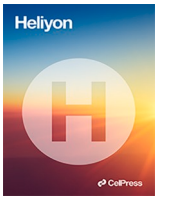
Heliyon 10 (2024) e38800

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Pachymic acid promotes ferroptosis and inhibits gastric cancer progression by suppressing the PDGFRB-mediated PI3K/Akt pathway

Jinlin Nie [a,b,1], Haoran Zhang [b,1], Xiaofeng Li [a], Jiawei Qin [a], Jiawei Zhou [a], Yuhui Lu [a], Nengjia Yang [a], Yanan Li [a], Hailiang Li [a,**], Cheng Li [a,*]

[a] *Department of Hepatobiliary Pancreatic Hernia Surgery, The Affiliated Guangdong Second Provincial General Hospital of Jinan University, Guangzhou, 510317, People's Republic of China*

[b] *Department of Gastrointestinal Surgery, The First Affiliated Hospital of Jinan University, Guangzhou, 510632, People's Republic of China*

A R T I C L E I N F O

Keywords:
Pachymic acid
Gastric cancer
Ferroptosis
PDGFRB
PI3K/Akt signalling pathway

A B S T R A C T

Gastric cancer (GC) is a common malignant tumour with high incidence and mortality rates worldwide. Despite current treatment modalities, including surgical resection and chemotherapy, challenges such as postoperative recurrence, metastasis and drug resistance persist. Therefore, investigating the feasibility and mechanism of traditional Chinese medicine in treating gastric cancer is crucial for discovering new anti-gastric cancer drugs or adjuvant therapies. Pachymic acid (PA) is a natural triterpenoid found in the traditional Chinese medicinal herb *Poria cocos* (PC) (Schw. Wolf). Recent studies have reported its inhibitory effects on various cancer cells, including liver, cervical, breast and gastric cancer. Our in vitro and in vivo experiments confirmed that PA inhibits the proliferation, migration and invasion of gastric cancer cells. The treatment of gastric cancer cells with various death inhibitors revealed that PA may suppress gastric cancer progression by inducing ferroptosis. Malondialdehyde, Fe^{2+}, reactive oxygen species and glutathione assays were performed to validate the effects of PA on ferroptosis in gastric cancer. High-throughput sequencing combined with analysis of the TCGA database identified PDGFRB as a potential downstream target of PA. In vivo experiments indicated that the PDGFRB overexpression could counteract the antitumour effects of PA, while ferroptosis induced by the PI3K/Akt signalling pathway may play a key role in this process. This study provides initial evidence that PA, through its interaction with PDGFRB, alters the PI3K/Akt signalling pathway, leading to ferroptosis in gastric cancer cells, thus manifesting its antitumour properties. This discovery holds promise for the development of novel therapeutic strategies for gastric cancer patients.

* Corresponding author. The Affiliated Guangdong Second Provincial General Hospital of Jinan University, 466 Middle Xingang Road, Guangzhou, 510317, Guangdong, People's Republic of China.

** Corresponding author. The Affiliated Guangdong Second Provincial General Hospital of Jinan University, 466 Middle Xingang Road, Guangzhou, 510317, Guangdong, People's Republic of China.

E-mail addresses: lihailiang5612@163.com (H. Li), 182838li@163.com (C. Li).

[1] Jinlin Nie and Haoran Zhang contributed equally to this study.

https://doi.org/10.1016/j.heliyon.2024.e38800
Received 26 June 2024; Received in revised form 27 September 2024; Accepted 30 September 2024
Available online 1 October 2024

Abbreviations

Akt	protein kinase B
CCK-8	Cell Counting Kit-8
DMSO	dimethyl sulfoxide
ECL	chemiluminescence
FBS	fetal bovine serum
GC	gastric cancer
GPX4	glutathione peroxi- dase 4
GSH	glutathione
KEGG	Kyoto Encyclopedia of Genes and Genomes
MDA	malondialdehyde
PA	pachymic acid
PC	poria cocos
PCD	programmed cell death
PDGFRB	Platelet-Derived Growth Factor Receptor Beta
PI3K	phosphoinositide 3-kinase
ROS	Reactive Oxygen Species
TCGA	The Cancer Genome Atlas

1. Introduction

Gastric cancer (GC) is one of the most prevalent malignant tumours globally and ranked as the third leading cause of cancer-related mortality. In 2020, statistics revealed an alarming incidence of over one million new cases of gastric cancer, culminating in 769,000 deaths [1]. Surgical resection is currently the cornerstone of treatment for gastric cancer. Nonetheless, a significant number of patients are diagnosed at an advanced stage, predisposing them to postooerative recurrence and metastasis [2]. Although chemotherapy contributes to prolonging patient survival and improving quality of life, the emergence of drug resistance and severe adverse reactions profoundly undermines the clinical utility of anticancer medications [3]. Consequently, there is an urgent need to identify and develop safe and effective therapeutic drugs and adjunct treatments.

Given the high costs and numerous side effects associated with traditional drugs, natural medicines have demonstrated significant advantages in the prevention and treatment of gastric cancer because of their low toxicity, safety and accessibility [4,5]. Pachymic acid (PA) is a natural triterpenoid compound found in the traditional Chinese medicinal herb *Poria cocos* (PC) (Schw. Wolf), and is also one of the major active ingredients in PC [6]. It exhibits diverse pharmacological activities, including antioxidant, anti-inflammatory and hypoglycemic effects [7]. Furthermore, it exerts inhibitory effects on various cancer cells, including liver, cervical, breast and gastric cancer [8–11]. Jeong et al. [12] indicated that poria acid could induce apoptosis in bladder cancer cells by regulating apoptotic protein inhibition and reactive oxygen species (ROS) production, thereby modulating the expression of the apoptosis factor Bcl-2 and initiating the caspase-regulated apoptotic pathway. Ma et al. [13] have suggested that poria acid induces ROS production in lung cancer cells. Earlier research has demonstrated that PA inhibits the proliferation and colony-forming abilities of gastric cancer cells [14]. Additionally, it reduces the migration and metastatic potential of these cells by decreasing matrix metalloproteinases expression and suppressing epithelial-mesenchymal transition process [15]. Although PA shows promise for cancer treatment, its precise mechanism of action remains unclear.

Ferroptosis, first reported by Stock in 2008, is an iron-dependent nonapoptotic process that regulates cell death [16]. It catalyses the buildup of ROS through the action of ferrous iron or lipoxygenases on highly expressed unsaturated fatty acids within the cell membrane. This process ultimately results in lipid peroxidation, leading to cell death [17–19]. Natural compounds exert antitumour effects through the ferroptosis pathway [20], however, there have been no reports on PA-induced ferroptosis in gastric cancer cells. Therefore, we hypothesised that PA plays a crucial role in inhibiting the proliferation and migration of gastric cancer cells and that its mechanism potentially involves the induction of ferroptosis. Further in vivo and in vitro experiments will be conducted to explore the specific pathways through which PA exerts its antitumour effects. We identified platelet-derived growth factor receptor beta (PDGFRB) as a significant downstream target of PA and found that the PI3K/Akt signalling pathway also playing a critical role in this process. These findings provide new scientific evidence for the application of PA in the treatment of gastric cancer.

2. Methods

2.1. Cell culture and Cell viability assay

Human gastric cancer cells (SGC-7901 and AGS) and human gastric mucosal epithelial cells (GES-1) were obtained from the Shanghai Cell Resource Centre for Life Sciences. The cells were cultured at 37 °C in a humidified 5 % CO_2 environment within a temperature-controlled incubator, with the media being refreshed every 3 days.

After cell culture, the cells were treated with various concentrations of PA (purchased from Chengdu Munster Biotechnology Co., Ltd.), with or without specific inhibitors for 48h. After adding 10 μL of cell counting kit-8 (CCK-8) reagent to each well, the absorbance was measured at 450 nm using an enzyme reader.

$$\text{Cell Viability} = \frac{(\text{Experimental Absorbance} - \text{Blank Well Absorbance})}{(\text{Control Well Absorbance} - \text{Blank Well Absorbance})} \times 100\%.$$

2.2. EdU labelling for Cell proliferation assay

Cells were seeded in a 96-well plate at a density of 5×10^3 cells per well and incubated for 24h. Subsequently, gradient doses of PA were added to each group, and the cells were incubated for an additional 48h. Staining was performed with a BeyoClick™ EdU-488 Cell Proliferation Assay Kit. Quantitative analysis was performed using ImageJ software after observing the newly proliferated cells under a fluorescence microscope.

2.3. Wound healing Experiment

The cells were plated in 6-well plates. When they reached about 80 % confluence, sterile 200 μl pipette tips were used to gently create scratches in the cell monolayer. The cells were then incubated for 48h in a serum-free medium. Wound healing was observed under an inverted microscope. Finally, the wound area was quantitatively analysed using ImageJ software to evaluate cell migration and healing capacity.

2.4. Transwell invasion assay

The Transwell inserts and the Matrigel (Corning, USA) were placed in the chamber at 4 °C for 12 h, and all subsequent steps were performed on an icebox. The inserts were placed in a 24-well plate, the Matrigel was mixed with the culture medium in a 1:4 ratio, 60 μl of the mixture was quickly added to each well of the inserts; and then the 24-well plate was placed in the incubator. After the Matrigel mixture solidified, the plate was removed. The inserts were removed, and 700 μL of culture medium containing 10 % serum was added to each well. After 48h of incubation, the invading cells in the upper chamber were fixed with a 4 % paraformaldehyde solution and stained with 0.1 % crystal violet. The number of infiltrated cells in the upper chamber was determined with an inverted microscope, and the cells were analysed using the ImageJ software.

2.5. Western blotting

A protein extraction kit was used to determine the overall protein content of the cells. The amount of protein was measured using a BCA assay kit. Afterward, the proteins were separated through electrophoresis and transferred onto membranes. After washing the membranes three times with tris-buffered saline containing Tween, they were incubated with the primary antibody overnight at 4 °C. Following incubation, the membranes were washed again incubated with the secondary antibody for a duration of 60 min. Finally, the bands were observed by using an improved chemiluminescence (ECL) reagent kit, and the outcomes were evaluated using ImageJ software.

2.6. Apoptosis assays

The percentage of apoptotic cells was measured following the instruction (Beyotime, C1062L). After cell culture, the cells were plated in 60 mm dishes and subjected to various treatments for 48h. Then, the cells were collected and resuspended in 300 μL of ice-cold 1 × binding buffer, and stained with PI and FITC Annexin V for 30 min at 4 °C in the dark. The results were analszed using a flow cytometer.

2.7. Cell transfection

Lentiviral constructs for transfection were procured from OBiO (Shanghai, China). These constructs were designed for the over-expression of PDGFRB (labelled oePDGFRB) and the knockdown of PDGFRB (termed shPDGFRB), along with standard control constructs (designated control and shNC). To establish stable colorectal cancer cell lines, cell selection was conducted using 400 μg/ml of neomycin.

2.8. RNA-Seq

In this study, two groups were established: the control and experimental groups. SGC-7901 cells were cultured in a regular medium for the control group and in a medium with 20 μM PA (for 48 h) for the experimental group. Three samples were collected from each group for subsequent transcriptome sequencing. Libraries were constructed after RNA extraction, and their quality was confirmed through quality control checks. Once the libraries passed the quality control, sequencing was performed using the Illumina sequencing platform—conducted by Haipulos Biotechnology Co. Ltd.

2.9. Molecular docking validation

The crystal structure of PDGFRB was obtained from the Protein Data Bank (PDB) database, and the molecular structure of PA was retrieved from the TCMSP database. The binding affinity of poria acid to PDGFRB was evaluated using AutoDockTools-1.5.7 software. The most favourable docking outcomes were visualised using the PyMol software. A binding energy below −5.0 kcal/mol indicates a strong binding affinity of the compound to the protein. The lower the binding energy, the more stable the interaction.

2.10. MDA and GSH measurement

To measure malondialdehyde (MDA) and **glutathione (GSH)** levels, cells in the logarithmic growth phase were plated at a density of 3×10^5 cells per well in 6-well plates. Following overnight adhesion, the cells were divided into the following groups: a control group, palmitic acid (PA) treatment groups (10 μM, 20 μM, 40 μM) and a ferroptosis inhibitor group (20 μmol/L PA + 10 μmol/L Fer-1). Cell lysates were collected, MDA and GSH detection kits were used to analyse the production of MDA and GSH in GC cells (purchased from Shanghai Beyotime Biotechnology Co., Ltd.).

2.11. Fe^{2+} content determination

Cancer cells were seeded in 6-well plates and incubated with various concentrations of PA and a ferroptosis inhibitor. Following the treatment, the cells were centrifuged, washed, and resuspended via sonication. The sonicated cell suspension was centrifuged, and the supernatant was collected for iron ion measurements.

2.12. Intracellular reactive oxygen species (ROS) detection

After cell culture, the cells were divided into the following groups: a control group, PA treatment groups (10 μmol/L, 20 μmol/L, 40 μmol/L) and a ferroptosis inhibitor group (20 μmol/L PA + 10 μmol/L Fer-1), these groups were treated for 48h. Subsequently, 300 μL of DCFH-DA probe solution was added to each well. The cells were then incubated in a light-protected incubator for 30 min. After incubation, the cells were harvested and analysed for fluorescence intensity using a flow cytometer.

2.13. qRT–PCR detection

After 48 h of treatment with PA, both PA-treated and control group gastric cancer cells were harvested. Total RNA was isolated from each sample using the TRizol method and its purity was assessed. Subsequently, the purified RNA was utilized as a template for cDNA synthesis (Table S1). GAPDH served as the internal reference gene.

2.14. In vivo tumor xenograft model

All animal experiments were approved by the Experimental Animal Center of the South China Agricultural University. To establish a xenograft tumour model, 5×10^6 SGC-7901 cells were injected into the right axilla of BALB/c nude mice. After a 14-day period, the mice were randomly divided into five groups and intraperitoneally injected with the following drugs: control (normal saline), PA (100 mg/kg/day), PA (200 mg/kg/day), PA (400 mg/kg/day) and PA (200 mg/kg/day) + Fer-1 (100 mg/kg/day). Tumor length and width, along with the weight of the mice, were measured every other day after the initial administration of the drug. The tumour volume was calculated using the following formula: (tumour length × tumour width^2)/2. On the 14th day after the initial treatment, the mice were euthanised, and all the tumours were collected.

2.15. Lentiviral transfection

The plasmids and viruses used in this study were provided by Shanghai Genechem Co., Ltd. Plasmids were constructed using the shRNA sequence CAACGAGTCTCCAGTGCTA targeting PDGFRB, along with overexpression plasmids containing the full-length cDNA of PDGFRB. The objective plasmids and helper packaging plasmids were mixed with OPTI-MEM and then co-incubated with FuGENE transfection reagent to form transfection complexes. These complexes were introduced into 293T cells, and after 48 h of culture, the supernatant containing lentiviral particles was collected, filtered through a 0.45 μm filter, aliquoted and stored at −80 °C for future use. Gastric cancer cells were subsequently co-cultured with lentiviruses, followed by selection and amplification to obtain stably transfected cell lines.

2.16. Statistical analysis

The experimental results, including western blotting, qRT–PCR, cell scratch, transwell migration and invasion assays, were analysed using the ImageJ software. Statistical analysis was conducted employing GraphPad Prism 8, and the data are expressed as the mean ± standard deviation from at least three independent experiments. Differences between two groups were assessed using t-tests. Statistical significance is indicated as *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, representing various levels of statistical significance. “ns” indicates no statistical significance.

3. Results

3.1. PA inhibits the proliferation, invasion and migration of gastric cancer cells

To determine the effects of PA on gastric cancer, we administered varying concentrations of PA to gastric cancer cells (AGS and SGC-7901) and gastric mucosal epithelial cells (GES-1). PA inhibited gastric cancer cell survival in a concentration-dependent manner while demonstrating relatively low toxicity towards normal gastric mucosal epithelial cells (Fig. 1A and B). This suggests that PA exerted selective cytotoxicity in gastric cancer cells. The morphology of the cells treated with different concentrations of PA is shown in Fig. 1C. Subsequently, the effect of PA on cellular proliferation was assessed using the EdU assay. The results revealed that PA inhibited the growth of gastric cancer cells in a dose-dependent manner (Fig. 1D and E). Wound healing assays demonstrated a notable reduction in the migration ability of AGS and SGC-7901 cells upon PA administration (Fig. 1F and G). Transwell assays were performed to demonstrate the diminished invasive ability of AGS and SGC-7901 cells in the presence of PA (Fig. 1H and I). As expected, a higher percentage of dead cells was observed in gastric cancer cells following PA treatment compared to the control. However, overall apoptosis levels were lower (Fig. 1J and K), possibly because apoptosis may not be the primary mechanism of cell death induced by PA.

3.2. PI3K/Akt pathway may be a key route through which PA exerts its antitumour effects

To further explore the molecular mechanisms underlying the effects of PA on gastric cancer progression, we conducted high-throughput sequencing to analyse the differentially expressed genes in gastric cancer cells treated with PA (Table S2). Subsequently, we merged these genes with differentially expressed and prognosis-related genes in gastric cancer from The Cancer Genome Atlas (TCGA) database, resulting in 114 differentially expressed and prognosis-related genes in PA-treated gastric cancer cells. These genes were visualised using Venn diagrams and volcano plots (Fig. 2A and B), annotating the top 10 upregulated and downregulated genes in the volcano plot. Additionally, KEGG pathway analysis revealed that these differentially expressed genes were mainly enriched in several critical pathways (Table S3), including proteoglycans in cancer and the PI3K/Akt, MAPK, cAMP, and cGMP-PKG signalling pathways (Fig. 2C). Thus, the PI3K/Akt pathway may be the key signalling pathway through which PA exerts its anticancer effects.

3.3. PDGFRB is a crucial downstream target for the anti-gastric cancer effects of PA

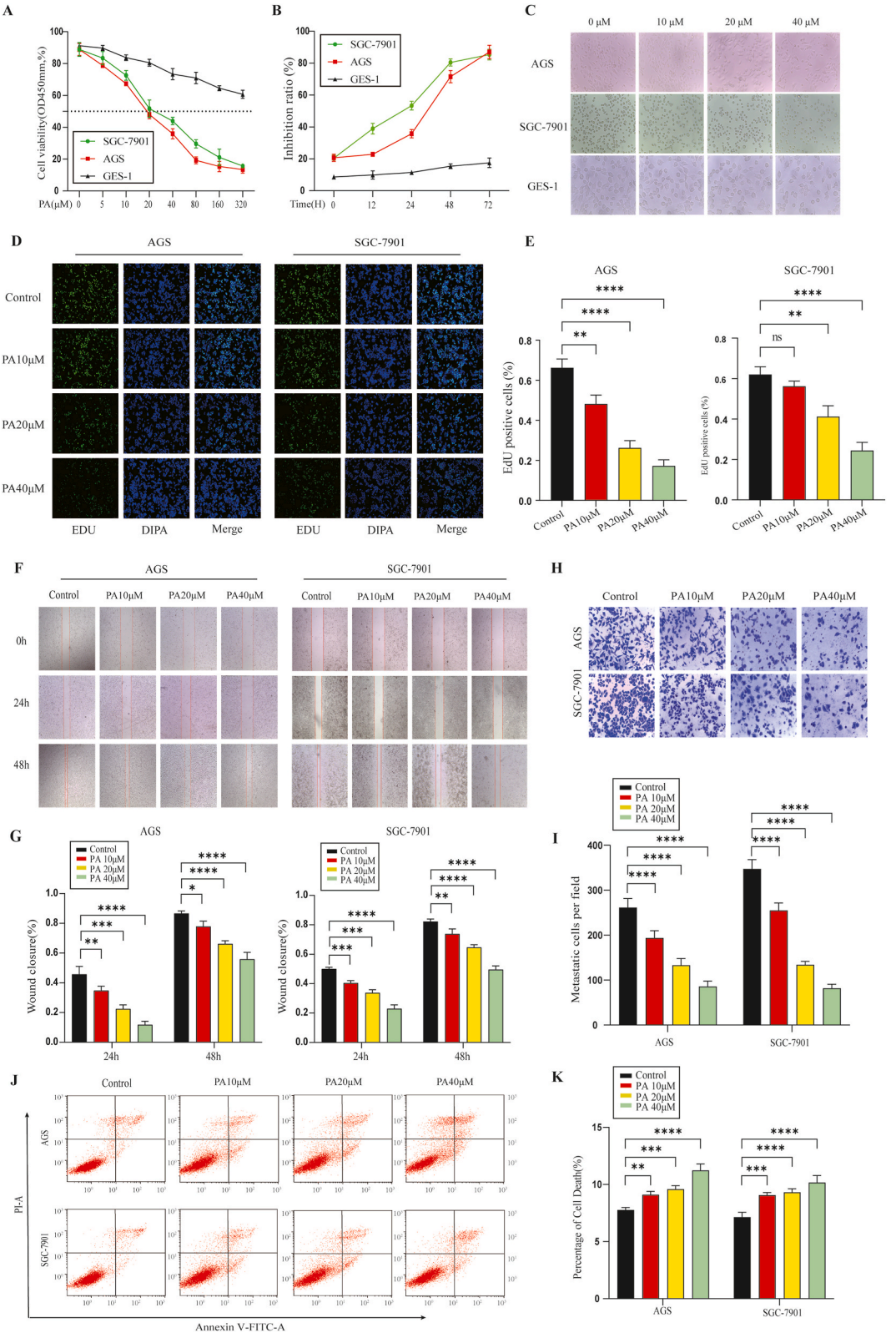
We then examined genes significantly downregulated according to the RNA sequencing results, particularly PDGFRB, which were strongly correlated with the PI3K/Akt signalling pathway. Through analysis of TCGA and the University of Alabama at Birmingham Cancer (UALCAN) databases [21], we found that PDGFRB was upregulated in gastric cancer tumor cells, with higher expression levels in more malignant gastric cancers (Fig. 2D–F). K–M survival analysis also revealed that higher PDGFRB expression in gastric cancer patients was associated with worse clinical outcomes (Fig. 2G). Molecular docking validation was used to verify whether the PA and PDGFRB could covalently bind to each other, and the results showed that PA binds stably to the PDGFRB protein (Fig. 2H), with a binding energy of −6.42 kcal/mol. Thus, PA may directly bind to the PDGFRB protein and subsequently regulate the expression of downstream proteins. Subsequent qRT–PCR and western blotting analyses confirmed that PA significantly reduced PDGFRB expression in both AGS and SGC-7901 cells (Fig. 2I–K).

3.4. PDGFRB can regulate the PI3K/Akt pathway, thereby affecting the proliferation, invasion and migration abilities of gastric cancer cells

To verify the effect of PDGFRB on gastric cancer cells, we utilized lentiviral transfection technology to construct AGS and SGC-7901 cell lines with PDGFRB overexpression (OV-PDGFRB) and PDGFRB knockdown (sh-PDGFRB), transfection efficiency was assesswd through western blotting (Fig. 3A and B). EdU experiments indicated that the proliferation efficiency of gastric cancer cells was significantly increased in the OV-PDGFRB group but significantly decreased in the sh-PDGFRB group (Fig. 3C and D). Transwell assays (Fig. 3E and F) and wound healing assays (Fig. 3G and H) showed that, compared to those of the control group, the invasion and proliferation abilities of the OV-PDGFRB group were significantly greater, while those of the sh-PDGFRB group exhibited the opposite results. Additionally, we assessed the expression levels of proteins in the PI3K/Akt signalling pathway and observed that PDGFRB activated the PI3K/Akt signalling pathway by increasing the levels of phosphorylated PI3K, Akt and mTOR (Fig. 3I and J).

3.5. Ferroptosis contributed to PA-induced death in gastric cancer cells

To determine the primary cell death pathway activated by PA, we used specific inhibitors, including necrostatin-1 (Nec-1, a necroptosis inhibitor), chloroquine (CQ, an autophagy inhibitor), and Z-VAD (a pancaspase inhibitor), along with ferroptosis inhibitors, such as deferoxamine (DFO), ferrostatin (Fer-1) and liproxstatin-1 (Lip-1). Our results indicated that Nec-1, Z-VAD and CQ did not significantly inhibit PA-induced cell death in AGS and SGC-7901 cells (Fig. 4A–C). However, the ferroptosis inhibitors DFO, Fer-1 and Lip-1 notably reversed the PA-induced cell death (Fig. 4D–F). These findings suggest that ferroptosis is the predominant mechanism underlying PA-induced cell death.



(caption on next page)

Fig. 1. Effect of PA on the proliferation, invasion and migration abilities of gastric cancer cells. (A) Viability of SGC-7901, AGS and GES-1 cells treated with different concentrations of PA. (B) Inhibition rates of SGC-7901, AGS and GES-1 cells after treatment with the IC50 of PA. (C) Observation of the morphology of SGC-7901, AGS and GES-1 cells treated with different concentrations of PA. (D, E) Quantitative analysis of EdU fluorescence to assess the effect of PA on the proliferative capacity of gastric cancer cells. (F, G) Wound healing experiments demonstrating the effect of PA on the migratory ability of gastric cancer cells. (H, I) Transwell experiments demonstrated the ability of PA to inhibit the invasion of gastric cancer cells. (J, K) Assessment of cell death in gastric cancer cells treated with PA using flow cytometry. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n = 3.

3.6. In vivo and In vitro experiments demonstrate that PA inhibits gastric cancer by promoting ferroptosis

GSH depletion, redox-active iron overload, lipid peroxidation and ROS accumulation are crucial events in ferroptosis. Consequently, we assessed the levels of Fe^{2+}, ROS, GSH and the MDA. As anticipated, following PA treatment, a decrease in GSH levels was observed (Fig. 5A), while MDA, ROS and Fe^{2+} levels were increased (Fig. 5B–E). Furthermore, the ferroptosis inhibitor Fer-1 alleviated PA-induced GSH depletion and mitigated the increase in the levels of MDA, ROS and Fe^{2+} induced by PA (Fig. 5A–E). To further ascertain whether PA induces cell death through ferroptosis, western blotting performed to examine ferroptosis-related proteins. Our results revealed a significant reduction in the expression levels of SLC7A11, ferritin and GPX4 in gastric cancer cells following PA treatment (Fig. 5F and G). We established a subcutaneous xenograft model of gastric cancer to evaluate the in vivo antitumour efficacy of PA. Our results demonstrated that PA significantly suppressed the growth of xenograft tumours, and this antitumour effect of PA was reversed by the ferroptosis inhibitor Fer-1 (Fig. 6H–J). Overall, our findings suggest that PA induces ferroptosis in gastric cancer cells.

3.7. PA can Counterct the promotive effect of overexpressed PDFGRB on gastric cancer

The proliferation, invasion and migration abilities of SGC-7901 cells were enhanced after the overexpression of PDGFRB. However, when SGC-7901 cells overexpressing PDGFRB were treated with PA, the promoting effect of PDGFRB on tumours was counteracted (Fig. 6A–C). Moreover, western blotting experiments revealed that treatment with PA in SGC-7901 cells counteracted the effects of PDGFRB on promoting the expression of key ferroptosis-related genes (SLC7A11, ferritin and GPX4) and the phosphorylation of genes related to the PI3K/Akt signalling pathway (Fig. 6D and E).

4. Discussion

Owing to the intricate pathogenic mechanisms of gastric cancer, drugs targeting a single factor may be insufficiently effective. This has led to a growing trend in gastric cancer treatment toward combination drug therapy that targets multiple factors. Scholars view traditional Chinese medicine as a collection of combinatory compounds [22,23]. Because of their ability to exert multi-target effects, natural products have emerged as a significant pharmaceutical resources for the treatment of gastric cancer [24]. Pachymic acid (PA), an important active component of PC, exhibits anti-inflammatory, antioxidant, and anticancer properties [6]. However, its efficacy in treating gastric cancer remains unclear. We found that PA effectively inhibited the proliferation, migration and invasion of human gastric cancer cells, suppressed tumourigenesis in vivo, and exerted dose-dependent effects. Additionally, PA caused minimal damage to normal gastric tissues, suggesting its potential as a therapeutic or adjunct agent for gastric cancer.

In the field of oncology, programmed cell death (PCD) may be a key component in cancer diagnosis and treatment [25]. Currently, the types of PCD identified in gastric cancer primarily include ferroptosis, pyroptosis, necroptosis and autophagy [26–29]. PA reduces the viability of gastric cancer cells and leads to cell death. However, the PCD type responsible for this effect remains unclear. Currently, there is limited research on the impact of PA on gastric cancer, with existing studies mainly indicating that PA primarily induces apoptosis in gastric cancer cells by inducing cell cycle arrest [30,31]. Therefore, we used various types of PCD inhibitors in combination with gradient concentrations of PA to treat gastric cancer cells. We found that PA exerted its antitumour effects by promoting ferroptosis in gastric cancer cells. However, its effect on necroptosis, pyroptosis and apoptosis was not as pronounced. Ferroptosis is characterized by intracellular iron dependence, abnormal elevation of lipid peroxidation and disruption of the redox balance, leading to cell death [32]. Currently, the regulation of ferroptosis in gastric cancer cells is primarily focused on increasing glutathione peroxidase 4 (GPX4) activity, preventing reactive oxygen species (ROS) generation, and inhibiting the ferroptosis process [33]. The results of our study support these findings. We observed that treatment with PA led to an increased GSH depletion and excessive accumulation of MDA, ROS and Fe^{2+} in gastric cancer cells, while iron metabolism regulatory proteins (SLC7A11, ferritin and GPX4) were downregulated, resulting in ferroptosis. Additionally, the ferroptosis inhibitor Fer-1 partially counteracted the ferroptosis-promoting and antitumour effects of PA, further validating our hypothesis.

PDGFRB is a transmembrane tyrosine kinase receptor encoded by the *PDGFRB* gene. It plays a crucial role in stimulating blood vessel formation upon activation by platelet-derived growth factors [34], as well as in regulating crucial cellular processes such as growth, proliferation, invasion, migration and survival [35–38]. Dysregulation of PDGFRB is closely associated with both carcinogenesis and cardiovascular diseases [38]. In the context of gastric cancer, PDGFRB may function as a valuable prognostic factor, as indicated in a 2017 study by Raja, who examined PDGFRB immunohistochemical expression [39]. Furthermore, a series of studies has consistently demonstrated that the overexpression of PDGFRB is linked to the progression of gastric cancer [40], with high epithelial PDGFRB expression correlating with differences in disease-free survival and overall survival among gastric cancer patients [41]. To elucidate the involvement of PDGFRB in the anticancer effects of PA, we conducted RNA sequencing. We observed a significant downregulation of PDGFRB in gastric cancer cells treated with PA compared to that in untreated gastric cancer cells. To investigate the

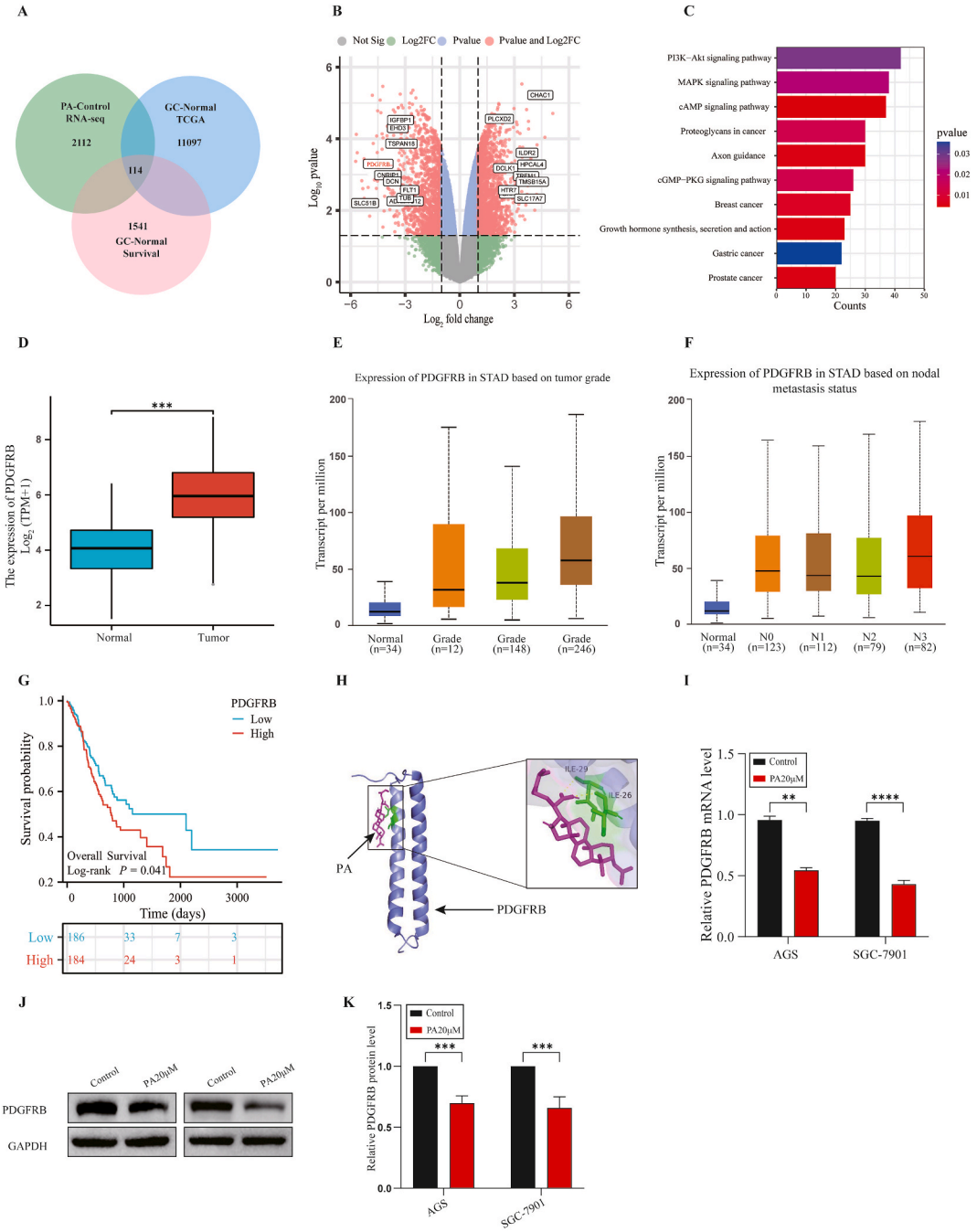


Fig. 2. PDGFRB is an important target for the anti-gastric cancer effects of PA. (A) Venn diagram showing the DEGs in gastric cancer cells induced by PA compared with those in the TCGA database and the genes associated with survival. (B) Volcano plot analysis of differentially expressed transcripts in gastric cancer cells treated with PA. (C) KEGG analysis of differentially expressed transcripts in gastric cancer cells treated with PA. (D, E, F) The expression of PDGFRB in GC and its relationship with tumor grade and lymph node metastasis grade (data were obtained from the UALCAN cancer database). (G) Kaplan–Meier curves of PDGFRB expression and overall survival. (H) Molecular docking of PA with PDGFRB. (I) qRT−PCR analysis of PDGFRB expression after treatment with PA (20 μmol/L). (J, K) Western blot analysis of PDGFRB protein expression levels in gastric cancer cells after treatment with PA (20 μmol/L) (Supplementary Material Fig. S1). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n = 3.

role of PDGFRB in PA-induced ferroptosis in gastric cancer, we modulated PDGFRB expression in AGS and SGC-7901 cells using lentiviral vectors. Knockdown of PDGFRB led to reduced proliferation, migration and invasion of gastric cancer cells, along with decreased levels of ferroptosis-regulating proteins. In contrast, PDGFRB overexpression increased these cellular processes.

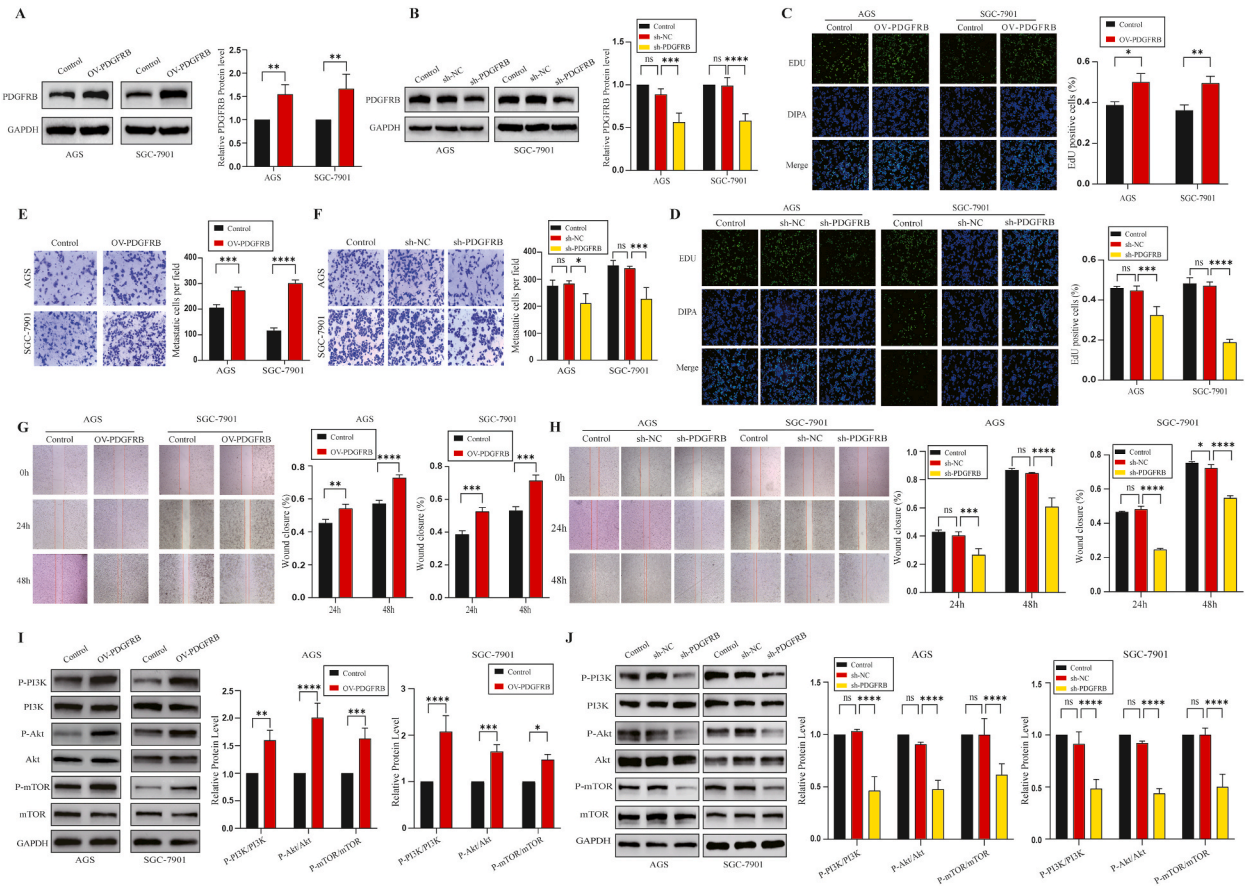


Fig. 3. Overexpression and knockdown of PDGFRB can regulate the progression of gastric cancer cells and the PI3K/Akt signalling pathway. (A, B) The efficiency of PDGFRB overexpression and knockdown was validated through Western blotting (Supplementary Material Fig. S2). (C, D) Quantitative analysis of EdU fluorescence to assess the effect of PDGFRB overexpression or knockdown on the proliferative capacity of gastric cancer cells. (E, F) The effect of PDGFRB overexpression or knockdown on the invasive capacity of gastric cancer cells was assessed using a Transwell assay. (G, H) Wound healing experiments demonstrating the effect of PDGFRB overexpression or knockdown on the migratory ability of gastric cancer cells. (I, J) Western blot analysis demonstrated that the expression of PI3K/Akt signalling pathway components (phosphorylated PI3K, Akt and mTOR) is regulated by PDGFRB (Supplementary Material Figs. S3–S4). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n = 3.

Interestingly, PA can counteract the promoting effects of PDGFRB overexpression on the proliferation, migration and invasion of gastric cancer cells. The mechanism by which PDGFRB regulates ferroptosis in gastric cancer cells involves multiple factors and requires further investigation. In terms of molecular regulatory mechanisms, we used molecular docking techniques to reveal that PA exhibits high binding affinity for PDGFRB. In addition, our high-throughput sequencing analysis suggested that the PI3K/Akt pathway may plays a key role in PA-induced ferroptosis in gastric cancer. These results collectively suggest that PA not only promotes ferroptosis in gastric cancer cells by directly binding to PDGFRB but may also indirectly regulate gastric cancer progression through the PI3K/Akt signalling pathway. We found that both PA treatment and PDGFRB knockdown inhibited the activation of the PI3K/Akt pathway and reduced the phosphorylation levels of PI3K, Akt and mTOR, further supporting our hypothesis. In addition, a subset of gastric cancer patients exhibits PI3K mutations, which can lead to abnormal activation of the PI3K/Akt pathway and promote cancer progression. However, our study did not analsze this aspect in clinical samples, which requires further discussion and validation.

5. Conclusions

Our study provides initial evidence that PA induces ferroptosis in gastric cancer cells by modulating the PI3K/Akt signalling pathway mediated by PDGFRB, thereby exerting its anticancer effects. These findings hold promise for the development of novel therapeutic agents for gastric cancer patients.

Funding information

This research was supported by Science and Technology Projects in Guangzhou, 2023A03J0274; The Projects of Traditional

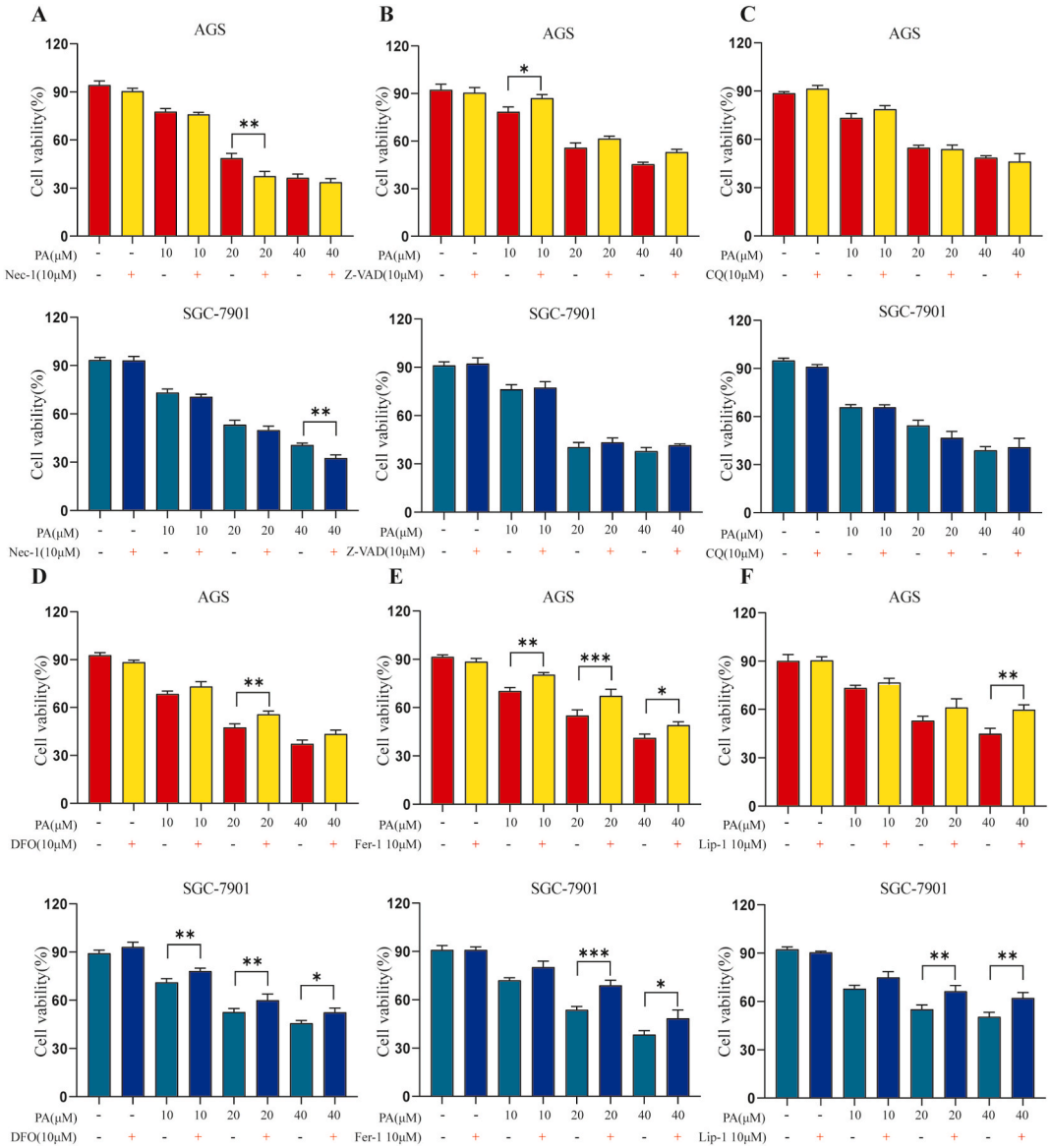


Fig. 4. Inhibitory effects of PA alone or in combination with other cell death inhibitors on the growth of gastric cancer cells. (A–F) Column chart illustrating changes in cell viability upon the addition of various cell death inhibitors (Nec-1, Z-VAD, CQ, DFO, Fer-1, and Lip-1) to AGS and SGC-7901 cells treated with gradient concentrations of PA. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n = 3. Nec-1, necrostatin-1; CQ, chloroquine; Z-VAD, carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]; DFO, deferoxamine; Fer-1, ferrostatin; Lip-1, liproxstatin-1.

Chinese Medicine Bureau of Guangdong Province, 20242005.

Date availability statement

The analysed data sets generated during the present study are available from the corresponding author on reasonable request.

Ethics statement

The Ethics Committee of South China Agricultural University approved our study (Approval Code: 2023H007). All the experimental methods abided by the Helsinki Declaration.

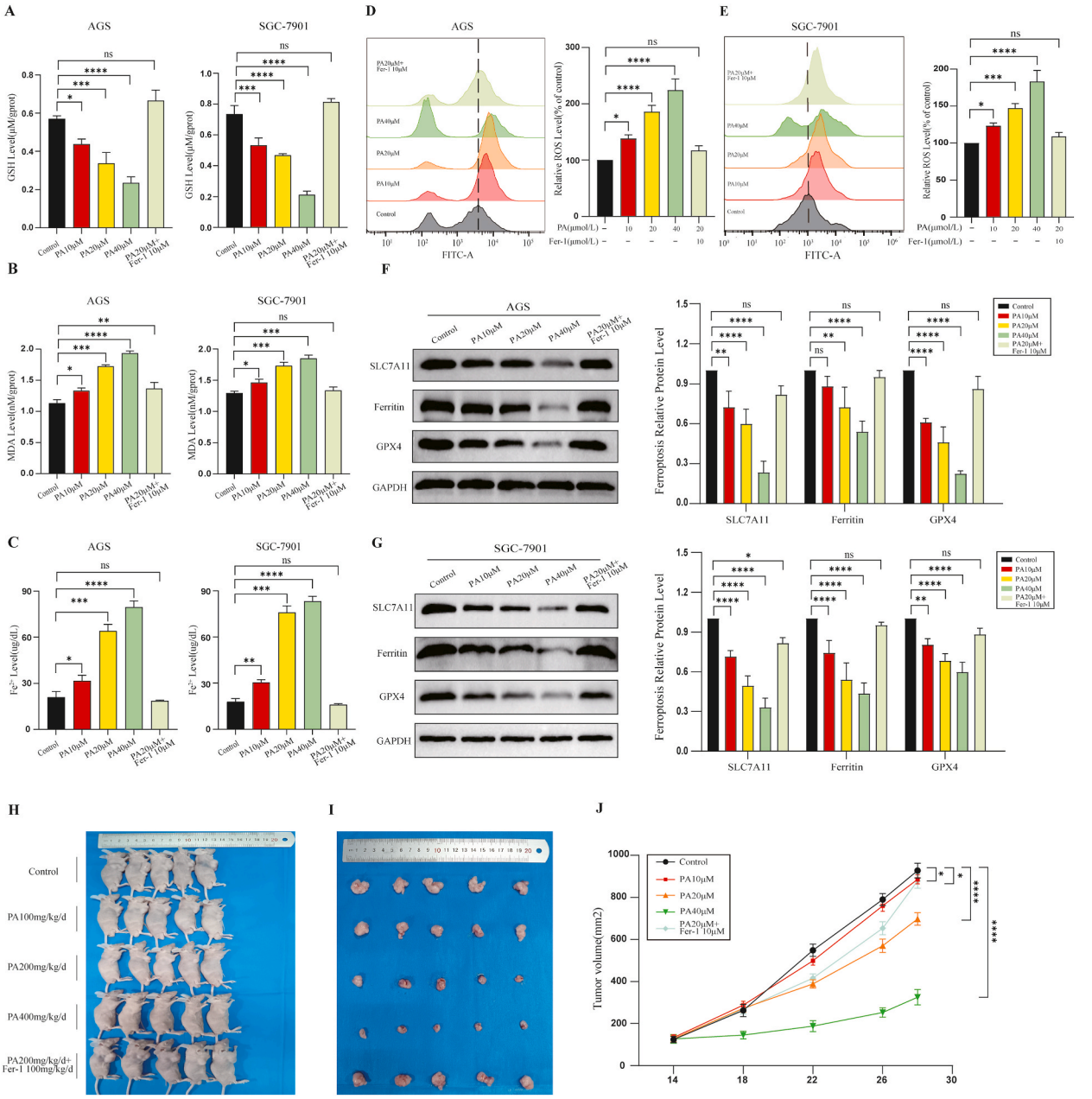


Fig. 5. In vivo and in vitro experiments confirmed that PA inhibits gastric cancer by promoting ferroptosis. (A) The effect of PA on intracellular GSH in gastric cancer cells. (B) Effect of PA on intracellular MDA in gastric cancer cells. (C) Effect of PA on intracellular Fe^{2+} in gastric cancer cells. (D, E) Bar chart validating the effect of PA on intracellular ROS levels in gastric cancer cells. (F, G) The impact of PA on ferroptosis-related protein levels in gastric cancer cells (Supplementary Material Fig. S5). (H, I) Photos of each group of mice and subcutaneous tumor specimens. (J) Curves showing the tumor volume of each group of mice. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n = 3. GSH, glutathione; MDA, malondialdehyde; ROS, reactive oxygen species.

CRediT authorship contribution statement

Jinlin Nie: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **Haoran Zhang:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation. **Xiaofeng Li:** Software, Resources, Methodology, Investigation. **Jiawei Qin:** Writing – review & editing, Validation, Supervision, Software. **Jiawei Zhou:** Validation, Supervision, Software, Resources, Methodology, Investigation. **Yuhui Lu:** Visualization, Software, Project administration, Investigation. **Nengjia Yang:** Validation, Resources, Project administration, Investigation, Data curation. **Yanan Li:** Writing – original draft, Project administration, Methodology. **Hailiang Li:** Writing – review & editing, Resources, Project administration, Funding

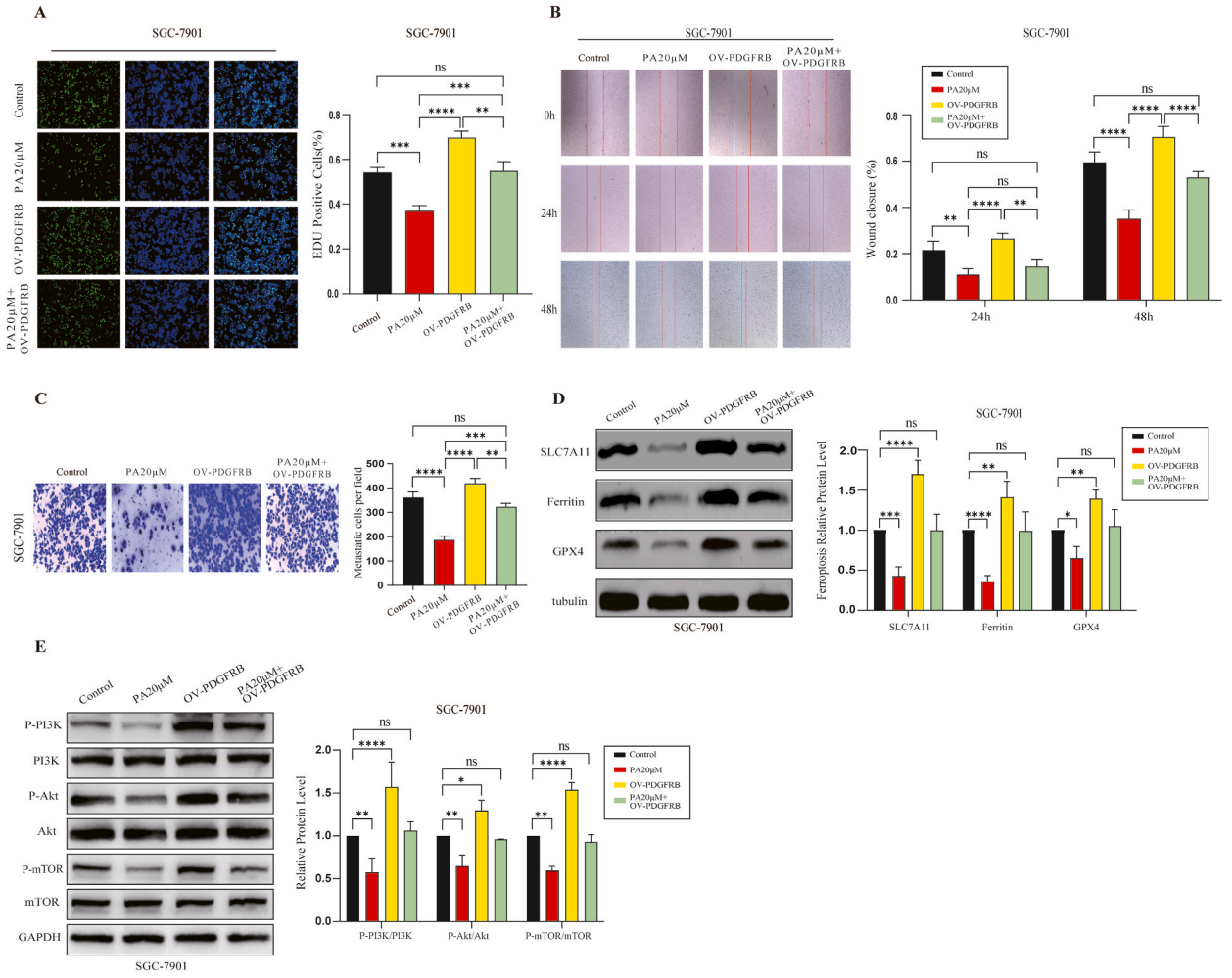


Fig. 6. PA can counteract the promoting effect of overexpressed PDFGRB on gastric cancer. (A) The EdU assay detected changes in cell proliferation among the different treatment groups. (B) The wound healing assay detected changes in cell migration abilities among the different treatment groups. (C) Transwell assays detected changes in cell invasion abilities among the different treatment groups. (D) Changes in key ferroptosis-related proteins in each group. Tubulin was used as a loading control (Supplementary Material Fig. S6). (E) Changes in the levels of PI3K/Akt signalling pathway proteins in each group. GAPDH was used as a loading control (Supplementary Material Fig. S7). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n = 3.

acquisition, Conceptualization. **Cheng Li:** Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge the Affiliated Guangdong Second Provincial General Hospital of Jinan University for funding this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38800.

References

[1] H. Sung, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality Worldwide for 36 cancers in 185 Countries, CA: a cancer journal for clinicians 71 (3) (2021) 209–249.
[2] Z. Li, et al., Multi-omics characterization of molecular features of gastric cancer correlated with response to neoadjuvant chemotherapy, Sci. Adv. 6 (9) (2020) eaay4211.
[3] Y. Xuan, Y.N. Wang, Hypoxia/IL-1α axis promotes gastric cancer progression and drug resistance, Journal of Digestive Diseases 18 (9) (2017) 511–520.
[4] J.W. Pepper, et al., Synthesis: cancer research meets evolutionary biology, Evolutionary applications 2 (1) (2009) 62–70.
[5] E. Yagüe, et al., Ability to acquire drug resistance arises early during the tumorigenesis process, Cancer Res. 67 (3) (2007) 1130–1137.
[6] C. Wei, et al., Pharmacological profiles and therapeutic applications of pachymic acid, Exp. Ther. Med. 24 (3) (2022) 1–13.
[7] X. Li, et al., Pachymic acid activates TP53INP2/TRAF6/caspase-8 pathway to promote apoptosis in renal cell carcinoma cells, Environ. Toxicol. (2024).
[8] S. Huang, et al., Progress in the pharmacological study of Pachymic acid, Chin. Med. 37 (12) (2015) 3.
[9] Chunwei, et al., Pachymic acid inhibits the tumorigenicity of gastric cancer cells by the mitochondrial pathway, Anti Cancer Drugs 28 (2) (2017) 170–179.
[10] Gapter, et al., Induction of apoptosis in prostate cancer cells by pachymic acid from Poria cocos, Biochem. Biophys. Res. Commun. 332 (4) (2005) 1153–1161.
[11] H. Ling, et al., Pachymic acid inhibits cell growth and modulates arachidonic acid metabolism in nonsmall cell lung cancer A549 cells, Mol. Carcinog. 49 (3) (2010) 271–282.
[12] J.W. Jeong, et al., Pachymic acid induces apoptosis of EJ bladder cancer cells by DR5 Up-regulation, ROS generation, modulation of Bcl-2 and IAp family members, Phytother Res. 29 (10) (2015) 1516–1524.
[13] J. Ma, et al., Pachymic acid induces apoptosis via activating ROS-dependent JNK and ER stress pathways in lung cancer cells, Cancer Cell Int. 15 (1) (2015) 78.
[14] C. Lu, J. Ma, D. Cai, Pachymic acid inhibits the tumorigenicity of gastric cancer cells by the mitochondrial pathway, Anti Cancer Drugs 28 (2) (2017) 170–179.
[15] H. Wang, et al., Poria acid, triterpenoids extracted from Poria cocos, inhibits the invasion and metastasis of gastric cancer cells, Molecules 27 (11) (2022) 3629.
[16] W.S. Yang, B.R. Stockwell, Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells, Chem. Biol. 15 (3) (2008) 234–245.
[17] S.J. Dixon, et al., Ferroptosis: an iron-dependent form of nonapoptotic cell death, Cell 149 (5) (2012) 1060–1072.
[18] B.R. Stockwell, J.P.F. Angeli, H. Bayir, Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease, Cell 171 (2) (2017) 273–285.
[19] W.S. Yang, et al., Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis, Proc. Natl. Acad. Sci. U. S. A. 113 (34) (2016) E4966.
[20] Y. Yuan, et al., Kaempferol ameliorates oxygen-glucose deprivation/reoxygenation-induced neuronal ferroptosis by activating Nrf2/SLC7A11/GPX4 Axis, Biomolecules 11 (7) (2021) 923.
[21] D.S. Chandrashekar, et al., UALCAN: an update to the integrated cancer data analysis platform, Neoplasia 25 (2022) 18–27.
[22] F. Yu, et al., Progress on the screening and analysis of bioactive compounds in traditional Chinese medicines by Biological Fingerprinting analysis, Comb. Chem. High Throughput Screen. 13 (10) (2010).
[23] C.Y. Wang, X.Y. Bai, C.H. Wang, Traditional Chinese medicine: a treasured natural resource of anticancer drug research and development, Am. J. Chin. Med. 42 (3) (2014) 543–559.
[24] A.-G. Zhao, et al., Effects of Chinese Jianpi herbs on cell apoptosis and related gene expression in human gastric cancer grafted onto nude mice, World J. Gastroenterol. 8 (5) (2002) 792.
[25] X. Tong, et al., Targeting cell death pathways for cancer therapy: recent developments in necroptosis, pyroptosis, ferroptosis, and cuproptosis research, J. Hematol. Oncol. 15 (1) (2022) 174.
[26] J.-Z. Liu, et al., BDH2 triggers ROS-induced cell death and autophagy by promoting Nrf2 ubiquitination in gastric cancer, J. Exp. Clin. Cancer Res. 39 (2020) 1–18.
[27] L. Rong, et al., Salidroside induces apoptosis and protective autophagy in human gastric cancer AGS cells through the PI3K/Akt/mTOR pathway, Biomed. Pharmacother. 122 (2020) 109726.
[28] H. Wang, et al., Cell death affecting the progression of gastric cancer, Cell death discovery 8 (1) (2022) 377.
[29] Y. Wang, et al., Wnt/beta-catenin signaling confers ferroptosis resistance by targeting GPX4 in gastric cancer, Cell Death Differ. 29 (11) (2022) 2190–2202.
[30] C. Lu, D. Cai, J. Ma, Pachymic acid sensitizes gastric cancer cells to radiation therapy by upregulating bax through hypoxia, Am. J. Chin. Med. 46 (4) (2018) 875–890.
[31] K.X. Sun, H.W. Xia, Pachymic acid inhibits growth and induces cell cycle arrest and apoptosis in gastric cancer SGC-7901 cells, Oncol. Lett. 16 (2) (2018) 2517–2524.
[32] W.S. Yang, et al., Regulation of ferroptotic cancer cell death by GPX4, Cell 156 (1–2) (2014) 317–331.
[33] S. Hao, et al., Cysteine dioxygenase 1 mediates erastin-induced ferroptosis in human gastric cancer cells, Neoplasia 19 (12) (2017) 1022–1032.
[34] J. Andrae, R. Gallini, C. Betsholtz, Role of platelet-derived growth factors in physiology and medicine, Cold Spring Harbor Laboratory 22 (10) (2008) 1276–1312.
[35] T. Donnem, et al., Co-expression of PDGF-B and VEGFR-3 strongly correlates with lymph node metastasis and poor survival in non-small-cell lung cancer - ScienceDirect, Ann. Oncol. 21 (2) (2010) 223–231.
[36] F. Calzolari, et al., Tumor progression and oncogene addiction in a PDGF-B-induced model of gliomagenesis, Neoplasia 10 (12) (2008) 1373. IN10.
[37] D.C. Lev, et al., Inhibition of platelet-derived growth factor receptor signaling restricts the growth of human breast cancer in the Bone of nude mice, Clinical Cancer Research An Official Journal of the American Association for Cancer Research 11 (1) (2005) 306–314.
[38] J. Yu, C. Ustach, H.R.C. Kim, Platelet-derived growth factor signaling and human cancer, J. Biochem. Mol. Biol. (1) (2003) 36.
[39] Raja, et al., Immunohistochemical expression and localization of cytokines/chemokines/growth factors in gastric cancer, Cytokine 89 (2017) 82–90.
[40] J. Chen, et al., Candidate genes in gastric cancer identified by constructing a weighted gene co-expression network, PeerJ 6 (2018) e4692.
[41] J.X. Wang, et al., GLI2 induces PDGFRB expression and modulates cancer stem cell properties of gastric cancer, Eur. Rev. Med. Pharmacol. Sci. 21 (17) (2017) 3857.