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Research article

Ciprofloxacin is a novel anti-ferroptotic antibiotic

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ABSTRACT

Cancer patients undergoing chemotherapy are susceptible to various bacterial infections, necessitating prompt and precise antimicrobial treatment with antibiotics. Ciprofloxacin is a clinically utilized broad-spectrum antimicrobial agent known for its robust antiseptic activity. While ferroptosis, an oxidative form of cell death, has garnered attention as a promising avenue in cancer therapy, the potential impact of ciprofloxacin on the anticancer effects of ferroptosis remains unclear. This study seeks to investigate the potential influence of antibiotics on ferroptosis in human pancreatic ductal adenocarcinoma (PDAC) cells. Here, we report a previously unrecognized role of ciprofloxacin in inhibiting ferroptosis in human PDAC cells. Mechanistically, ciprofloxacin suppresses erastin-induced endoplasmic reticulum (ER) stress through the activating transcription factor 6 (ATF6) and ER to nucleus signaling 1 (ERN1) pathway. Excessive ER stress activation can trigger glutathione peroxidase 4 (GPX4) degradation through autophagic mechanisms. In contrast, ciprofloxacin enhances the protein stability of GPX4, a crucial regulator that suppresses ferroptosis by inhibiting lipid peroxidation. Thus, our study demonstrates the anti-ferroptotic role of ciprofloxacin, highlighting the importance of careful consideration when contemplating the combination of ciprofloxacin with specific ferroptosis inducers in PDAC patients.

1. Introduction

Ferroptosis is a form of regulated cell death characterized by the iron-dependent accumulation of lipid peroxides within cell membranes [1–3]. This unique form of cellular demise distinguishes itself from other cell death mechanisms, such as apoptosis, and is characterized by specific molecular ecosystems [4]. The small molecule erastin is the first reported ferroptosis activator known to target system xc^-, an amino acid antiporter responsible for importing cystine [1]. Inside the cell, cystine is rapidly reduced to cysteine, which then participates in the synthesis of the antioxidant glutathione (GSH). GSH, in turn, serves as a cofactor for glutathione peroxidase 4 (GPX4), an enzyme that plays a pivotal role in inhibiting lipid peroxidation, thereby preventing ferroptosis [5–8]. Furthermore, multiple GPX4-independent pathways have been reported to bolster the defense against ferroptosis in a context-dependent manner [4]. Despite ongoing research, the full scope of ferroptosis in health and disease remains incompletely

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understood, rendering it a vital focus in tumor therapy [9–12].

Ciprofloxacin is a broad-spectrum antibiotic belonging to the fluoroquinolone class of antibiotics [13]. It is commonly used to treat various bacterial infections, including urinary tract infections, respiratory tract infections, skin infections, and gastrointestinal infections [14]. In the context of cancer patients, particularly those undergoing chemotherapy or radiation therapy, weakened immune systems increase susceptibility to infections [15]. Ciprofloxacin may be prescribed prophylactically or as a therapeutic intervention to address bacterial infections that may arise during cancer treatment or disease progression [16–19]. Additionally, ciprofloxacin exhibits anticancer potential primarily by disrupting organelles, inducing DNA damage, and subsequently impeding tumor growth [20–23]. However, the effect of ciprofloxacin on the susceptibility of cancer cells to ferroptosis remains uncertain.

2. Materials and methods

2.1. Reagents

ZVAD-FMK (S7023), staurosporine (S1412), ferrostatin-1 (S7243), erastin (S7242), cycloheximide (S7418), hydroxychloroquine (S4430), MG132 (S2619), ciprofloxacin (S5208), thioflavin-T (S6873), JTC801 (S2722), 4μ8C (S7272), melatonin (S1204), necrostatin-1 (S8037), ampicillin sodium (S3170), LDC7559 (S9622), and kanamycin sulfate (S2315) were procured from Selleck Chemicals. Antibodies targeting PARP (13371-1-AP), ATP6V0D1 (18274-1-AP), TFRC (10084-2-AP), ACSL4 (22401-1-AP), AIFM2 (20886-1-AP), DHODH (14877-1-AP), ACTB (81115-1-RR), ERN1 (27528-1-AP), EIF2AK3 (20582-1-AP), and ATF6 (24169-1-AP) were sourced from Proteintech Biotechnology. The antibody to GPX4 (ab252833) was obtained from Abcam. The antibody to *p*-MLKL (37333) was purchased from Cell Signaling Technology. The antibodies to MAP1LC3A/B-II (A5179) and SQSTM1/p62 (A5180) were purchased from Bimake.

2.2. Cell culture

Human PDAC cell lines SW1990 (CRL-2172), CFPAC1 (CRL-1918), AsPC1 (CRL-1682), PANC1 (CRL-1469), and MIAPaCa2 (CRL-1420) were obtained from the American Type Culture Collection. All cell lines were maintained in complete Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, 11995073) supplemented with 10 % fetal bovine serum (FBS; Thermo Fisher Scientific, A3840001) and 1 % streptomycin/penicillin (Yeasen, 60162ES76) at 37 °C in a 5 % CO_2 atmosphere. Routine testing for mycoplasma contamination was performed, and contaminated cultures were promptly eliminated.

2.3. Western blot assay

RIPA lysis buffer supplemented with phosphatase inhibitor cocktail (Cell Signaling Technology, 5872) was employed to lyse cells, followed by quantitative determination of protein concentration using the bicinchoninic acid assay (BCA; Thermo Fisher Scientific, 23225) [24,25]. Proteins were resolved using 10 % or 12.5 % polyacrylamide gel electrophoresis gels (Epizyme, PG112) and subsequently transferred onto polyvinylidene difluoride membranes (Millipore, IPVH00010). Blocking was achieved with 5 % skim milk at room temperature for 1 h, followed by overnight incubation with various primary antibodies (1:1000) and subsequent incubation with secondary antibodies (1:2000) at room temperature for 1 h. Visualization of the resulting signals was accomplished using enhanced chemiluminescence (Thermo Fisher Scientific, 34095).

2.4. Cell viability assay

The assessment of cell viability was conducted using a CCK8 kit (Yeasen Biotechnology, 40203ES80) following the manufacturer's instructions [26,27]. Cells were seeded in 96-well plates, and after drug treatment for the specified duration, the medium was removed. A DMEM medium devoid of FBS was utilized to prepare a solution containing 10 % CCK8, which was then added to the cells and incubated for 1 h. Absorbance at 450 nm was measured using a multifunctional microplate reader.

2.5. Propidium iodide/hoechst33342 staining and thioflavin-T staining

Propidium iodide (PI) and Hoechst 33342 (BestBio, BB-4131-1) commercial reagents were employed to conduct cell death experiments in accordance with the manufacturer's instructions. Briefly, the treated cells were directly supplemented with a 1:100 dilution of PI and Hoechst 33342 and subsequently incubated at room temperature for 30 min. Fluorescence microscopy was employed for visualization, and ImageJ software was utilized for statistical analysis [28].

For thioflavin-T (Selleck Chemicals, S6873) staining [29], thioflavin-T was prepared in a PBS solution at a dilution of 1:3000. The cells were then cultured in the staining solution within an incubator for 30 min, after which they were observed under a fluorescence microscope.

2.6. Colony formation assay

Cells in logarithmic growth phase were seeded in 6 or 12-well plates at a density of approximately 2000 cells per well [30,31]. Following 24 h of drug treatment, the cells were detached and cultured for an additional 10–14 days. After washing with PBS, the cells

were fixed with 4 % paraformaldehyde and stained with 0.4 % crystal violet solution (Solarbio, C8470). Subsequently, the number of colonies per well was quantified using ImageJ software, with the number of colonies in the untreated group set as 100 %.

2.7. qPCR assay

RNA was extracted utilizing the conventional Trizol method, followed by reverse transcription of 1 μg RNA into first-strand cDNA using PrimeScript RT Master Mix (#RR036A, Takara). Real-time fluorescence quantitative polymerase chain reaction (qPCR) was employed to amplify the cDNA from diverse cell samples. The results were analyzed using the $2^{-\Delta\Delta Ct}$ method [32,33].

2.8. RNAi

Human *GPX4* shRNA and control empty shRNA (pLKO.1) were obtained from Sigma. 293FT cells (Thermo Fisher Scientific, R70007) were utilized to generate high-titer lentiviral particles [34,35]. These lentiviral particles, carrying the specified shRNA, were

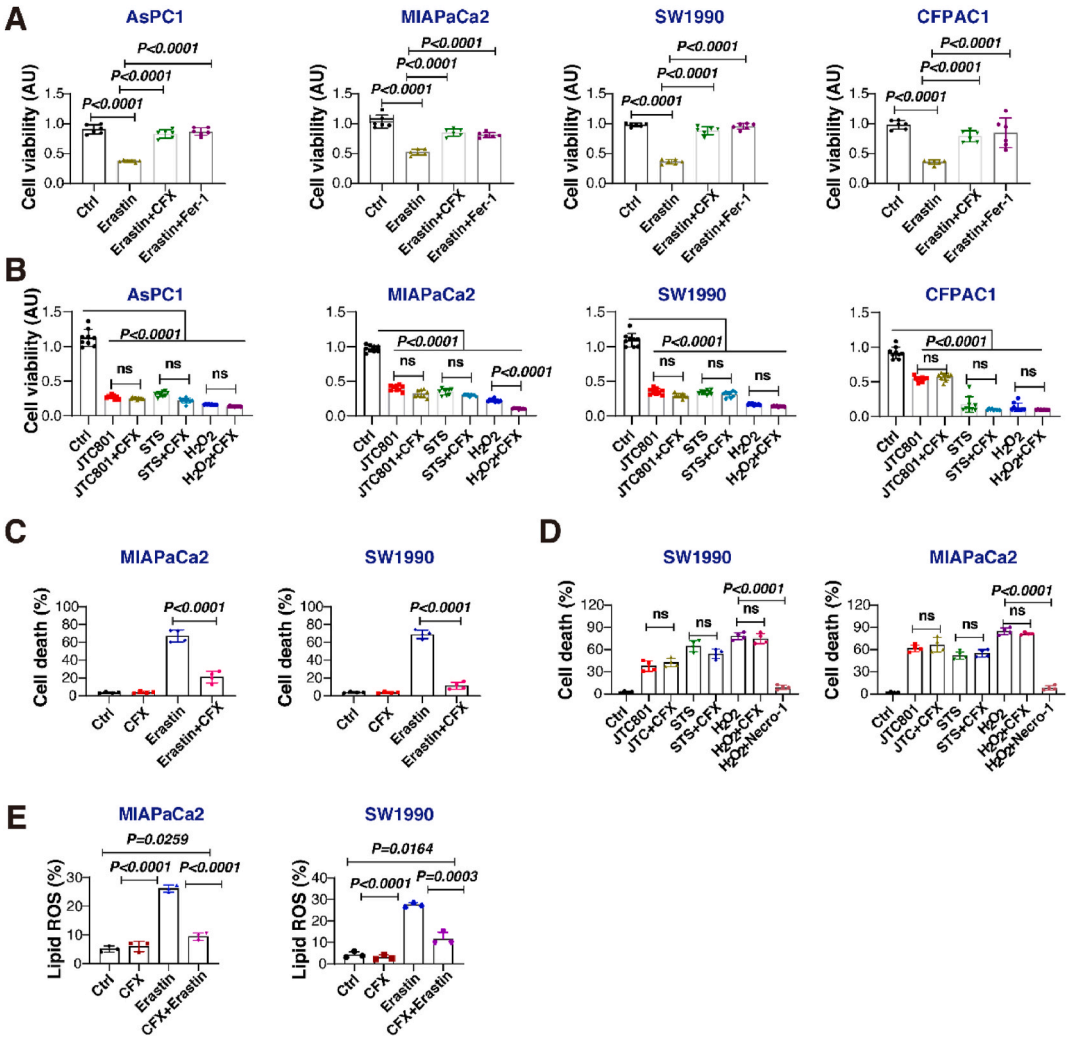

Fig. 1. Ciprofloxacin inhibits erastin-induced ferroptosis

(A) Cell viability assay of indicated PDAC cells following treatment with erastin (20 μM) in the absence or presence of ciprofloxacin (CFX; 50 μg/mL) or ferrostatin-1 (0.5 μM) for 24 h

(B) Cell viability assay of indicated PDAC cells following treatment with JTC801 (3.5 μM) or staurosporine (STS; 0.5 μM) or H_2O_2 (500 μM) in the absence or presence of ciprofloxacin (50 μg/mL) for 24 h

(C) Cell death assay of indicated PDAC cells following treatment with erastin (20 μM) in the absence or presence of ciprofloxacin (50 μg/mL) for 24 h

(D) Cell death assay of indicated PDAC cells following treatment with JTC801 (3.5 μM) or STS (0.5 μM) or H_2O_2 (500 μM) in the absence or presence of ciprofloxacin (50 μg/mL) or necrostatin-1 (0.5 μM) for 24 h

(E) Lipid ROS levels of PDAC in the indicated groups were detected using C11-BODIPY 581/591.

employed to transduce the cells for 48 h, followed by selection with puromycin (2 μg/ml; YEASEN, 60210ES72).

2.9. Immunofluorescence assay

Following drug treatment, cells were initially fixed with 4 % paraformaldehyde, permeabilized with 0.5 % Triton X-100, and subsequently blocked with 5 % BSA [36,37]. The cells were then incubated overnight with various primary antibodies, followed by a 1-h incubation with fluorescent secondary antibodies of different species at room temperature. Fluorescence confocal microscopy was then employed for observation.

2.10. Lipid peroxidation assay

The lipid peroxidation MDA assay kit (Beyotime, S0131) was employed to assess ferroptosis indicators following the manufacturer's instructions. Additionally, the lipid ROS level was analyzed using C11-BODIPY via flow cytometry [38–40].

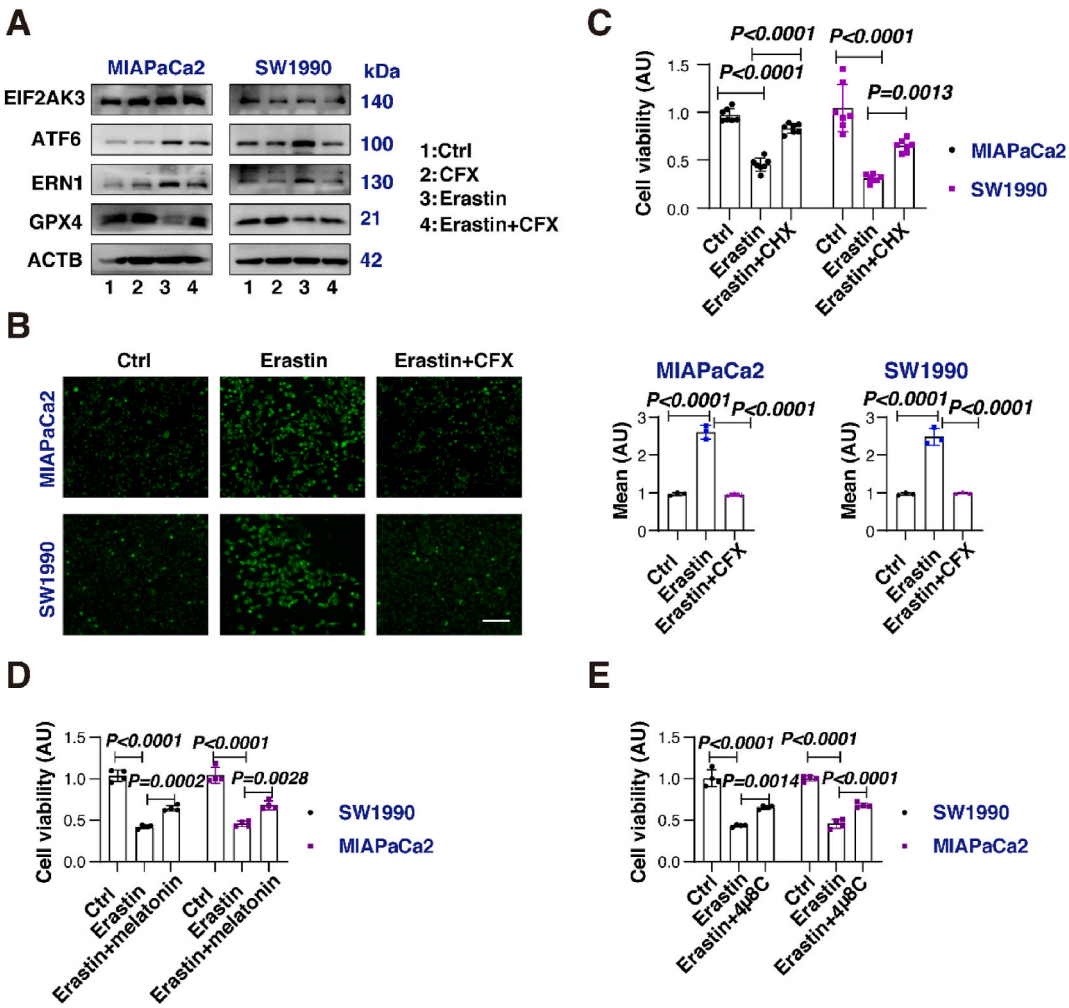

Fig. 2. Ciprofloxacin inhibits erastin-induced ER stress during ferroptosis

(A) Western blot detection of protein expression in PDAC cells in the indicated treatment groups [erastin (20 μM); ciprofloxacin (50 μg/mL)] for 24 h

(B) Amyloid deposition in PDAC cells was detected using the fluorescent dye thioflavin-T (green; 1:2000). Mean fluorescence intensity quantification using Image J software. Bar = 200 μm

(C) Cell viability assay of indicated PDAC cells following treatment with erastin (20 μM) in the absence or presence of cycloheximide (CHX; 10 μM) for 24 h

(D–E) Cell viability assay of indicated PDAC cells following treatment with erastin (20 μM) in the absence or presence of 4μ8C (500 nM), melatonin (20 μM) for 24 h.

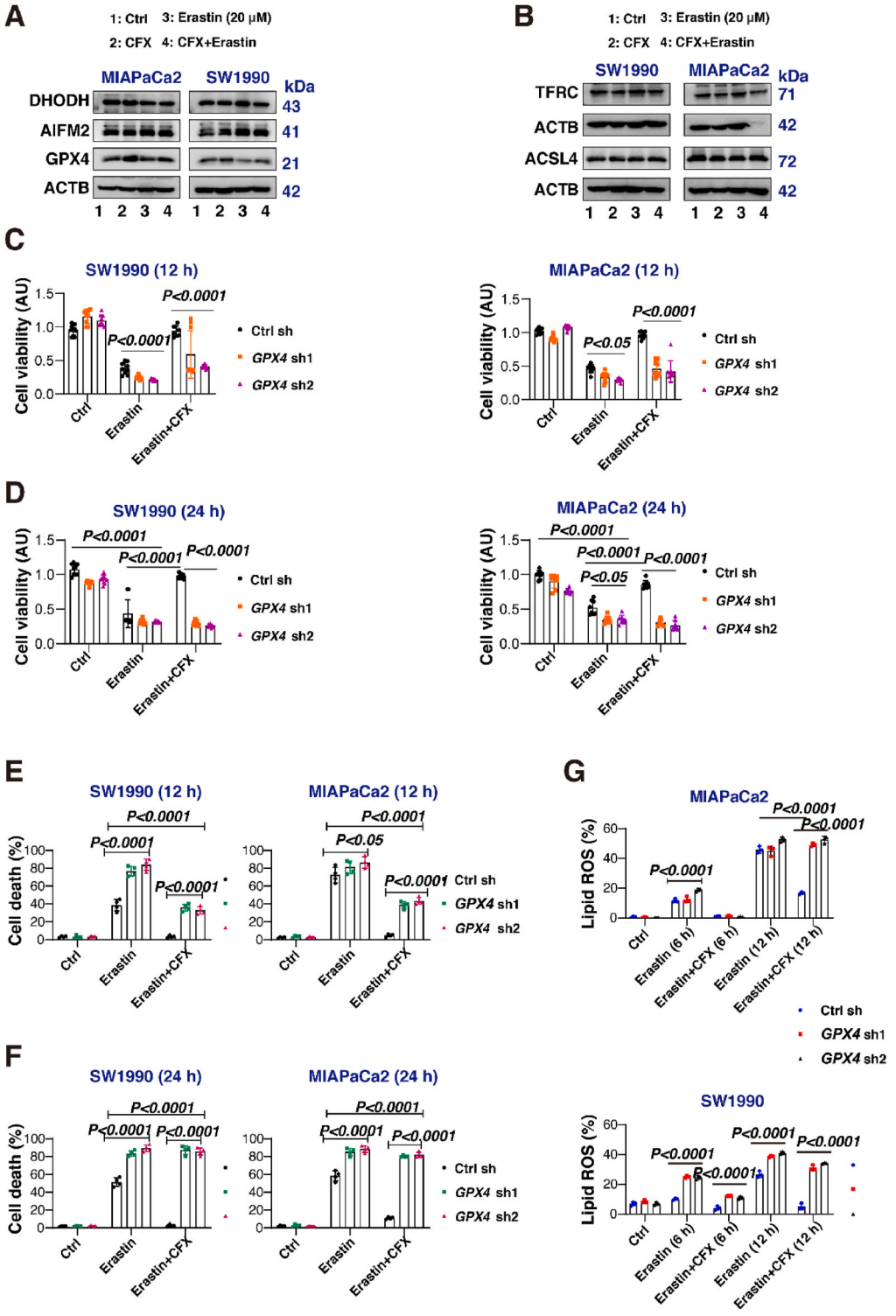

Fig. 3. Ciprofloxacin induces GPX4 upregulation

(A-B) Western blot detection of protein expression in PDAC cells in the indicated treatment groups [erastin (20 μM); ciprofloxacin (50 μg/mL)] for 24 h

(C) Cell viability assay of indicated control and *GPX4* knockdown PDAC cells following treatment with erastin (20 μM) in the absence or presence of ciprofloxacin (50 μg/mL) for 12 h

(D) Cell viability assay of indicated control and *GPX4* knockdown PDAC cells following treatment with erastin (20 μM) in the absence or presence of ciprofloxacin (50 μg/mL) for 24 h

(E) Cell death assay of indicated control and *GPX4* knockdown PDAC cells following treatment with erastin (20 μM) in the absence or presence of ciprofloxacin (50 μg/mL) for 12 h
(F) Cell death assay of indicated control and *GPX4* knockdown PDAC cells following treatment with erastin (20 μM) in the absence or presence of ciprofloxacin (50 μg/mL) for 24 h
(G) Lipid ROS levels of PDAC in the indicated groups [erastin (20 μM); ciprofloxacin (50 μg/mL)] were detected using C11-BODIPY 581/591.

2.11. Statistical analysis

Statistical analysis was conducted using GraphPad Prism 8.02. An unpaired *t*-test was employed to compare the difference between the means of two groups, while a one-way or two-way analysis of variance (ANOVA) with Tukey's multiple comparisons test was utilized for comparisons among various groups.

3. Results

3.1. Ciprofloxacin inhibits erastin-induced ferroptosis

Ciprofloxacin, a synthetic third-generation quinolone antibacterial antibiotic renowned for its broad antimicrobial spectrum (Fig. S1A), was investigated for its potential influence on cell death. We first assessed the impact of ciprofloxacin on the cell viability of four different human PDAC cell lines (SW1990, MIAPaCa2, AsPC1 and PANC1). As expected, a high dose of ciprofloxacin at 80 μg/mL exhibited the ability to inhibit cell growth. Conversely, ciprofloxacin at a concentration of 50 μg/mL failed to hinder cell growth, induce cell death, or disrupt cell colony formation (Fig. S1B-S1D). Thus, we employed the 50 μg/mL concentration of ciprofloxacin in subsequent experiments.

Subsequently, we treated these PDAC cell lines to representative inducers of various cell death pathways, including ferroptosis (erastin), alkaliptosis (JTC801), necroptosis (H_2O_2), and apoptosis (staurosporine), both in the presence and absence of 50 μg/mL ciprofloxacin. The cell viability assay revealed that ciprofloxacin inhibited erastin-induced growth inhibition (Fig. 1A). In contrast, it had no significant impact on the anticancer activities of JTC801, staurosporine, or H_2O_2 (Fig. 1B). This selective protective effect of ciprofloxacin against ferroptosis was further confirmed by propidium iodide (PI) staining. In addition, erastin-induced inhibition of cell viability was not rescued by the inhibitor of necroptosis (necrostatin-1), apoptosis (Z-VAD-FMK), and pyroptosis (LDC7559), but was reversed by the inhibitor of ferroptosis (ferrostatin-1), which confirmed its differential influence on various cell death pathways (Fig. 1C and D, and S2A-S2C).

Next, we proceeded to investigate the effect of ciprofloxacin on lipid peroxidation using the BODIPY 581/591 C11 probe. This assay demonstrated that ciprofloxacin inhibited erastin-induced lipid ROS production (Fig. 1E). In contrast, other antibacterial antibiotics, such as ampicillin and kanamycin, did not demonstrate a comparable effect on erastin-induced growth inhibition (Fig. S1E and S1F).

3.2. Ciprofloxacin inhibits erastin-induced ER stress during ferroptosis

ER stress occurs when there is an accumulation of unfolded or misfolded proteins within the ER, leading to the activation of the unfolded protein response (UPR). The UPR aims to restore ER homeostasis by reducing the protein load and enhancing protein folding capacity. However, if ER stress is prolonged or severe, it can lead to cell dysfunction and cell death, including ferroptosis [41]. To further investigate whether ciprofloxacin inhibits ferroptosis by modulating ER stress, we evaluated its effect on key ER stress-related pathways, including the eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3), activating transcription factor 6 (ATF6), and endoplasmic reticulum to nucleus signaling 1 (ERN1) pathway.

Indeed, Western blot assays demonstrated that erastin induced an increase in the protein expression of ATF6 and ERN1, while EIF2AK3 remained unaffected (Fig. 2A). However, when PDAC cells were co-treated with ciprofloxacin, ATF6 and ERN1 were inhibited (Fig. 2A). Additionally, we utilized the thioflavin-T assay to detect intracellular unfolded proteins, and the results demonstrated that ciprofloxacin reduced the accumulation of unfolded proteins induced by erastin (Fig. 2B). The use of cycloheximide, a widely recognized inhibitor of protein synthesis in eukaryotic cells, alleviated the erastin-induced inhibition of cell viability (Fig. 2C). Furthermore, the use of pharmacological approaches (e.g., 4μ8C and melatonin) to inhibit ATF6 or ERN1 similarly improved the erastin-induced inhibition of cell viability (Fig. 2D and E).

3.3. Ciprofloxacin induces GPX4 upregulation

To further determine the impact of ciprofloxacin on ferroptosis, we assessed the levels of GPX4 along with other key anti-ferroptotic proteins, including dihydroorotate dehydrogenase (DHODH) and apoptosis-inducing factor mitochondria-associated 2 (AIFM2). Additionally, we evaluated the expression of pro-ferroptotic effector proteins such as transferrin receptor (TFRC) and acyl-CoA synthetase long-chain family member 4 (ACSL4).

Western blot results demonstrated that ciprofloxacin treatment alone resulted in the up-regulation of GPX4 (Fig. 3A and B). Ciprofloxacin also prevented the erastin-induced degradation of GPX4 (Fig. 3A and B). In contrast, the protein expression levels of DHODH, AIFM2, ACSL4, and TFRC remained unaffected by ciprofloxacin treatment (Fig. 3A and B). Furthermore, ciprofloxacin had no impact on the expression of poly(ADP-Ribose) polymerase (PARP), ATPase H^+ transporting V0 subunit D1 (ATP6V0D1), and

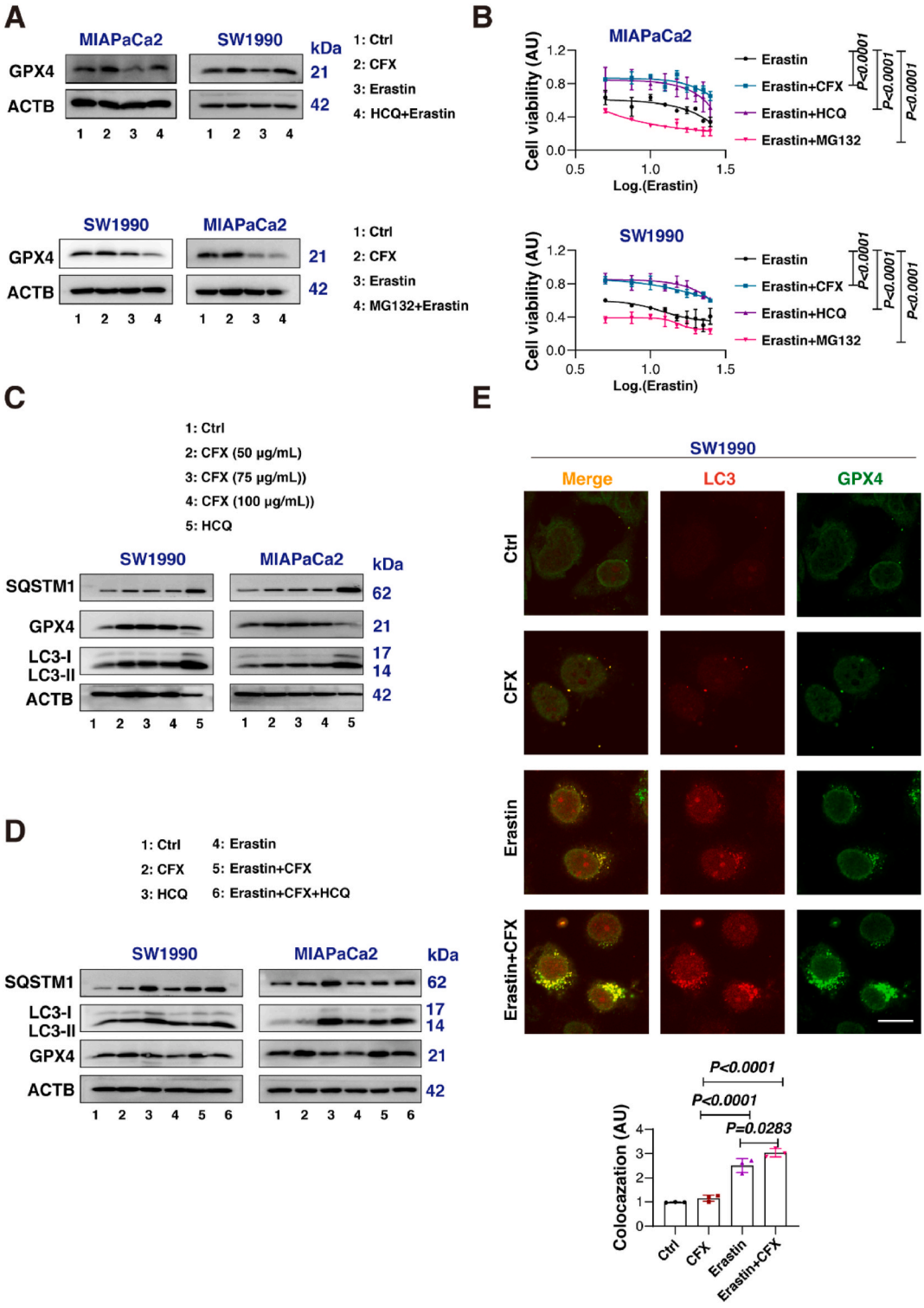

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Fig. 4. Ciprofloxacin inhibits the autophagic degradation of GPX4
(A) Western blot detection of protein expression in PDAC cells in the indicated treatment groups [erastin (20 μM); ciprofloxacin (50 μg/mL); hydroxychloroquine (HCQ; 20 μM)] for 24 h
(B) Cell viability assay of indicated PDAC cells following treatment with erastin (20 μM) in the absence or presence of ciprofloxacin (50 μg/mL), MG132 (300 nM) or HCQ (20 μM) for 24 h
(C–D) Western blot detection of protein expression in PDAC cells in the indicated treatment groups for 24 h
(E) Colocalization of endogenous MAP1LC3A/B with GPX4 in SW1990 cells treated with erastin (20 μM) in the presence or absence of ciprofloxacin (50 μg/mL) for 24 h. Scale bar: 5 μm.

phosphorylated mixed lineage kinase domain-like pseudokinase (*p*-MLKL), which are key effector proteins associated with apoptosis, alkaliptosis, and necroptosis, respectively (Fig. S3A-S3C). These findings suggest a selective role of ciprofloxacin in modulating GPX4 protein levels during erastin-induced ferroptosis.

Next, we determined whether the ciprofloxacin-mediated increase in GPX4 protein expression is a crucial factor in the inhibition of ferroptosis. We employed shRNA-mediated RNA interference to knock down *GPX4* in SW1990 and MIAPaCa2 cells (Fig. S3D). In the absence of *GPX4*, erastin induced cell viability inhibition and cell death, and notably, ciprofloxacin failed to prevent erastin-induced growth inhibition and cell death at 12 and 24 h (Fig. 3C–F, S4A, and S4B). Consistently, ciprofloxacin also failed to prevent lipid ROS production in the *GPX4*-knockdown cells (Fig. 3G).

3.4. Ciprofloxacin inhibits the autophagic degradation of GPX4

To delve deeper into the mechanisms underlying the downregulation of GPX4 during ferroptosis, we initially conducted quantitative PCR (qPCR) experiments, which revealed that ciprofloxacin-induced GPX4 expression is not contingent on transcriptional regulation (Fig. S3E). Considering the influence of the ubiquitin-proteasome system and lysosome-dependent autophagy on GPX4 protein levels in a context-dependent manner [7,42–50], PDAC cells were treated with either the proteasome inhibitor MG132 or the autophagy inhibitor hydroxychloroquine in combination with erastin.

Our Western blot analysis revealed that only hydroxychloroquine effectively inhibited erastin-induced GPX4 degradation (Fig. 4A). Furthermore, erastin-induced cell growth inhibition was attenuated in the presence of hydroxychloroquine, whereas MG132 exacerbated erastin-induced viability inhibition (Fig. 4B). To further test how ciprofloxacin inhibits GPX4 degradation, we examined the protein levels of microtubule-associated protein 1 light chain 3-II (MAP1LC3-II), which indicated that ciprofloxacin had a similar effect to hydroxychloroquine in increasing erastin-induced MAP1LC3-II levels (Fig. 4C and D). Furthermore, the co-localization of the autophagosome marker MAP1LC3A/B-II and GPX4 puncta increased in SW1990 cells treated with ciprofloxacin in combination with erastin (Fig. 4E).

4. Discussion

Ciprofloxacin is widely recognized for its potent antibacterial properties, making it an essential agent for treating a broad range of bacterial infections [13]. In this study, we revealed a unique characteristic of ciprofloxacin among fluoroquinolone antibiotics: its ability to selectively inhibit ferroptosis in PDAC cells, distinguishing it from other forms of cell death [28,51–53]. Given the extensive utilization of ciprofloxacin in cancer patients grappling with bacterial infections, the co-administration of ciprofloxacin alongside a ferroptosis inducer raises the prospect of potential attenuation in the anticancer effectiveness of the latter. Consequently, the selective behavior exhibited by ciprofloxacin and other antibiotic families implies that other antibiotics may not manifest such counterproductive effects.

Our present study has shed light on ciprofloxacin's pivotal role in shielding against ER stress—an integral initiation event in ferroptosis [30,54–56]. ER stress can be triggered by a multitude of cellular stressors, encompassing the aggregation of misfolded proteins within the endoplasmic reticulum, oxidative stress, nutrient insufficiency, and perturbations in calcium homeostasis [57]. When these stressors overwhelm the endoplasmic reticulum's capacity for protein folding, it precipitates the UPR, designed to rectify ER homeostasis [58]. Our investigations have indicated that ciprofloxacin inhibits erastin-induced ER stress, with a reliance on ATF6 and ERN1, as opposed to other ER stress pathways [59,60]. Further investigation is warranted to elucidate the upstream signaling mechanisms involved in ciprofloxacin-mediated inhibition of ER stress.

It's worth noting that the inhibition of ATF6 and ERN1 by ciprofloxacin might not be the direct cause of ferroptosis inhibition but rather a consequence of cell response following ferroptosis remission. Given ciprofloxacin's purported ability to target mitochondria [61], it could disrupt calcium homeostasis, which plays a role in the exchange between the ER and mitochondria, impacting ferroptosis occurrence [62]. Hence, ciprofloxacin may inhibit ferroptosis by modulating ER-mitochondrial calcium homeostasis [63].

A prominent feature of ferroptosis is the accumulation of lipid peroxides within cell membranes, leading to membrane dysfunction [64,65]. ER stress can modulate the degradation of GPX4 [66–68], which, in turn, impacts the cell's ability to inhibit lipid peroxidation. Our research has unveiled a hitherto unacknowledged role of ciprofloxacin—its ability to inhibit the ER stress-induced autophagy pathway. Autophagy represents a lysosomal-dependent degradation pathway proficient in selectively eliminating proteins or organelles via distinct autophagy receptors [66,69,70]. Prior studies have proposed sequestosome 1 (SQSTM1/p62) and tax1 binding protein 1 (TAX1BP1) as autophagy receptors governing GPX4 degradation under diverse conditions [45]. Ciprofloxacin appears to not only affect the fusion of autophagosomes with lysosomes, but also likely interferes with the binding of GPX4 to autophagy receptors. Although ciprofloxacin does not affect the expression of other ferroptosis regulators, it is still important to investigate

whether prolonged inhibition of GPX4 expression could activate GPX4-independent pathways [71].

Both ER stress and autophagy demonstrate dual effects in ferroptosis, which necessitates consideration of the stimuli type and cancer types involved [72]. Thus, prolonged treatment of erastin in combination with ciprofloxacin may increase the susceptibility of PDAC cells to ferroptosis. In addition, further investigation is needed to determine whether ciprofloxacin plays a similar role with other ferroptosis inducers (e.g., RSL3 and FIN56) in PDAC and other tumor types. Furthermore, our data indicates that ciprofloxacin induced a slight upregulation of GPX4 in a transcript-independent manner. Given the complex interplay between autophagy and the ubiquitin-proteasome system pathway in regulating protein degradation, further investigation is warranted to elucidate the mechanism and action of ciprofloxacin in enhancing GPX4 protein stability. Additionally, it is necessary to evaluate how ciprofloxacin regulates cell death sensitivity in the context of the interplay between ferroptosis and non-ferroptotic cell death, which is frequently encountered in patient treatment.

In summary, our study has highlighted ciprofloxacin's ability to inhibit erastin-induced ferroptosis in PDAC cells, emphasizing the need for careful consideration when combining therapeutic strategies. Future research should delve into the exact timing and specific mechanisms underlying this protective phenomenon.

Data availability statement

Data will be made available upon reasonable request.

CRediT authorship contribution statement

Fangquan Chen: Writing – original draft, Visualization, Validation, Software, Formal analysis, Data curation. **Hu Tang:** Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Junhao Lin:** Validation, Software, Resources, Formal analysis. **Rui Kang:** Writing – review & editing, Validation, Supervision, Formal analysis, Data curation. **Daolin Tang:** Writing – original draft, Validation, Supervision, Formal analysis, Data curation, Conceptualization. **Jiao Liu:** Writing – review & editing, Validation, Supervision, Software, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32571.

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