The migration index was calculated based on the measurement of wound closure at both time points. This experiment was performed in triplicate and repeated five times to ensure the consistency and reproducibility of the results. The wound healing assay was analyzed using the ImageJ software (National Institutes of Health, USA) for quantification of migration and wound closure.

2.5 Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from BxPC-3 cells using TRIzol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. RNA (1 µg) was reverse transcribed into cDNA using a PrimeScript RT Reagent Kit (RR047A; TaKaRa, Shiga, Japan). TP53 mRNA levels were quantified by RT-qPCR using the StepOnePlusTM Real-Time PCR System (Applied Biosystems, USA), with GAPDH as the internal control. Primers: TP53 forward 5′-GTTGAGTGGAAAGTACGGAACG-3′, reverse 5′-TGTGGGTGCTTGTGTAACCAG-3′; GAPDH forward 5′-TGTGGGCATCAATGGATTTGG-3′, reverse 5′-ACACCATGTATTCCGGGTCAAT-3′. Relative quantities were determined using the comparative 2−ΔΔCt method.

2.6 Determination of intracellular ferrous iron (Fe^{2+}) content

Intracellular Fe^{2+} levels were determined using a ferrous iron colorimetric (E-BC-K304-S; Wuhan ElLerite Biotechnology Co., Ltd., Wuhan, China) following the manufacturer's instructions. Cells, 48 h after transfection, were collected, washed twice

with PBS, and lysed with cell lysis buffer. Samples were centrifuged at 13,000 × g for 10 minutes at 4 °C, and the supernatants were collected.The Fe^{2+}content was measured by adding the working reagent to the samples, followed by incubation at room temperature for the specified reaction time. The absorbance was recorded at 593 nm using a microplate reader. The Fe^{2+} concentration was calculated based on the standard curve, and the results were normalized to the total protein content, which was determined using a BCA protein assay kit.

2.7 Reactive oxygen species (ROS) assay

Total ROS activity was measured using the Intracellular Total ROS Activity Assay Kit (CA1420; Beijing Solarbio Science & Technology Co., Ltd.). Cells were seeded into 6-well plates, and 48 h after transfection, the original medium was removed. Cells were stained with fresh culture medium containing 10 μmol/L DCFH-DA dye in the dark at 37°C for 30 minutes. After incubation, cells were harvested, washed, and suspended in PBS. ROS levels were then analyzed using a flow cytometer (BD FACSCanto™; USA). The data were processed using FlowJo software, and the percentage of ROS-positive cells and relative fluorescence intensity were quantified. Data are presented as mean ± SEM.

2.8 Detection of intracellular lipid ROS levels using fluorescent probe

To detect intracellular lipid ROS levels, cells were incubated with BODIPY 581/591 C11 fluorescent probe in serum-free DMEM/F12 medium. The probe was diluted to a final concentration of 3 μM. The existing culture medium was removed, and the cells were incubated with the fluorescent probe at 37 °C for 30 min in the dark. After incubation, the cells were washed three times with serum-free medium to remove any residual background fluorescence. Subsequently, PBS was added, and fluorescence images were captured using a fluorescence microscope at emission wavelengths of 581 nm and 500 nm.

2.9 Western blot

Protein was extracted using RIPA lysis buffer containing a protease inhibitor, and the concentration was determined using a Q5000 UV-Vis Spectrophotometer (Quawell, USA). Equal amounts of protein (20 μg) were separated by SDS-PAGE and transferred to PVDF membranes (Whatman Westran, Sigma). Membranes were blocked with 5% skim milk for 1 h, then incubated overnight at 4 °C with primary antibodies: HSPB1 (ab79868, Abcam), TP53 (ab79868, Abcam), GPX4 (#59735, CST), SLC7A11 (R382036, ZEN BIO), and Tubulin (ab7291, Abcam) (1:1000). Membranes were incubated with HRP-conjugated secondary antibodies for 1 h at room temperature, washed, and signals detected using electrochemiluminescence reagents. Gel and blot images were processed and cropped to highlight relevant protein bands. The cropped areas focused on the target proteins, ensuring clear presentation of the data. ImageJ software was used to quantify protein band intensities, and the ratio of target protein to tubulin expression was used as a measure of relative protein levels.

2.10 Statistical analysis

Data were analyzed using GraphPad Prism 9.0 (GraphPad Inc., USA). Measurement data were presented as mean ± standard deviation ($\bar{x} \pm s$); counting data were expressed as frequency (%). One-way ANOVA with Tukey's multiple comparison test was used for multiple group comparisons, and the t-test for two-group comparisons. A significance level of $P < 0.05$ was considered statistically significant.

3 Results

3.1 Expression of HSPB1 in pancreatic cancer

HSPB1 expression in different human organs was investigated. BioGPS database analysis showed intermediate to low HSPB1 expression in pancreatic tissues compared to other tissues (Fig. 1A). GEPIA database revealed significantly upregulated HSPB1 expression in pancreatic cancer (Fig. 1B). Protein level analysis in pancreatic tumor tissues supported these findings (Fig. 1C and D, $P = 0.0153$). qRT-PCR analysis showed elevated HSPB1 transcription levels in multiple pancreatic cancer cell lines compared to normal pancreatic cells (HPDE) (Fig. 1E), further supported by

protein level analysis (Fig. 1F and G). These results suggest that upregulated HSPB1 expression in pancreatic cancer may play a significant role in its pathogenesis and progression.

3.2 Effect of HSPB1 on pancreatic cancer cell proliferation and migration

Stable BxPC-3 cell lines with overexpressed ($P = 0.0303$) and silenced ($P = 0.0066$) HSPB1 were generated, confirmed by quantitative analysis (Fig. 2A and B). Cell proliferation was assessed using the CCK-8 assay. The HSPB1 overexpression group had significantly higher proliferative capacity than the control group starting from 24 h ($P = 0.0049$ & 48 h: $P = 0.0016$). The HSPB1 knockdown group had significantly lower proliferation capacity at 48 h ($P = 0.0008$) (Fig. 2C). Cell migration assays showed that reduced HSPB1 expression resulted in slower migration compared to control cells ($P = 0.004$ and $P = 0.0142$), whereas enhanced HSPB1 expression led to significant scratch width reduction after 24 h ($P = 0.0075$) and near confluence after 48 h ($P = 0.0018$) (Fig. 2D and E). These observations suggest that HSPB1 is a positive regulator of proliferation and migration in BxPC-3 cells.

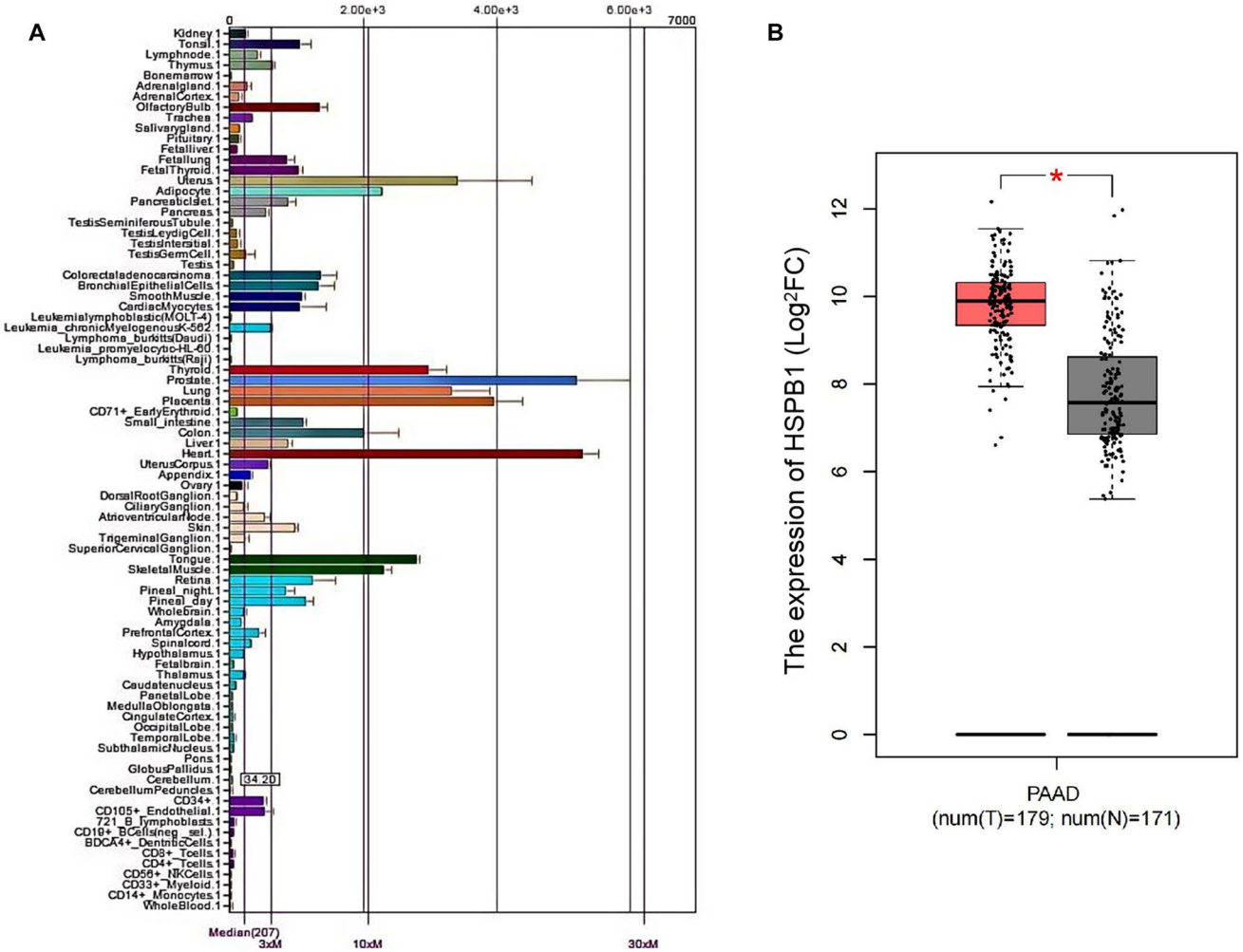


Fig. 1 The expression of HSPB1 was up-regulated in pancreatic cancer. **A** BioGPS database showing HSPB1 expression in different human tissues. **B** The mRNA expression level of HSPB1 in patients with pancreatic cancer in the GEPIA database. Left (red): tumor (N = 179); right (gray): normal (N = 171), $P < 0.05$; error bars denote the mean ± SEM. **C** Pancreatic tumor tissues and paracarcinoma tissues were collected from three patients clinically diagnosed with pancreatic cancer. The tissues were homogenized in RIPA buffer containing complete protease inhibitor, and then Western blot analysis was performed to measure the protein expression of HSPB1 and tubulin. **D** Densitometry was used to determine the fold change in the expression of HSPB1 relative to the expression of tubulin. **E** mRNA expression levels of HSPB1 in pancreatic cancer cell lines. **F** HSPB1 protein expression levels in normal pancreatic cells and pancreatic cancer cell lines. **G** Densitometry was used to determine the fold change in the expression of HSPB1 relative to the expression of tubulin (n = 6 for each group)

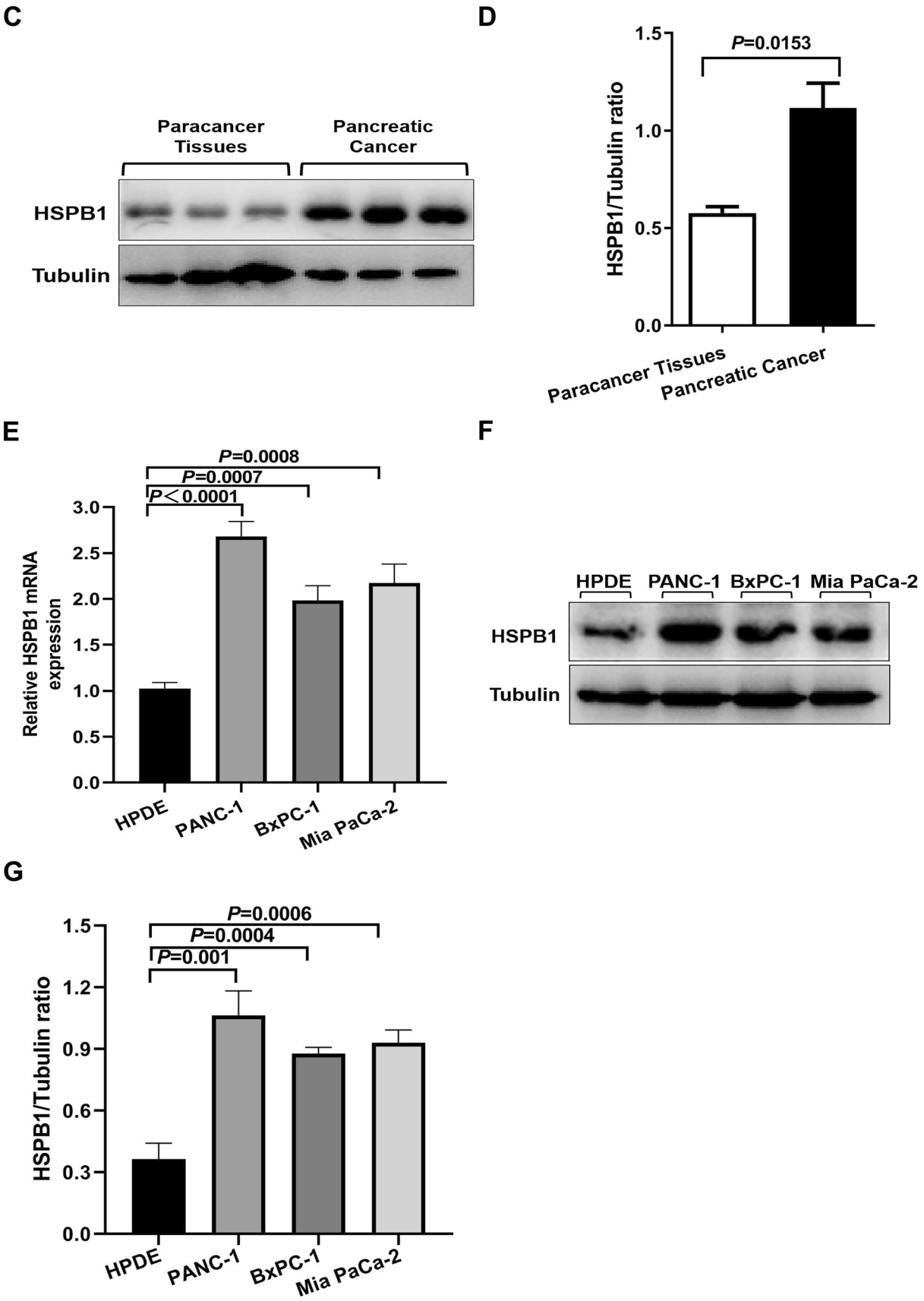


Fig. 1 (continued)

3.3 HSPB1 regulates ferroptosis in BxPC-3

High HSPB1 expression is related to pancreatic cancer development and drug resistance [11]. HSPB1 plays a crucial role in iron metabolism and ferroptosis-mediated cancer therapy [16]. HSPB1 overexpression augments GPX4 and SLC7A11 levels in hypoxic-ischemic rat hippocampus tissues [19]. We validated HSPB1 overexpression or silencing on GPX4 and SLC7A11 levels. HSPB1 overexpression increased GPX4 and SLC7A11 expression, while downregulation attenuated their expression (Fig. 3A–D). Erastin-induced ferroptosis was inhibited by HSPB1 overexpression, as cells co-treated with erastin and pCMV-Cmyc-HSPB1 had more intact mitochondrial structures and reduced damage (Fig. 3E). As shown in Fig. 3F, high HSPB1 expression partially reversed the decrease in cell viability induced by Erastin, whereas HSPB1 silencing significantly enhanced cell death induced by Erastin. These results suggest that HSPB1 protects mitochondrial structure, mitigates Erastin-induced damage, and enhances cell survival in the context of ferroptosis, highlighting its potential role in protecting cells from ferroptosis-related stress.

3.4 The role of TP53 in HSPB1-mediated ferroptosis

TP53 inhibits SLC7A11 expression and cystine uptake, decreasing GPX4 activity and leading to ferroptosis [20]. HSPB1 alleviates H_2O_2-induced premature senescence by reducing TP53 in vascular endothelial cells [21]. We assessed the effect

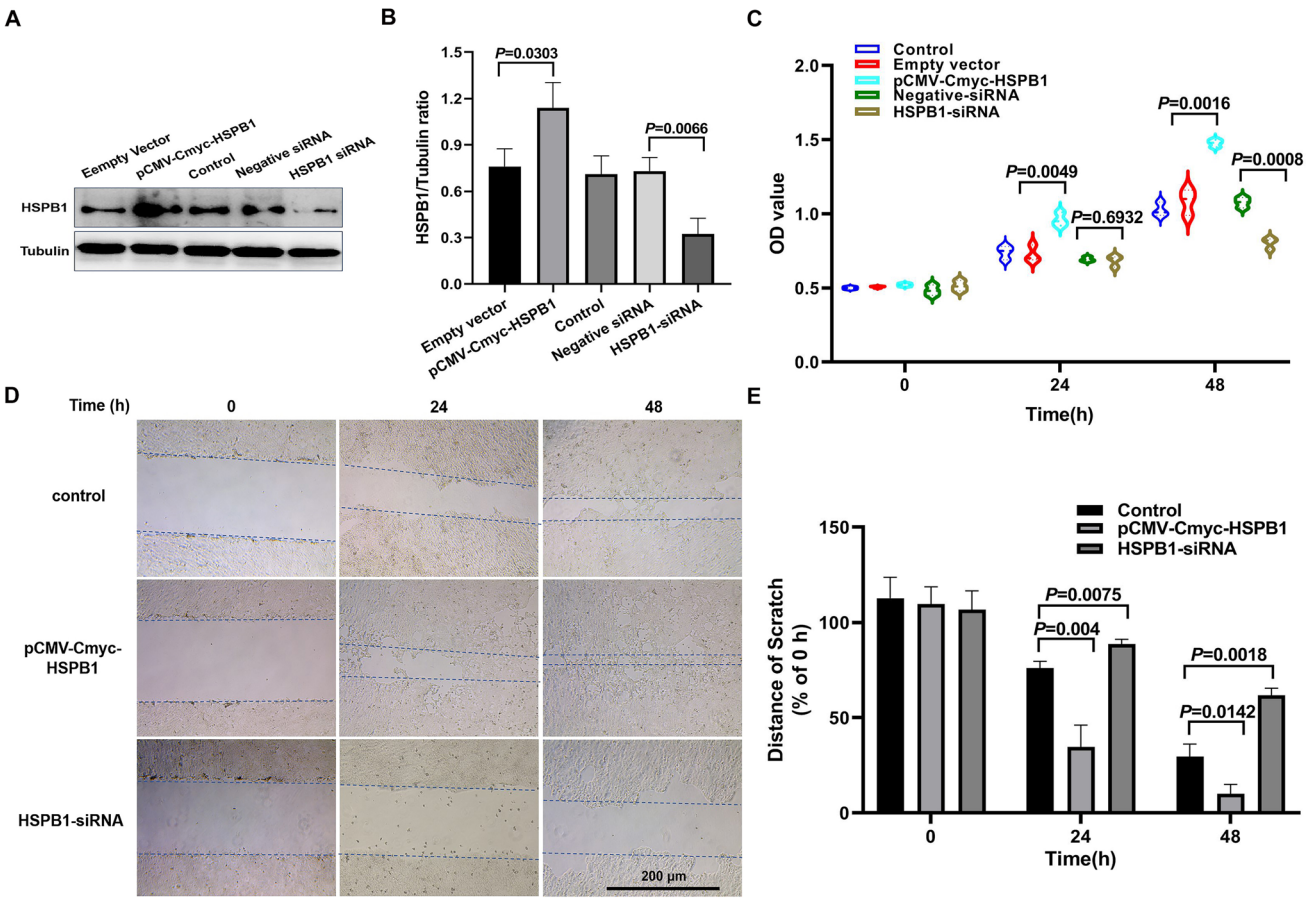


Fig. 2 HSPB1 promotes BxPC-3 progression. **A** The overexpression efficiency and knockdown efficiency of HSPB1 in BxPC-3 cells were detected via Western Blotting. **B** Densitometry was used to determine the fold change in the expression of HSPB1 relative to that of tubulin (n = 6 for each group). **C** Changes in the proliferation rate of BxPC-3 cells after the overexpression or downregulation of HSPB1 were detected via a CCK-8 assay. The error bars denote the means ± SEMs. **D** Representative images showing the migration of BxPC-3 cells with different HSPB1 levels via a scratch migration assay (scale bar, 200 μm). The wound healing assay was analyzed using the ImageJ software for quantification of migration and wound closure. **E** Quantification of the distance traveled by the scratch at 0, 24, and 48 h. The values are given as mean ± S.D.s. Statistical significance was tested by two-way ANOVA (n = 6, n.s.: not significant)

of HSPB1 on TP53 expression and its involvement in ferroptosis. In our analysis, HSPB1 overexpression led to a significant decrease in TP53 protein expression ($P = 0.0021$), whereas HSPB1 knockdown resulted in a significant upregulation of TP53 ($P < 0.0001$) (Fig. 4B and C). No significant differences in mRNA levels were found (Fig. 4A). In cells with reduced HSPB1 expression, TP53 protein levels progressively increased at 1-h ($P = 0.0101$) and 2-h ($P = 0.0213$) time points (Fig. 4D and E), suggesting that HSPB1 may reduce the degradation rate of TP53.

To further evaluate HSPB1's role in TP53-dependent ferroptosis, we used Kevetrin hydrochloride (TP53 promoter) and Pifithrin-α hydrobromide (TP53 inhibitor). The effects of HSPB1 overexpression on SLC7A11 and GPX4 expression were significantly attenuated by Kevetrin hydrochloride ($P = 0.0001$ and $P < 0.0001$) (Figs. 5A–C and 7A–C). Additionally, the HSPB1-induced decrease in TP53 expression was partially reversed by Kevetrin hydrochloride ($P = 0.0017$) (Figs. 5D and 7D). High ROS ($P < 0.0001$) (Figs. 5E and 7F) and free iron ($P < 0.0001$) (Figs. 5F and 7G) levels induced by TP53 were reversed by HSPB1 overexpression. Furthermore, as illustrated in Fig. 7E, PANC-1 cells treated with Kevetrin hydrochloride exhibited higher lipid ROS fluorescence signals (green). In contrast, cells with HSPB1 overexpression showed lower lipid ROS signals, further supporting the regulatory role of HSPB1 in iron metabolism.

Conversely, PFT reversed the decrease in SLC7A11 and GPX4 expression and increase in TP53 expression caused by HSPB1 downregulation, reducing high ROS and free iron levels (Figs. 6A–F and 7A–D, F, G).These results suggest that TP53 is a critical target of HSPB1 in pancreatic cancer cells, regulating ferroptosis and cell survival.

4 Discussion

Ferroptosis, a novel form of cell death proposed by Dixon in 2012 [22], depends on iron and ROS changes. It includes mitochondrial cristae reduction or loss, mitochondrial outer membrane rupture, and membrane condensation [23]. Ferroptosis serves as an adaptive mechanism for eliminating tumor cells, inhibiting tumor initiation and progression [24].

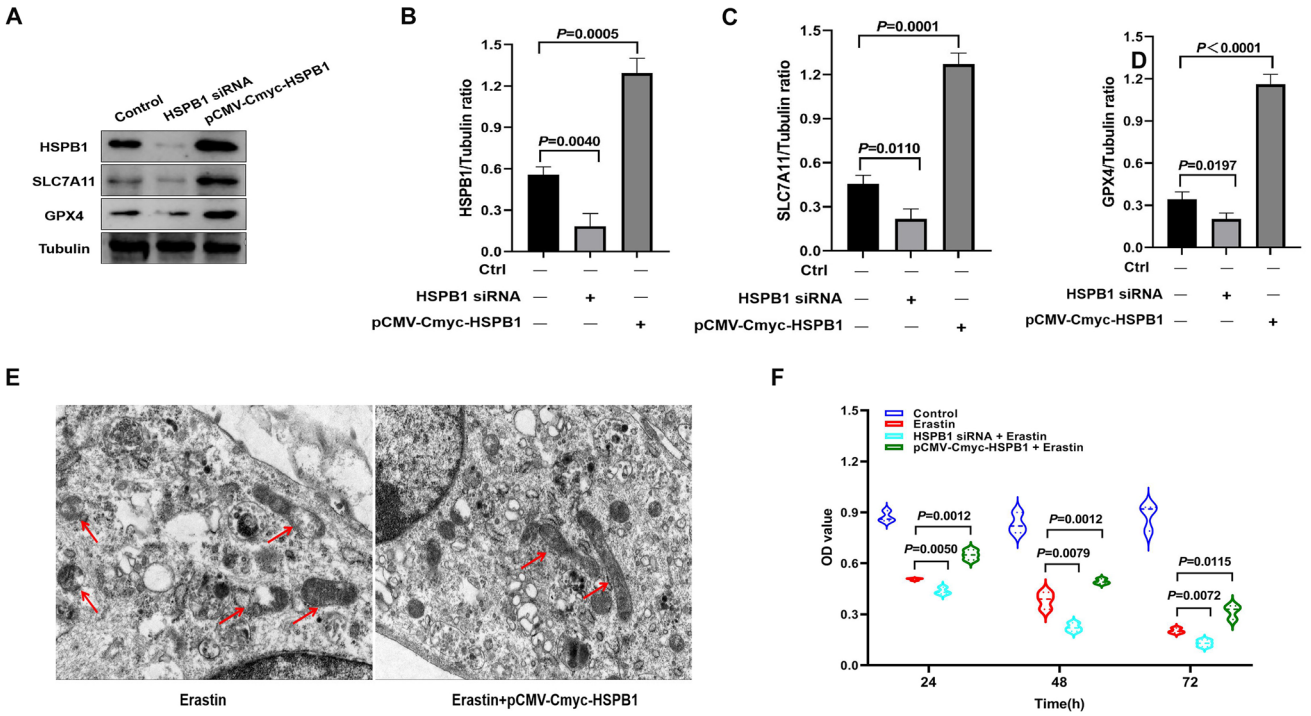


Fig. 3 HSPB1 is involved in the regulation of ferroptosis in BxPC-3 cells. **A**–**D** BxPC-3 cells were transfected with HSPB1 siRNA or pCMV-Cmyc HSPB1 for 48 h. **A** Protein samples were immunoblotted with anti-HSPB1, anti-SLC7A11, anti-GPX4 or anti-tubulin antibodies. (**B**–**D**) Densitometry was used to determine the fold changes in the expression of HSPB1, SLC7A11, and GPX4 relative to that of Tubulin. **E** BxPC-3 cells were treated with pCMV-Cmyc-HSPB1 for 48 h, followed by erastin (15 μM) for 24 h, and then TEM was used to observe mitochondrial morphology. Scale bar, 1 μm (×6.0k). **F** BxPC-3 cells were transfected with HSPB1 siRNA or pCMV-Cmyc HSPB1 for 48 h. Changes in the proliferation rate of BxPC-3 cells after the overexpression or downregulation of HSPB1 were detected via a CCK-8 assay. Values represent the means ± standard deviations (n = 6 for each group)

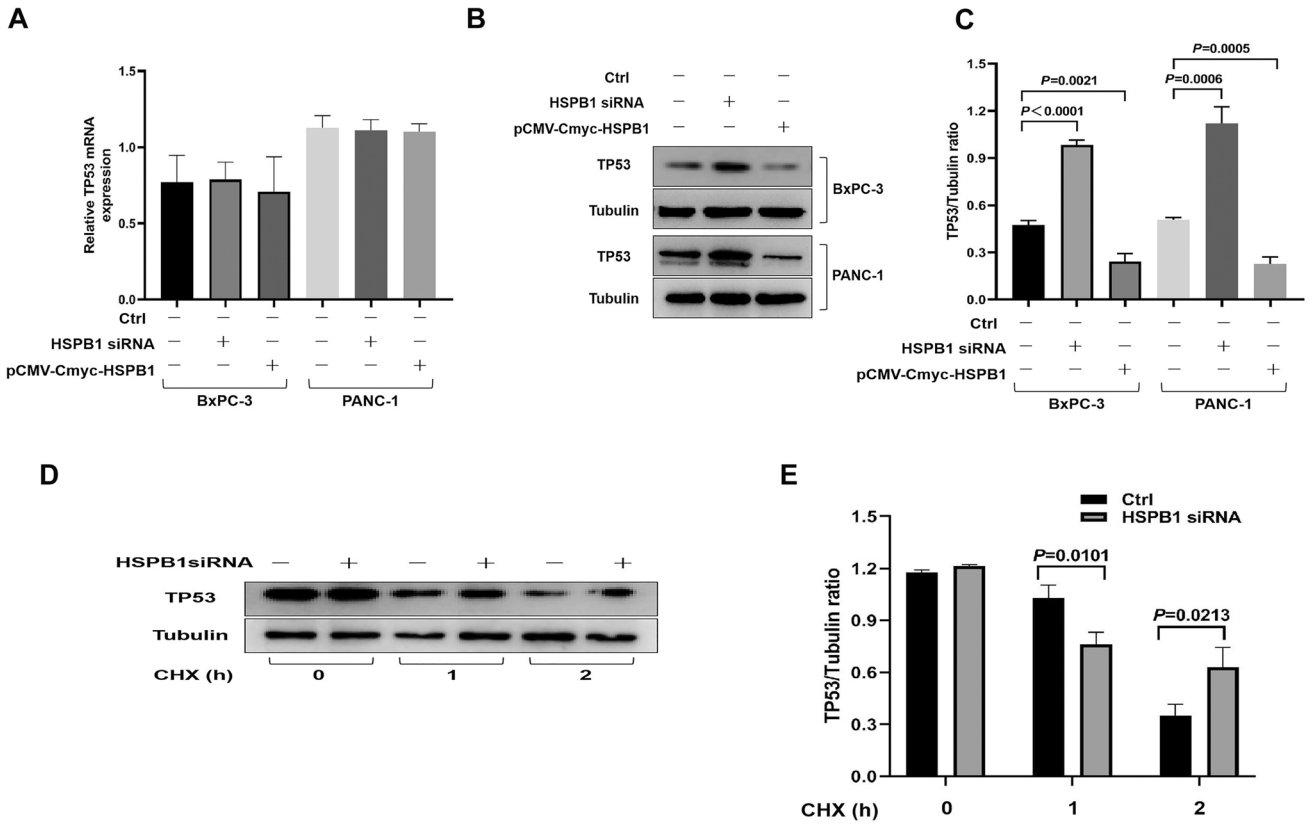


Fig. 4 HSPB1 stabilizes the expression of TP53 in BxPC-3 cells. BxPC-3 cells and PANC-1 cells were transfected with HSPB1 siRNA (silencing), pCMV-Cmyc-HSPB1 (overexpression), or left untreated (Mock control). **A** TP53 gene expression was measured by real-time quantitative PCR. Total RNA was extracted, and cDNA was synthesized. The TP53 mRNA levels were quantified using specific primers for TP53 (forward: 5′-GTTGAGTGGAAAGTACGGAACG-3′, reverse: 5′-TGTGGGTGCTTGTGTAACCAG-3′) and GAPDH as an internal control. The relative expression was calculated using the 2−ΔΔCt method. No significant differences in TP53 mRNA levels were found between the experimental groups. **B** Protein samples were immunoblotted with anti-TP53 or anti-Tubulin antibodies. **C** Densitometry was used to determine the fold change in the expression of TP53 relative to the expression of tubulin. **D** and **E** BxPC-3 cells were transfected with HSPB1 siRNA for 48 h. **D** BxPC-3 cells were treated with 20 mg/mL of CHX for 0, 1, and 2 h, and the membrane TP53 levels were assayed by Western immunoblotting assays. **E** Densitometry was used to determine the fold change in the expression of TP53 relative to the expression of tubulin.Values represent the means ± standard deviations (n = 6 for each group)

However, ferroptosis and its regulatory mechanisms in tumors remain contradictory, with different mechanisms driving cancer progression and inhibiting tumor growth [25].

GPX4, an antioxidant enzyme, breaks down hydrogen peroxide and lipid peroxide, safeguarding cells against oxidative stress and regulating ferroptosis [26]. SLC7A11 contributes to glutathione biosynthesis, collaborating with GPX4 to mitigate oxidative damage and modulate ferroptosis [11]. HSPB1 is a type of HSP that is constitutively expressed in many cells or tissues and is highly expressed in a variety of tumors [27]. It is an ATP-independent molecular chaperone induced by heat shock or other chemical stimuli. HSPB1 can inhibit programmed cell death such as apoptosis [28]. In recent years, HSPB1 has been shown to be involved in the process of ferroptosis [5], but how it affects the ferroptosis of tumor cells is still unclear. Our study found high HSPB1 expression in pancreatic cancer, indicating its involvement in cancer development. Silencing HSPB1 decreased pancreatic cancer cell viability and proliferation, increased ROS and Fe^{2+} levels, and decreased SLC7A11 and GPX4 expression. HSPB1 overexpression promoted proliferation, increased ROS and Fe^{2+} levels, and increased ferroptosis-related proteins. These results suggest that HSPB1 promotes pancreatic cancer by inhibiting ferroptosis.

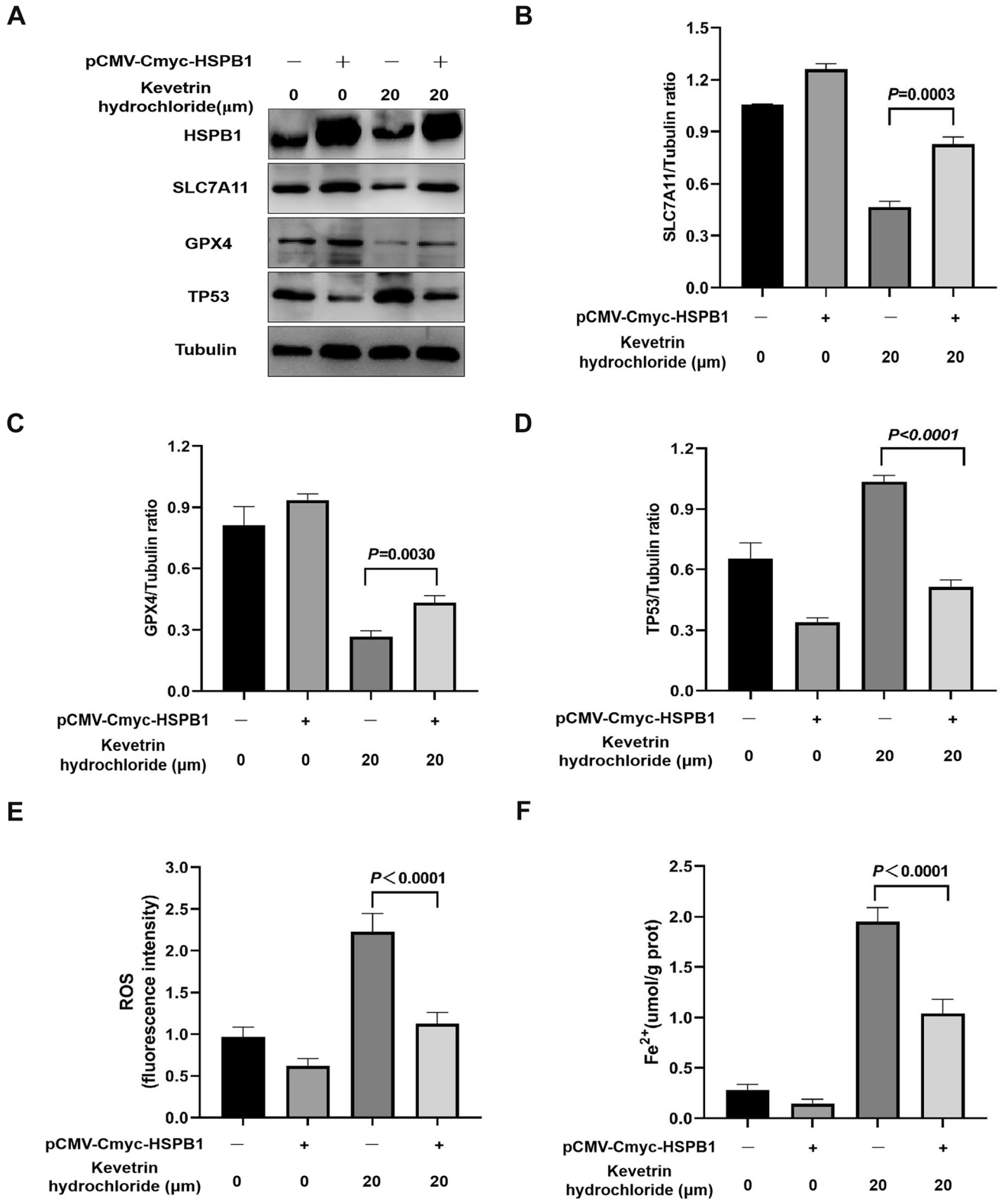


Fig. 5 Overexpression of HSPB1 combats TP53 agonist-induced cell ferroptosis in BxPC-3 cells. BxPC-3 cells were transfected with pCMV-Cmyc HSPB1 for 48 h, after which the cells were treated with 20 μM kecetrin hydrochloride for 24 h. **A** Protein samples were immunoblotted with anti-SCL7A11, anti-GPX4, anti-TP53, or anti-Tubulin antibodies. **B**–**D** Densitometry was used to determine the fold change in the expression of SCL7A11, GPX4, and TP53 relative to that of tubulin. **E** ROS levels were measured by flow cytometry, and the data are presented as the means ± standard deviations. **F** The level of Fe^{2+} was examined via an Iron Assay Kit. Values represent the means ± standard deviations (n = 6 for each group)

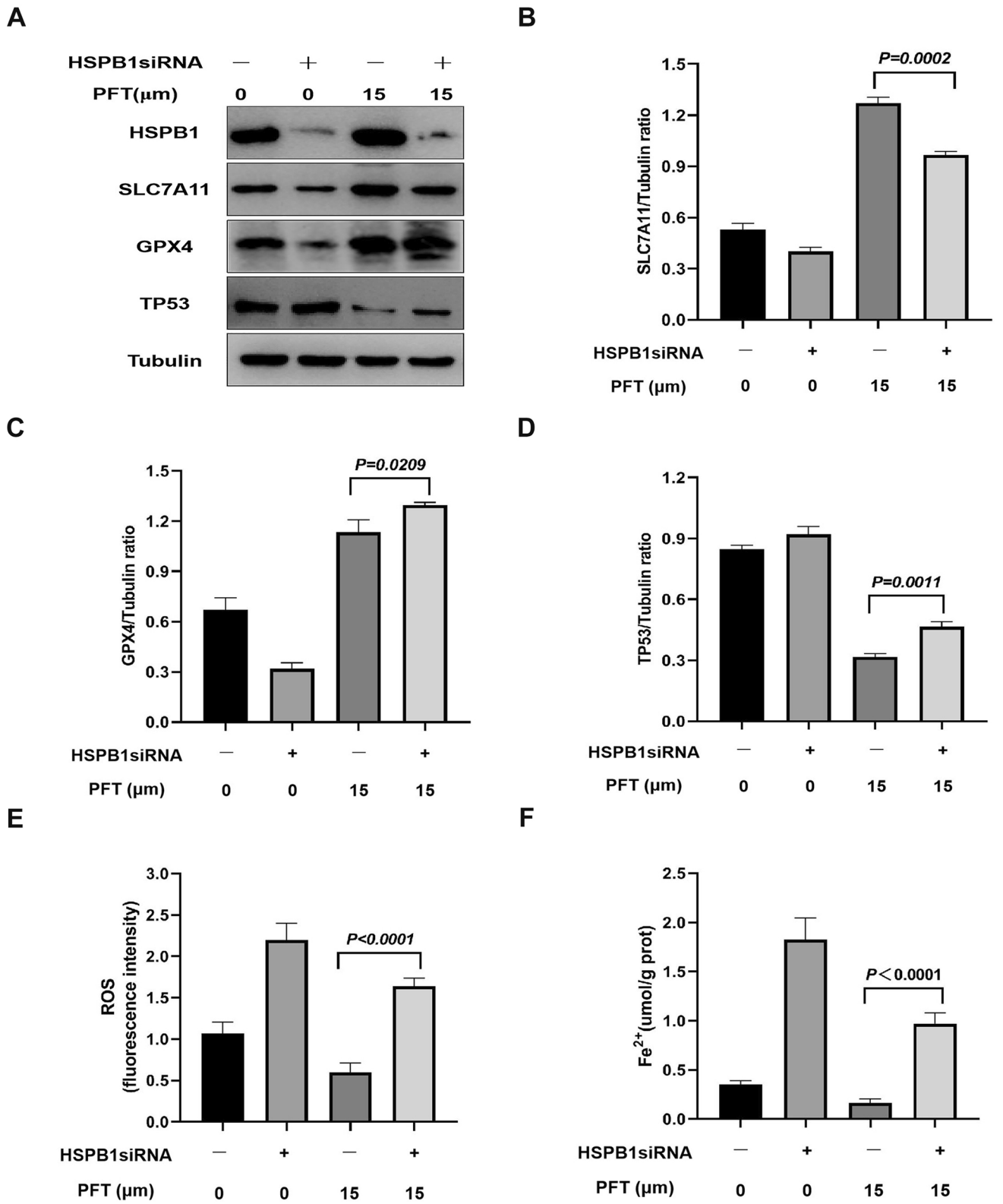


Fig. 6 TP53 inhibitor combats down-expression of HSPB1-induced cell ferroptosis in BxPC-3 cells. BxPC-3 cells were transfected with HSPB1 siRNA for 48 h, after which cells were treated with 15 μM PFT for 24 h. **A** Protein samples were immunoblotted with anti-SCL7A11, anti-GPX4, anti-TP53, or anti-Tubulin antibodies. **B**–**D** Densitometry was used to determine the fold changes in the expression of SCL7A11, GPX4, and TP53 relative to that of tubulin. **E** ROS levels were measured by flow cytometry, and the data are presented as the means ± standard deviations. **F** The level of Fe^{2+} was examined via an Iron Assay Kit.Values represent the means ± standard deviations (n = 6 for each group)

Fig. 7 The Role of HSPB1 in TP53-Mediated Ferroptosis in PANC-1 Cells. **A**–**D**) PANC-1 cells were transfected with HSPB1 siRNA and pCMV-Cmyc HSPB1 for 48 h respectively, after which cells were treated with 15 μM PFT and 20 μM kecetrin hydrochloride for 24 h. **A** Protein samples were immunoblotted with anti-SCL7A11, anti-GPX4, anti-TP53, or anti-Tubulin antibodies. **B**–**D** Densitometry was used to determine the fold changes in the expression of SCL7A11, GPX4, and TP53 relative to that of tubulin. **E** PANC-1 cells were transfected with pCMV-Cmyc HSPB1 for 48 h, after which cells were treated with 20 μM kecetrin hydrochloride for 24 h. Afterward, lipid ROS levels were analyzed using immunofluorescence. **F** and **G** PANC-1 cells were transfected with HSPB1 siRNA and pCMV-Cmyc HSPB1 for 48 h respectively, after which cells were treated with 15 μM PFT and 20 μM kecetrin hydrochloride for 24 h. **F** ROS levels were measured by flow cytometry, and the data are presented as the means ± standard deviations. **G** The level of Fe^{2+} was examined via an Iron Assay Kit. Values represent the means ± standard deviations (n = 6 for each group)

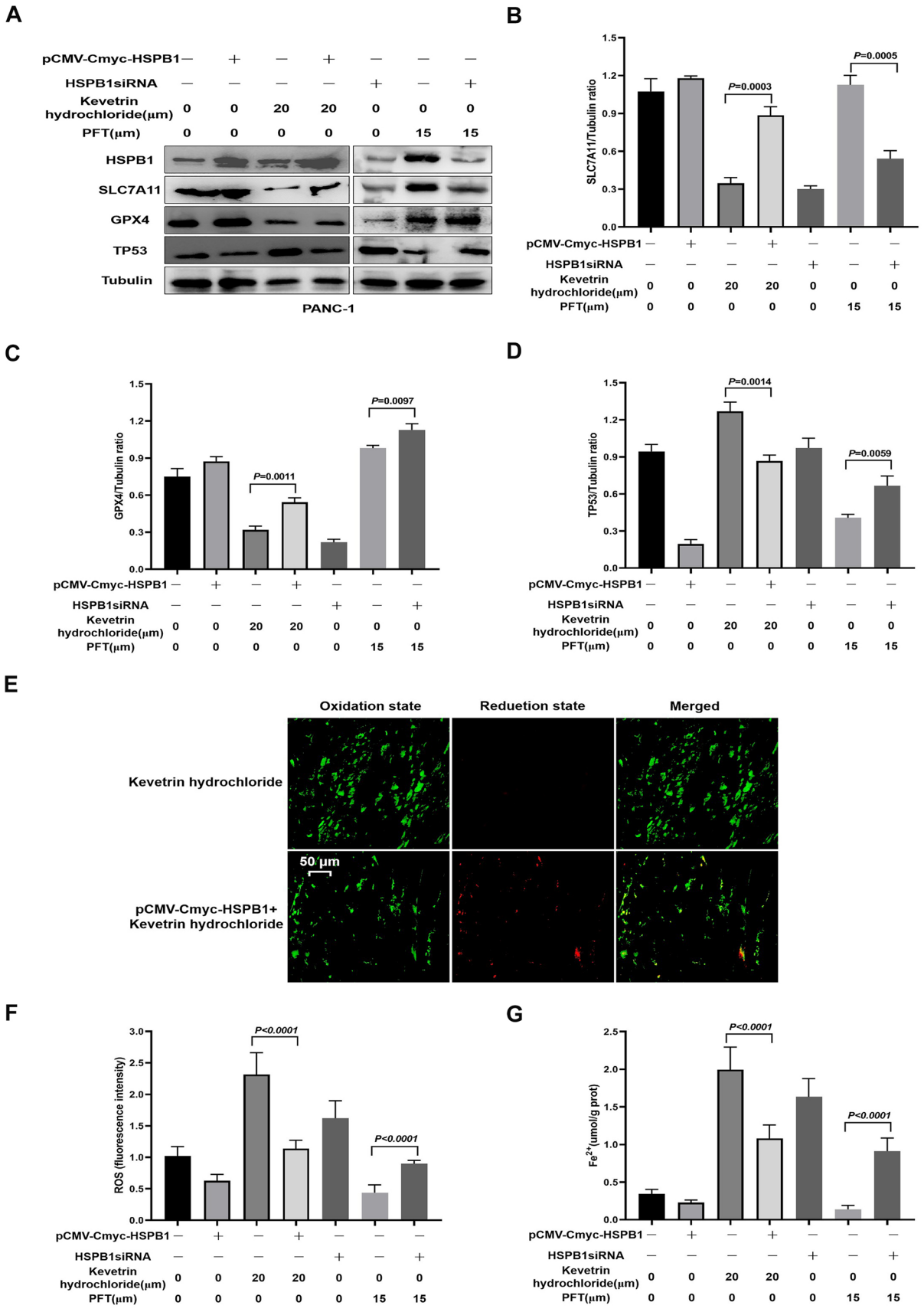
Previous studies have identified HSPB1 as a key regulator of ferroptosis in pancreatic cancer cells, particularly through its interaction with the RNA-binding protein FUS, which stabilizes Nrf2 mRNA [29]. This interaction is significant because Nrf2 plays a crucial role in the cellular response to oxidative stress, a key feature of ferroptosis. In our study, we build upon these findings by investigating how HSPB1 contributes to the regulation of ferroptosis in pancreatic cancer cells, specifically through its interaction with TP53.

The TP53 gene, located in chromosome 17p13.1, encodes the 53 kD tumor suppressor protein TP53, a crucial nuclear transcription factor [30]. TP53 controls the cell cycle, apoptosis, genomic stability, cell growth, differentiation, aging, and disease prevention. TP53 protein levels in cells are low and have a short half-life, making it easily hydrolyzed [31]. Pancreatic cancer has a wide spectrum of gene mutations, with four significant genes: KRAS, CDKN2A, SMAD4, and TP53 [32]. Our study demonstrated that HSPB1 promoted pancreatic cancer by reducing ferroptosis through regulating TP53 protein stability.

However, one limitation of our study is the use of mutant p53 cell lines (Panc-1 and BxPC-3), which carry p53 mutations (R273H and Y220C, respectively). While these cell lines are widely used in pancreatic cancer research and are representative of the common p53 mutations found in pancreatic cancer, the role of wild-type p53 in ferroptosis regulation is not fully addressed. Future studies should incorporate wild-type p53 cell lines to better elucidate the role of wild-type p53 in ferroptosis and further validate our findings.

Building upon these findings, our study further investigates the role of HSPB1 in pancreatic cancer progression and its regulation of ferroptosis through interactions with TP53. These results provide new insights into the molecular mechanisms of pancreatic cancer and suggest that HSPB1 could be a promising therapeutic target for modulating ferroptosis pathways in cancer treatment.

In summary, our study offers compelling evidence that HSPB1 significantly influences pancreatic cancer progression by regulating cell proliferation, migration, and ferroptosis. The understanding of the HSPB1-TP53 interaction enriches the current molecular knowledge of pancreatic cancer, laying the groundwork for future therapeutic interventions targeting HSPB1 and its related pathways. Future research should focus on developing specific HSPB1 inhibitors or modulators, evaluating their efficacy in preclinical and clinical settings, and exploring their potential as novel pancreatic cancer treatment strategies.


A
pCMV-Cmyc-HSPB1
HSPB1siRNA
Kevetrin hydrochloride(μm)
PFT(μm)
HSPB1
SLC7A11
GPX4
TP53
Tubulin
PANC-1
B
SLC7A11/Tubulin ratio
P=0.0003
P=0.0005
C
GPX4/Tubulin ratio
P=0.0011
P=0.0097
D
TP53/Tubulin ratio
P=0.0014
P=0.0059
E
Oxidation state
Reduetion state
Merged
Kevetrin hydrochloride
pCMV-Cmyc-HSPB1+ Kevetrin hydrochloride
50 μm
F
ROS (fluorescence intensity)
P<0.0001
P<0.0001
G
Fe2+(umol/g prot)
P<0.0001
P<0.0001

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Author contributions Tian ZY was responsible for ensuring the integrity of the entire study and contributed to the editing and reviewing of the manuscript. Jing Q contributed to the conception and design of the study, as well as the development of intellectual content and literature research. Liu Q and Luo JH conducted the experiments. Tian ZY and Ning N were responsible for data acquisition and analysis. Jing Q and Ning N confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

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Data availability The data generated in the present study may be requested from the corresponding author.

Declarations

Ethical approval and consent to participate Not applicable.

Patient consent for publication Not applicable.

Competing interests The authors have declared that no competing interests exist.

References

1. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115–32.
2. Alian OM, Philip PA, Sarkar FH, et al. Systems biology approaches to pancreatic cancer detection, prevention and treatment. Curr Pharm Des. 2014;20(1):73–80.
3. Harada K, Baba Y, Shigaki H, et al. Prognostic and clinical impact of PIK3CA mutation in gastric cancer: pyrosequencing technology and literature review. BMC Cancer. 2016;16:400.
4. Marin F, Bonet C, Munoz X, et al. Genetic variation in MUC1, MUC2 and MUC6 genes and evolution of gastric cancer precursor lesions in a long-term follow-up in a high-risk area in Spain. Carcinogenesis. 2012;33(6):1072–80.
5. Saito H, Kono Y, Murakami Y, et al. Highly activated PD-1/PD-L1 pathway in gastric cancer with PD-L1 expression. Anticancer Res. 2018;38(1):107–12.
6. Oue N, Sentani K, Sakamoto N, et al. Clinicopathologic and molecular characteristics of gastric cancer showing gastric and intestinal mucin phenotype. Cancer Sci. 2015;106(8):951–8.
7. Boger C, Kruger S, Behrens HM, et al. Epstein-Barr virus-associated gastric cancer reveals intratumoral heterogeneity of PIK3CA mutations. Ann Oncol. 2017;28(5):1005–14.
8. Stockwell B, Friedmann Angeli JP, Bayir H, et al. Ferroptosis: A regulated cell death linking metabolism, redox biology, and disease. Cell. 2017;171(2):273–85.
9. Yuan H, Li X, Zhang X, et al. Identification of ACSL4 as a biomarker and contributor of ferroptosis. Biochem Biophys Res Commun. 2016;478(3):1338–43.
10. Gao M, Yi J, Zhu J, et al. Role of mitochondria in ferroptosis. Mol Cell. 2019;73(2):354–63.
11. Sun X, Ou Z, Xie M, et al. HSPB1 as a novel regulator of ferroptotic cancer cell death. Oncogene. 2015;34(5):5617–25.
12. Duan H, Li L, He S. Advances and prospects in the treatment of pancreatic cancer. Int J Nanomed. 2023;18:3973–88.
13. Schäfer C, Seeliger H, Bader DC, et al. Heat shock protein 27 as a prognostic and predictive biomarker in pancreatic ductal adenocarcinoma. J Cell Mol Med. 2012;16(8):1776–91.
14. Shi Y, Wang Y, Niu K, et al. How CLSPN could demystify its prognostic value and potential molecular mechanism for hepatocellular carcinoma: a crosstalk study. Comput Biol Med. 2024;172: 108260.
15. Okuno M, Adachi S, Kozawa O, et al. The clinical significance of phosphorylated heat shock protein 27 (HSPB1) in pancreatic cancer. Int J Mol Sci. 2016;17(1):137.
16. Dai Y, Hu L. HSPB1 overexpression improves hypoxic-ischemic brain damage by attenuating ferroptosis in rats through promoting G6PD expression. J Neurophysiol. 2022;128(5):1507–17.
17. Wang SJ, Li D, Ou Y, et al. Acetylation is crucial for p53-mediated ferroptosis and tumor suppression. Cell Rep. 2016;17(2):366–73.
18. Hou CM, Liu GS, Jiang Y, et al. Small heat shock protein HSPB1 alleviates H2O2-induced premature senescence of human vascular endothelial cells through up-regulation of SIRT1. Chin J Pathophysiol. 2022;38(10):1929–37.
19. Dixon SJ. Ferroptosis: Bug or feature? Immunol Rev. 2017;277(1):150–7.
20. Ajoolabady A, Asikhodapasandhokmabad H, Libby P, et al. Ferritinophagy and ferroptosis in the management of metabolic diseases. Trends Endocrinol Metab. 2021;32(6):444–62.
21. Mou Y, Wang J, Wu J, et al. Ferroptosis, a new form of cell death: opportunities and challenges in cancer. J Hematol Oncol. 2019;12(1):34.

22. Friedmann AJ, Krysko DV, Conrad M. Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. Nat Rev Cancer. 2019;19(7):405–14.
23. Yang WS, SriRamaratnam R, Welsch ME, et al. Regulation of ferroptotic cancer cell death by GPX4. Cell. 2014;156(1–2):317–31.
24. Bridges RJ, Natale NR, Patel SA. System xc-cystine/glutamate antiporter: an update on molecular pharmacology and roles within the CNS. Br J Pharmacol. 2012;165(1):20–34.
25. Yun CW, Kim HJ, Lim JH, et al. Heat shock proteins: agents of cancer development and therapeutic targets in anti-cancer therapy. Cells. 2019;9(1):60.
26. Shan R, Liu N, Yan Y, et al. Apoptosis, autophagy and atherosclerosis: Relationships and the role of Hsp27. Pharmacol Res. 2021;166: 105169.
27. Majewski IJ, Kluijt I, Cats A, et al. An α-E-catenin (CTNNA1) mutation in hereditary diffuse gastric cancer. J Pathol. 2013;229(4):621–9.
28. Abe H, Maeda D, Hino R, et al. ARID1A expression loss in gastric cancer: pathway-dependent roles with and without Epstein-Barr virus infection and microsatellite instability. Virchows Arch. 2012;461(4):367–77.
29. Cicenas J, Kvederaviciute K, Meskinyte I, et al. KRAS, TP53, CDKN2A, SMAD4, BRCA1, and BRCA2 Mutations in Pancreatic Cancer. Cancers (Basel). 2017;9(5):42.
30. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000;408(6810):307–10.
31. Foroutan B. A Narrative Review of the TP53 and Its Product the p53 Protein. OBM Genetics. 2023;7(3):185.
32. Stefanoudakis D, Frountzas M, Schizas D, et al. Significance of TP53, CDKN2A, SMAD4 and KRAS in Pancreatic Cancer. Curr Issues Mol Biol. 2024;46(4):2827–44.