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Abstract

Objective: To investigate the inhibitory effect of the natural polyphenol Agrimol B on pancreatic ductal adenocarcinoma (PDAC) and its underlying molecular mechanisms.

Methods: The effects of Agrimol B on PDAC cell proliferation and apoptosis were assessed using Cell Counting Kit-8, colony formation, and flow cytometry assays. An *in vivo* PDAC xenograft mouse model was established for evaluation. Label-free quantitative proteomics, western blotting, immunofluorescence, and transmission electron microscopy were employed to analyze mitochondrial function, autophagy, and related signaling pathways. A patient-derived organoid model was used to evaluate the synergistic effects of Agrimol B with first-line chemotherapy drugs.

Results: Agrimol B significantly inhibited PDAC growth and induced apoptosis both *in vitro* and *in vivo*. Mechanistically, Agrimol B downregulated the expression of mitochondrial transcription termination factor 3, and promoted the accumulation of PTEN induced kinase 1 (PINK1) in mitochondria and Parkin translocation, thereby excessively activating PINK1/Parkin-dependent mitophagy. Concurrently, Agrimol B blocked lysosome biogenesis, leading to autophagosome accumulation and impaired autophagic flux. This dysfunctional autophagy ultimately mediated the anti-PDAC effect of Agrimol B. Furthermore, in PDAC patient-derived organoids, Agrimol B exhibited synergistic effects with first-line chemotherapy drugs such as gemcitabine and nab-paclitaxel.

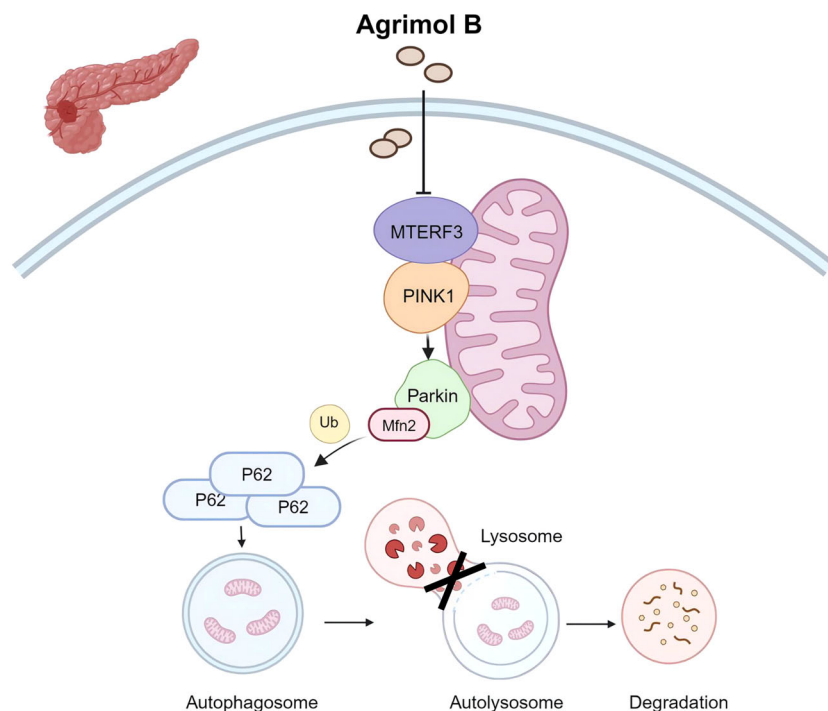
Conclusion: Agrimol B exerts its anti-PDAC effects by downregulating mitochondrial transcription termination factor 3, hyper-activating PINK1/Parkin-mediated mitophagy, and obstructing autophagic flux. Its synergistic effect with chemotherapy drugs provides experimental evidence supporting its potential clinical translation.

Keywords: pancreatic ductal adenocarcinoma, Agrimol B, mitophagy, MTERF3, PINK1/Parkin

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Graphical abstract



Agrimol B upregulates PINK1 by destabilizing the MTERF3 protein to initiate mitophagy, while also inhibiting autophagosome-lysosome fusion and reducing lysosomal activity to block autophagic flux; the resulting excessive autophagosome accumulation ultimately leads to PDAC cell death.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is currently the leading cause of cancer death worldwide [1, 2]. The mortality of patients with PDAC is expected to surpass that of patients with colorectal cancer by 2040, making it second only to lung cancer [3]. Due to the pancreas's location as a retroperitoneal organ and the nonspecific nature of its clinical symptoms, PDAC is often diagnosed at advanced stages, typically after distant metastasis has occurred. Consequently, surgical resection is feasible for only ~12% of patients, with 5-year survival rates remaining alarmingly low [4]. The PDAC tumour microenvironment is characterized by unique features that differentiate it from other malignancies. Firstly, the dense stromal component hinders the efficacy of chemotherapeutic agents. Secondly, there is a significant imbalance in the number and function of immune cells involved in both innate and adaptive immunity within this microenvironment. Moreover, the specific anatomical challenges associated with PDAC limit the application of radical radiotherapy doses, complicating effective tumour eradication. Therefore, there is an urgent need for novel treatment strategies that can improve patient outcomes [5].

Mitochondria are vital in the response to cellular stress. Under the action of external stimuli such as reactive oxygen species (ROS) stress, nutritional deficiency, and cell aging, the gradual accumulation of mitochondrial DNA (mtDNA) mutations occurs, reducing the intracellular mitochondrial membrane potential and causing depolarization damage [6]. To maintain the homeostasis of mitochondria and cells, damaged mitochondria are specifically

encapsulated in autophagosomes and fused with lysosomes for degradation, in a process known as mitophagy [7, 8]. In short, mitophagy is a type of selective autophagy that removes dysfunctional mitochondria from the cytoplasm in order to maintain mitochondrial integrity and homeostasis. However, impaired mitophagy can disrupt cell homeostasis and lead to cell death [9].

The attempt to find new antitumour active substances from natural resources (plants, animals, minerals, marine organisms, endogenous active substances, etc.) is a major research focus in the development of anticancer drugs [10–12]. Anticancer drugs derived from plants are widely used in clinical practice. For example, camptothecin (a topoisomerase inhibitor) is extensively used as an anticancer drug worldwide, and among camptothecin derivatives, hydroxycamptothecin has shown clinical efficacy for head and neck tumours, liver cancer, gastric cancer, and bladder cancer [13, 14]. Additionally, paclitaxel, an antimetastatic drug, promotes tubulin condensation and stabilizes microtubules [15]. Phenolic compounds are characterized by one or more aromatic rings combined with one or more hydroxyl groups [16]. The hydroxyl group on the benzene ring of phenolic compounds readily donates hydrogen electrons [16]. Recent studies have shown that phenolic compounds exhibit antioxidant, anti-inflammatory, and anticardiovascular disease effects [17]. Research into the role of polyphenols is also underway. In recent years, a series of studies has confirmed that polyphenols such as curcumin, epigallocatechin gallate, and resveratrol, exhibit antitumour activity by regulating autophagy, DNA methylation, histone modification, and long non-coding RNAs [18–21]. Previous studies have reported that Agrimol

B can also exert anticancer effects in prostate, colon, and liver cancer through different pathways [22–24]. However, its role in pancreatic cancer remains uncertain.

In our previous work, by screening a library of polyphenols, Agrimol B was found to have a potent inhibitory effect on the proliferation of PDAC cells. In this study, we present evidence that Agrimol B exhibits significant anti-PDAC activity by inducing a lethal blockade of autophagy. This process involves the degradation of mitochondrial transcription termination factor 3 (MTERF3) and the negative regulation of PTEN induced kinase 1 (PINK1) expression, thereby promoting the classical PINK1/Parkin mitophagy pathway. The blocked fusion of mitophagosomes and decreased lysosomal enzyme activity led to the accumulation of autophagosomes, which triggers mitophagy and further enhanced the cytotoxicity of Agrimol B in PDAC cells. Together, these results demonstrate that Agrimol B exhibits potent anti-PDAC effects, which are characterized by dysregulated mitophagy. Moreover, in a patient-derived organoid (PDO) model [25], Agrimol B enhanced the sensitivity of PDAC cells to first-line chemotherapy drugs. These findings provide direct preclinical and experimental evidence supporting the potential application of Agrimol B in PDAC treatment.

Materials and methods

Human specimens and ethics

PDAC tissue samples were acquired from patients who underwent surgical resection at West China Hospital of Sichuan University (Chengdu, China). All experiments involving these specimens were approved by the ethics committee of West China Hospital (CAT.201907V3, trial registration No. ChiCTR2100047942) and performed in strict accordance with the relevant regulations of the Declaration of Helsinki. The patients provided written informed consent. All samples were identified as tumour (including histopathologic type and stage) or normal tissues by a pathologist.

Cell culture

The PDAC cell lines PANC-1, AsPC-1, and CFPAC-1 were kindly provided by the Stem Cell Bank, Chinese Academy of Sciences. These cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM, Procell) or RPMI-1640 medium (Procell), supplemented with 10% foetal bovine serum (Vazyme), 2 mmol/l glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin in a 5% CO₂ atmosphere at 37°C. All cell lines used in this study were cultured for <2 months before being reinitiated from authentic stocks, and were routinely monitored via microscopic morphological observation and tested for mycoplasma contamination.

Reagents, antibodies, and plasmids

The polyphenol natural product library (L6100) and Agrimol B (T4S1173) were purchased from TargetMol. Hydroxychloroquine (HCQ) (HY-W031727), wortmannin (WORT) (HY-10197), Mdivi-1 (HY-15886), N-acetylcysteine (HY-B0215), Nab-Paclitaxel (HY-P99974), irinotecan (HY-16562), 5-fluorouracil (5-FU) (HY-107856), and oxaliplatin (HY-17371) were purchased from MedChem Express. Gemcitabine (S1714) and MG132 (S2619) were purchased from Selleck. Flag-MTERF3 plasmid was purchased from Fu-

lenGen. Primary antibodies against caspase 9 (680095), HADHA (R24526), superoxide dismutase 2 (SOD2) (306 028), autophagy related 7 (ATG7) (R23498), Sequestosome-1 (P62) (380612), cathepsin D (CTSD) (380946), and voltage dependent anion channel 1 (VDAC1) (R26067) were acquired from Zenbio. An anti-microtubule-associated protein light chain 3 (LC3) antibody (NB100-2220) was purchased from Novus, while anti-MTERF3 (EM1701-29) and lysosomal associated membrane protein 2 (LAMP2) (M1603-5) antibodies were purchased from HuaBio. The anti-Parkin (14060-1-AP) and mitochondrial import inner membrane translocase subunit 23 (TIM23) (11123-1-AP) antibodies were purchased from Proteintech, and an anti-PINK1 antibody (ab216144) was purchased from Abcam.

PDAC organoid construction

Patient-derived PDAC organoids were constructed using a MasterAim PDAC Organoid Kit (AIMINGMED). PDAC organoids were then dissociated into single cells, and 400 viable cells per well were plated in 384-well plates in 30 ml of 10% Matrigel (Corning) in complete human medium. Different drugs were added according to the experimental design after the regenerated PDAC organoids were observed under a microscope.

RNA interference

Scrambled small interfering (si) RNAs were custom synthesized chemically by GenePharma and resuspended according to the manufacturer's instructions. Cells were transfected at ~80% confluence using Lipofectamine 3000 reagent (Thermo Fisher Scientific). The experiments were performed 48 h after transfection, and the effects of siRNAs were verified through immunoblot analysis. siRNA sequences used in this study are listed in [supplementary Table 1](#), see online supplementary material.

Cell proliferation assay

Cell viability was measured using Cell Counting Kit-8 (CCK-8; Orisience). Cells were resuspended, counted, plated at ~5000 cells/well in a 96-well plate, and treated with different concentrations of Agrimol B for 24 h. Subsequently, 10 µL of CCK-8 solution was added to each well, and the cells were incubated for an additional 2–3 h. The optical density of cells was measured at a wavelength of 450 nm via a PE multifunctional microplate reader (BioTek Instruments).

Colony formation assay

The long-term effects on cell growth were determined using plate colony formation assays. Cells (1000/well) were plated in 12-well plates and the medium was changed every 4 days. After 1 week, cells were treated with the indicated concentration of Agrimol B for 24 h. One week later, colonies were fixed with 4% paraformaldehyde for 30 min and stained with crystal violet for 30 min. Images were captured after colonies had been washed three times.

EdU incorporation assay

EdU assays were performed according to the manufacturer's protocol (RIBOBIO). Following treatment, the cells were stained with

50 mmol/l 5-ethynyl-20-deoxyuridine for 4 h at 37°C. After fixation in 4% paraformaldehyde and permeabilization with 0.5% Triton X-100, the cells were stained with Apollo 488 reaction cocktail for 30 min followed by Hoechst 33342 for another 30 min. The Hoechst-positive cells and EdU-positive cells were immediately analysed using an inverted fluorescence microscope (NIKON).

Fluo-4 AM measurement

After different transfections, PANC-1 and AsPC-1 cells were seeded in 6-well plates and maintained for 24 h. Fluo-4 AM measurements were then performed according to the manufacturer's instructions (Beyotime). The data were analysed using FlowJo software.

ROS measurement

Following different transfections, PANC-1 and AsPC-1 cells were seeded in 6-well plates and maintained for 24 h. ROS were subsequently measured following the manufacturer's instructions (Beyotime), and the data were analysed using FlowJo software.

Mitochondrial membrane potential measurement

After different transfections, PANC-1 and AsPC-1 cells were seeded in 6-well plates and maintained for 24 h. Mitochondrial membrane potential was measured according to the manufacturer's instructions (Beyotime).

ATP measurement

After different transfections, PANC-1 and AsPC-1 cells were seeded in 6-well plates and maintained for 24 h. ATP levels were then measured following the manufacturer's instructions (Beyotime).

mtDNA measurement

After different transfections, PANC-1 and AsPC-1 cells were seeded in 6-well plates and maintained for 24 h. DNA was extracted according to the manufacturer's instructions (NucleoSpin Tissue) and mtDNA was detected using the Human Mitochondrial DNA Monitoring Primer Set (Takara).

Cell mitochondrial fraction

Mitochondrial fraction was isolated using a Cell Mitochondria Isolation Kit (Beyotime) according to the manufacturer's instructions. Protein concentration in the supernatant was measured using an instant BCA protein assay (Oriscience).

Label-free quantitative proteomics

PANC-1 and AsPC-1 cells were harvested and lysed after treatment with DMSO or 10 μ M Agrimol B. Protein lysates were subsequently reduced and alkylated with Tris (2-carboxyethyl) phosphine and iodoacetamide. After digestion with trypsin, samples were pooled and dried. After desalting using C18 Stage Tips, 2% acetonitrile and 0.1% trifluoroacetic acid were added into tube and the result-

ing peptides were analysed by Liquid Chromatograph-Mass Spectrometer (LC-MS) (Thermo Fisher Scientific Q Exactive Plus).

Observation of mitochondria and autophagosomes under transmission electron microscopy

Samples were prefixed with 3% glutaraldehyde, fixed with 1% osmium tetroxide, dehydrated in an acetone gradient, permeabilized, and embedded. Ultrathin sections (~60-90 nm) were prepared using an ultrathin sectioning machine, expanded, and mounted on copper mesh. The sections were stained with uranyl acetate for 10-15 min and then with lead citrate for 1-2 min. Photographs were taken with a JEM-1400 FLASH transmission electron microscope produced by Japan Electronics Corporation (JEOL).

Immunoblot assays

Cells were seeded in 6-well plates. After treatment, cells were harvested into an EP tube and digested in nondenaturing lysis buffer (NLB) buffer in the presence of a 1% protease inhibitor cocktail (Selleck). Protein concentration in the supernatant was quantified using an instant BCA protein assay. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a PVDF membrane. After the membrane was blocked with 5% skim milk for 90 min, it was incubated with an appropriate primary antibody overnight at 4°C, followed by incubation with a secondary antibody at room temperature for 90 min. Immunoreactive bands were detected using a chemiluminescence reagent (Proteintech), with β -actin serving as the internal control.

Immunohistochemistry assay

Formalin-fixed tissues were embedded in paraffin and consecutive sections were cut. The paraffin-embedded sections were dewaxed, rehydrated, and incubated in 3% H₂O₂ for 10 min to quench endogenous peroxidase activity. After antigen retrieval in citrate buffer and incubation with normal goat serum for 60 min at room temperature, the tumour sections were incubated with the indicated antibodies, followed by reaction with diaminobenzidine (Fuzhou Maixin Biotechnology) and counterstaining with Mayer's haematoxylin (Beyotime). Imaging was performed with a Leica DM2500 microscope. Two professional pathologists independently scored the immunohistochemical staining.

Immunofluorescence staining

Cells were plated on glass coverslips in 24-well plates. After various treatments, cells were fixed with 4% paraformaldehyde for 30 min and washed three times with phosphate-buffered saline. After permeabilization with 0.5% Triton X-100, cells were incubated with goat serum (Beyotime) for 40 min to block nonspecific binding sites of the primary antibody. After incubation with primary antibodies overnight at 4°C, the treated cells were incubated with Alexa Fluor secondary antibodies for 1 h at room temperature. The cells were incubated with DAPI (Solarbio) for 15 min to stain the nuclei. Images were captured using a confocal laser scanning microscope (Carl Zeiss, Oberkochen).

RNA isolation and quantitative real-time PCR

Total RNA was extracted from the cells after treatment, and cDNA was synthesized via a TaKaRa PrimeScript RT reagent kit (Takara) according to the manufacturer's protocol. The expression status of the candidate genes was determined using a real-time PCR system (Bio-Rad).

Flow cytometry

The apoptotic ratio was determined via an Annexin V-FITC/PI Apoptosis Detection Kit (4A BIOTECH) according to the manufacturer's protocol. The data were analysed via FlowJo software.

Generation of a tumour xenograft model

NCG mice (6 weeks old) were purchased from GemPharmatech and raised under SPF conditions. For the generation of a subcutaneous xenograft model, PANC-1 cells (1×10^7 cells/mouse) were injected subcutaneously into the mice. After the tumour volume reached $\sim 100 \text{ mm}^3$, the mice were divided into two groups and intraperitoneally injected with Agrimol B (20 mg/kg/day) or vehicle (5% DMSO, 10% ethanol, 10% Ricinus oil, or 75% physiologic saline). Tumour volume was measured every day and calculated using the following formula: $(\text{length} \times \text{width}^2/2)$. The mice were euthanized after 1 month and the xenograft tumours were harvested. All animal studies in this work were approved by the relevant committee of Sichuan University.

Statistical analysis

Statistical analysis was performed with GraphPad Prism v8.0.1 (La Jolla, CA, USA; <https://www.graphpad.com>). One- or two-way ANOVA or Student's t-test was used to determine statistical significance. All the data are presented as the mean (SD). $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ were considered statistically significant; ns means no significance.

Results

Agrimol B inhibits PDAC growth both *in vitro* and *in vivo*

First, we performed an unbiased, comparative analysis of therapeutic responses among 62 polyphenolic compounds (TOPSCIENCE L6100) in two human PDAC cell lines (PANC-1 and AsPC-1) via CCK-8 assays to identify potential alternatives (Fig. 1A and B). Agrimol B emerged as the top candidate with the highest cell inhibition rate (Fig. 1C and D). We then examined cell viability following Agrimol B exposure in multiple PDAC cell lines (PANC-1, AsPC-1, and CFPAC-1) through additional CCK-8 assays (Fig. 1E and F; [supplementary Fig. 1A-C](#), see online supplementary material). Consistent with these findings, Agrimol B treatment resulted in decreased clonogenicity and proliferative capacity, as demonstrated by colony formation and EdU incorporation assays (Fig. 1G and H; [supplementary Fig. 1D-F](#)). The results from flow cytometry analysis revealed that Agrimol B caused PDAC cell apoptosis in a dose-dependent manner (Fig. 1I). Western blot analy-

sis of apoptosis-related protein cleaved caspase 9, indicated that Agrimol B may mediate PDAC cell death by promoting apoptosis (Fig. 1J). Furthermore, to better mimic a clinical scenario to evaluate the effectiveness and safety of Agrimol B *in vivo*, we next established a cell line (PANC-1)-derived xenograft mouse model through subcutaneous implantation. As expected, tumour burden estimated volumetrically revealed a significant growth-inhibitory effect of Agrimol B without obvious weight loss following intraperitoneal administration for a 7-day period (Fig. 1K, M, and N). Hematoxylin-eosin staining (HE) showed no significant differences in the heart, liver, spleen, lung, or kidney between the control group and the treatment group ([supplementary Fig. 1G](#)), suggesting that Agrimol B has anti-pancreatic cancer activity *in vivo* without obvious toxic side effects. Immunohistochemical staining for the proliferation-related protein Ki67 revealed that the percentage of Ki67-positive cells in the Agrimol B group was significantly decreased (Fig. 1L and O). Hence, these results demonstrate that Agrimol B exhibits strong antitumour potency against PDAC both *in vitro* and *in vivo*, and this effect tends not to be followed by serious adverse reactions or even suspected toxicity, i.e. the therapeutic benefits of Agrimol B alone outweigh the risks.

Agrimol B induces mitochondrial damage in PDAC cells

To explore the mechanism underlying the antitumour activity of Agrimol B in PDAC cells, PANC-1 and AsPC-1 cells were treated with $10 \mu\text{mol/l}$ Agrimol B for 24 h, followed by label-free quantitative proteomics. The results revealed that the most significant changes caused by Agrimol B were in mitochondrial component proteins (Fig. 2A; [supplementary Fig. 2A-C](#), see online supplementary material). Therefore, we hypothesized that Agrimol B primarily induces PDAC cell death through mitochondrial damage. To test this hypothesis, we analysed several mitochondrial proteins after Agrimol B treatment, such as hydroxyacyl-CoA dehydrogenase alpha subunit (HADHA), TIM23, and SOD2, by western blot. The results showed that the mitochondrial protein expression gradually decreased as Agrimol B concentration increased (Fig. 2B). Mitochondria serve as reservoirs for calcium ions, and damage to mitochondria results in an increased calcium ion level in cytoplasm. Detected by flow cytometry, and observed under fluorescence microscopy, we found that the intracellular calcium level increased after treating PDAC cells with Agrimol B (Fig. 2C and D). Additionally, mitochondria are the primary source of ROS within cells. ROS accumulation can lead to mitochondrial damage, eventually resulting in cell death. This process is often accompanied by a decrease in mitochondrial membrane potential. By measuring ROS levels via flow cytometry, we found that ROS level in the Agrimol B group significantly increased (Fig. 2E). Through a combination of mitochondrial membrane potential-dependent red fluorescent probe MitoTracker Red CMXRos and the apoptotic green fluorescent probe Annexin V-FITC staining, Agrimol B was found to decrease the mitochondrial membrane potential in PANC-1 and AsPC-1 cells (Fig. 2F). As the relative number of mitochondria can be used to predict the mtDNA copy number, we measured the mtDNA content and found that Agrimol B treatment led to a decrease in the mtDNA copy number (Fig. 2G). Mitochondria are the primary sites of energy production in the body. When mito-

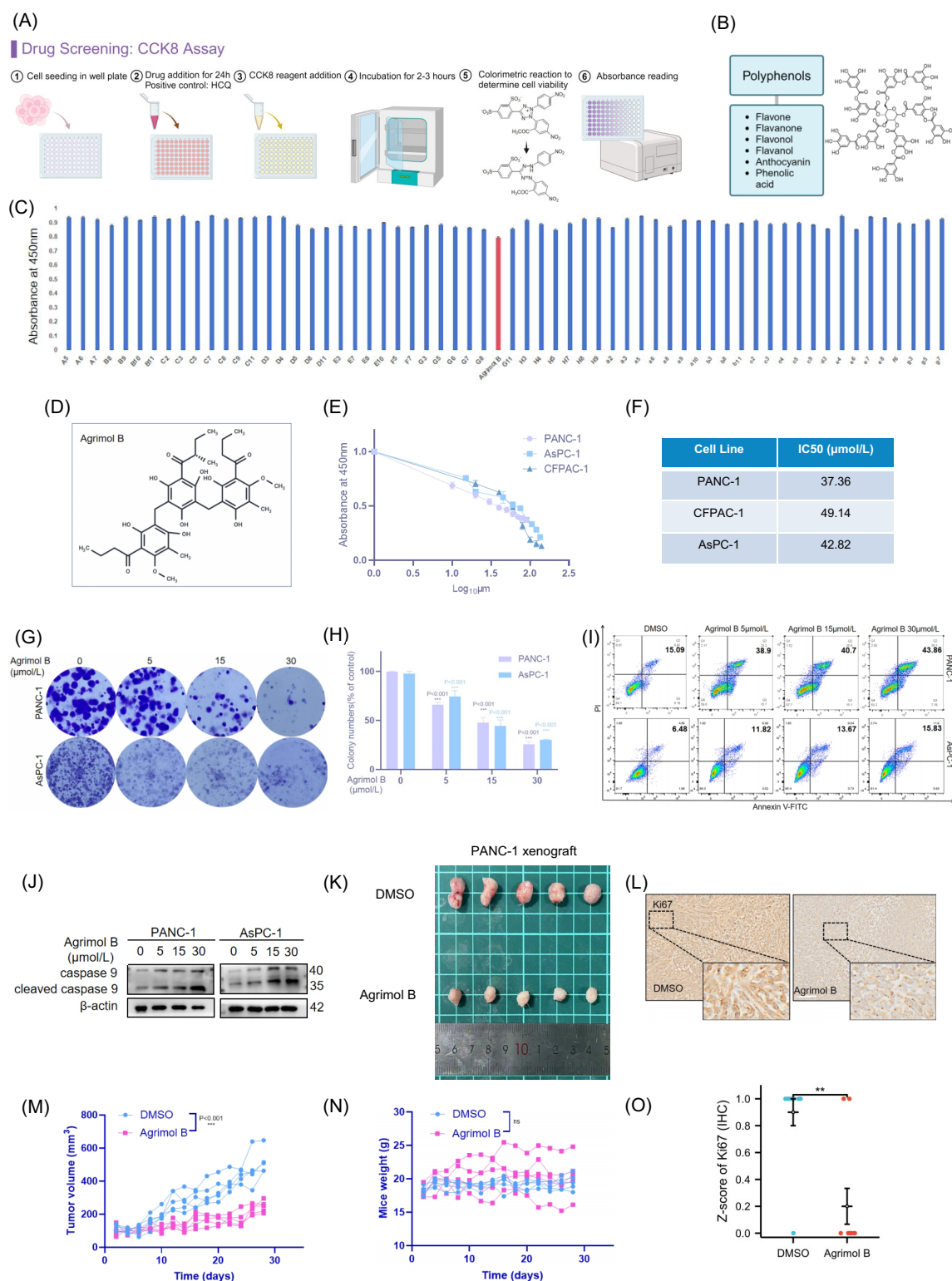


Figure 1 Agrimol B inhibits PDAC growth both *in vitro* and *in vivo*. (A) CCK-8 assay was used to evaluate the pharmacodynamic patterns of 62 natural polyphenols. (B) Polyphenol classification and chemical structure. (C) Viability of PANC-1 cells treated with 62 drugs (10 μmol/L). (D) Chemical structure of Agrimol B. (E) PANC-1, CFPAC-1, and AsPC-1 cell lines were treated with various concentrations of Agrimol B for 24 h. (F) IC₅₀ of Agrimol B in PDAC cells. (G, H) PDAC cells were treated with the indicated concentrations of Agrimol B. Cell proliferation was measured by a colony formation assay. (I) Flow cytometric analysis of PDAC cells treated with different concentrations of Agrimol B for 24 h. (J) Western blot analysis of caspase 9 and cleaved caspase 9 in PDAC cells treated with the indicated concentrations of Agrimol B for 24 h. (K) Tumours from the xenograft model treated with Agrimol B or vehicle. (M, N) Tumour volume and weight in mice in the cohorts treated with Agrimol B or vehicle. (L, O) Immunohistochemical staining of Ki67 in a xenograft model from the vehicle group or the Agrimol B group. Relative immunohistochemical scores were determined by multiplying the range of positively stained cells. Scale bars, 100 μm.

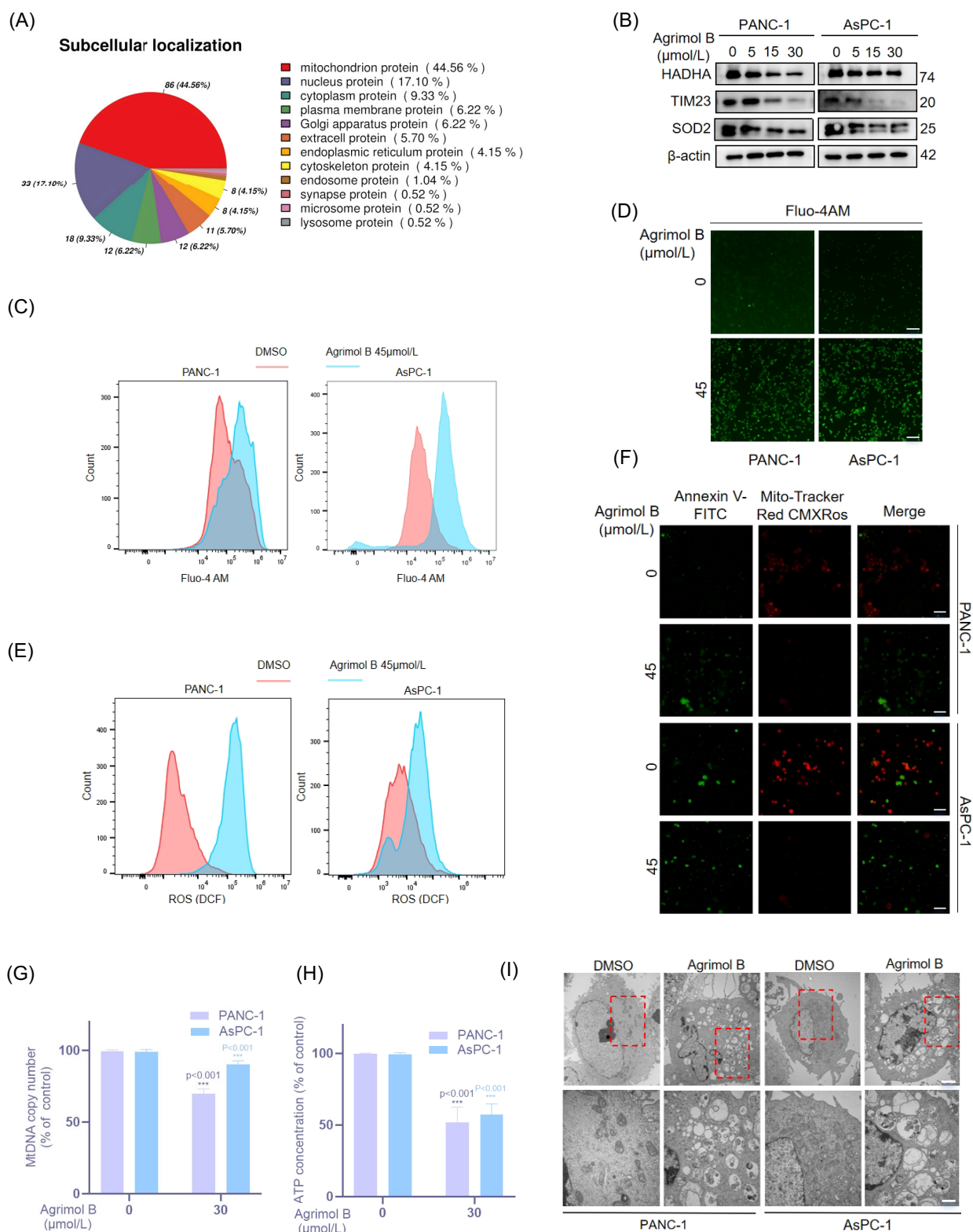


Figure 2 Agrimol B induces mitochondrial damage in PDAC cells. (A) Results of label-free quantitative proteomics after Agrimol B treatment for 24 h. (B) Western blot analysis of HADHA, TIM23, and SOD2 in PANC-1 and AsPC-1 cells. (C) Flow cytometric analysis of Fluo-4 AM accumulation in cells treated with or without 45 μmol/l Agrimol B. (D) Representative images of Fluo-4 AM accumulation in PANC-1 and AsPC-1 cells treated with or without 45 μmol/l Agrimol B for 24 h. Scale bars, 10 μm. (E) Flow cytometric analysis of mitochondrial ROS accumulation in cells treated with or without 45 μmol/l Agrimol B. (F) Representative images of mitochondrial morphology stained with Annexin V-FITC and MitoTracker Red CMXRos in PANC-1 and AsPC-1 cells treated with or without 45 μmol/l Agrimol B for 24 h. Scale bars, 10 μm. (G) Quantitative RT-PCR analysis of mtDNA copies. (H) ATP levels in PANC-1 and AsPC-1 cells treated with or without 45 μmol/l Agrimol B for 24 h. (I) Mitochondrial morphology was observed via transmission electron microscopy after treatment with or without Agrimol B for 24 h. Scale bars, 500 nm.

chondria are damaged, the ability to produce ATP is affected and ATP content significantly decreases. A luminometer revealed that the ATP content in PDAC cells treated with Agrimol B was significantly decreased (Fig. 2H). Finally, mitochondrial ultrastructure was observed under transmission electron microscopy, revealing that mitochondrial swelling, mitochondrial crista fracture, and mitochondrial hypertrophy, resulting in giant mitochondria or mitochondrial membrane rupture, and mitochondrial pyknosis occurred in Agrimol B-treated PDAC cells (Fig. 2I). These results indicate that Agrimol B induces mitochondrial damage in PDAC cells.

Agrimol B induces PINK1/Parkin pathway-dependent mitophagy initiation in PDAC cells

Mitophagy is a cellular process that selectively degrades excessive or damaged mitochondria. Given that the results above confirmed mitochondrial dysfunction in response to Agrimol B treatment, we investigated whether Agrimol B induces mitophagy in PDAC cells. During mitophagy, cells target specific mitochondria for degradation in order to maintain mitochondrial quality control. To verify whether Agrimol B actually causes mitophagy, we first examined the classical PINK1/Parkin pathway, which includes a mitochondrial serine/threonine kinase that normally occurs at low levels in normal mitochondria. PINK1 is hydrolysed by protein-dependent proteases in normal mitochondria. However, in the presence of mitochondrial damage or dysfunction, PINK1 stabilizes and accumulates within the mitochondria. This accumulation serves as a signal for mitophagy activation, triggering the selective removal of damaged mitochondria to maintain mitochondrial quality control and cellular homeostasis. Supporting our hypothesis, western blot analysis of isolated mitochondria from PDAC cells showed that Agrimol B treatment increased the protein levels of both PINK1 and Parkin in a dose-dependent manner (Fig. 3A). We also investigated the expression changes of the autophagosome marker LC3 and the autophagy-related protein ATG7 in PDAC cells after Agrimol B treatment. The results showed a dose-dependent increase in the expression of LC3 and pro-autophagic proteins, including ATG7, which was accompanied by the cytoplasmic accumulation of characteristic autophagic vacuoles and LC3 puncta (supplementary Fig. 3A, E and F, see online supplementary material). In further support of an autophagy-promoting role for Agrimol B, this induction was also validated by transmission electron microscopy, which visually demonstrates the formation of autophagosomes by Agrimol B use (supplementary Fig. 3D). Additionally, *in vivo* analysis of LC3 expression in subcutaneous tumours from NCG mice, assessed by immunohistochemical staining, revealed a significantly higher LC3-positive rate in the Agrimol B-treated group (supplementary Fig. 3B and C). Since Parkin translocation to the mitochondria is a hallmark of mitophagy, we further confirmed that Agrimol B induced Parkin's translocation from the cytoplasm to the mitochondria (Fig. 3B). Agrimol B treatment increased LC3 expression, and this effect was reversed by cotreatment with the mitophagy inhibitor Mdivi-1 (Fig. 3C). Moreover, PANC-1 and AsPC-1 cells transfected with siRNAs targeting PINK1 and Parkin (siPINK1 and siParkin) showed inhibited LC3 expression following Agrimol B treatment (Fig. 3D and E). Moreover, the contri-

butions made by Agrimol B to autophagic induction could be significantly attenuated through pharmacological inhibition using the phosphatidylinositol-3-kinase inhibitor wortmannin, also an early autophagy inhibitor (Fig. 3F-I). Thus, these findings collectively suggest that Agrimol B treatment can induce excessive mitophagy initiation in PDAC cells. Interestingly, PDAC cells exhibited potent cytotoxicity in response to Agrimol B treatment, which was closely linked to a strong induction of mitophagy, as expected from the correlation between mitochondrial damage and cell death.

Agrimol B blocks autophagic flux in PDAC cells

Normal autophagy depends not only on the smooth progress of autophagy initiation, but also on the smooth fusion of autophagosomes. To test Agrimol B-induced autophagic flux in PDAC cells, we measured the protein expression of P62, an autophagy-specific substrate that plays a crucial role in autophagy by binding to LC3 and delivering its substrate to lysosomes for degradation. Our results indicated that P62 expression was triggered by Agrimol B in a dose-dependent manner, suggesting a blockage of autophagic flux (Fig. 4A). Normal digestive enzyme activity and an acidic environment in lysosomes are essential for maintaining autophagic flux. The expression of CTSD, an important lysosomal digestive enzyme, decreased in both its precursor and mature forms in the Agrimol B-treated PDAC cell lines, suggesting that lysosomal hydrolytic enzymes were impaired and that the normal function of autophagy at the later stage was affected (Fig. 4B). As predicted, an in-depth study using a tandem GFP-RFP-LC3 construct revealed that autophagic flux was largely blocked in Agrimol B-treated PDAC cells, as evidenced by increased numbers of autophagosomes (yellow dots, RFP⁺ GFP⁺) and decreased numbers of autolysosomes (red dots, RFP⁺ GFP⁻) compared with those in the rapamycin-treated positive control groups (Fig. 4C, E, F). The possible involvement of attenuated autophagic flux was further identified by the infrequent but observable colocalization of LC3B with LAMP2 in PDAC cells upon Agrimol B treatment (Fig. 4D, G-L) and helped to explain why this blockage occurred. That is, the insufficient autophagic clearance induced by Agrimol B might be explained by decreased lysosomal biogenesis rather than attributed to altered lysosomal activity. What is noteworthy is that more and more LC3 puncta were formed in the group co-treated with HCQ (a late autophagy inhibitor) and Agrimol B (Fig. 4M-O; supplementary Fig. 3G). In summary, these data underscore that Agrimol B boosts autophagy initiation but blocks lysosome biogenesis, i.e. impaired autophagic flux, in PDAC cells.

Accumulation of mitophagosomes contributes to the anti-PDAC effects of Agrimol B

As the novel role of Agrimol B in treating PDAC has emerged, it is of great importance to perform in depth analyses of its anti-PDAC activity. Based on our previous findings, the impairment of autophagic flux has emerged as a critical factor in Agrimol B-induced cytotoxicity. To address this issue, we employed inhibitors tar-

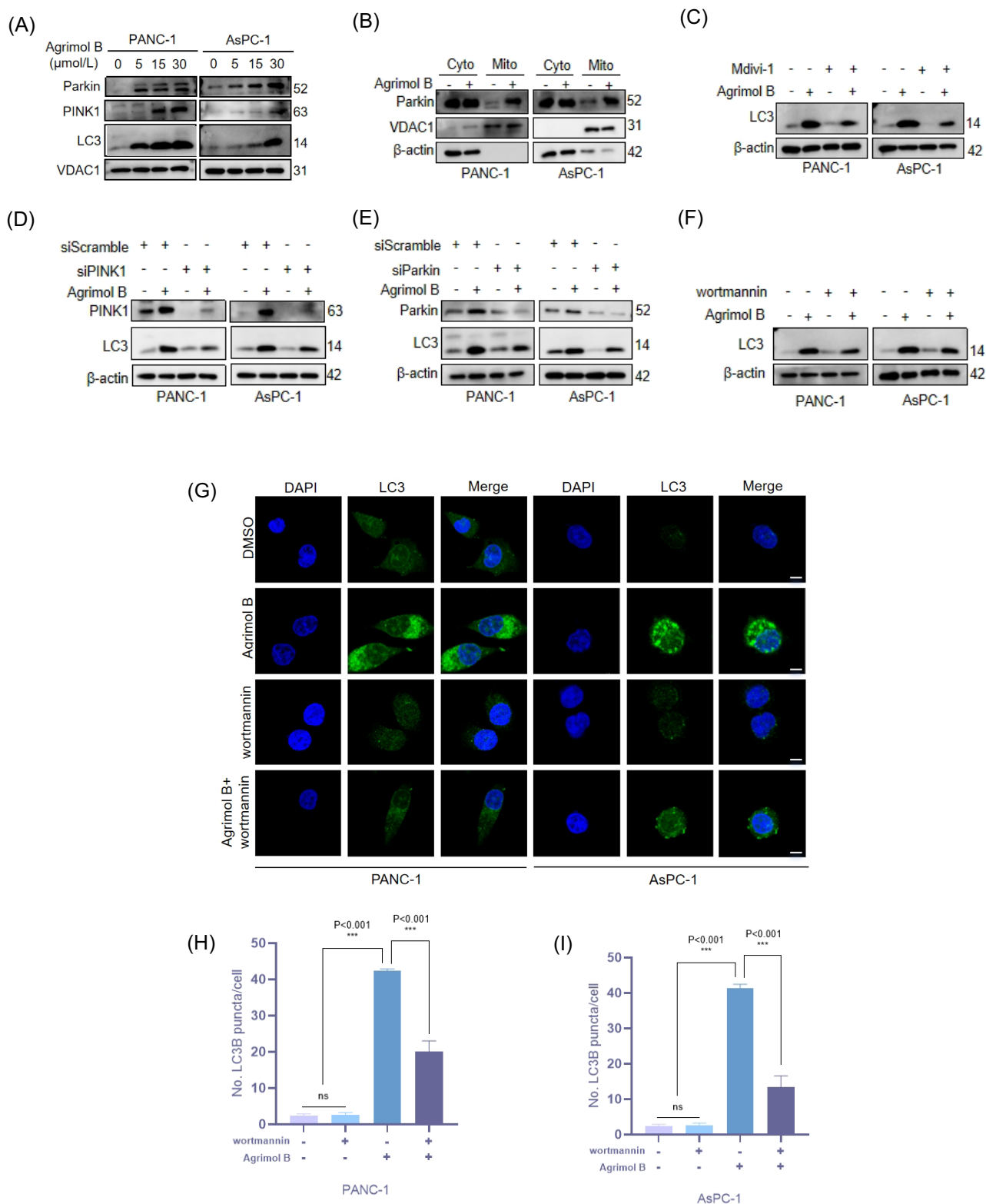


Figure 3 Agrimol B induces PINK1/Parkin pathway-dependent mitophagy initiation in PDAC cells. (A) Western blot analysis of PINK1, Parkin, and LC3 in the mitochondria of PANC-1 and AsPC-1 cells. (B) Western blot analysis of Parkin in the mitochondria and cytoplasm of PANC-1 and AsPC-1 cells. (C) Western blot analysis of LC3 in the presence or absence of Agrimol B in the presence or absence of Mdivi-1 for 24 h. (D, E) Western blot analysis of LC3 in PDAC cells transfected with siScramble, siPINK1, or siParkin following treatment with or without Agrimol B. (F) Western blot analysis of LC3 in PANC-1 and AsPC-1 cells with or without Agrimol B in the presence or absence of wortmannin. (G-I) Immunofluorescence analysis of LC3 in PDAC cells treated with or without Agrimol B in the presence or absence of wortmannin. Scale bars, 10 μm.

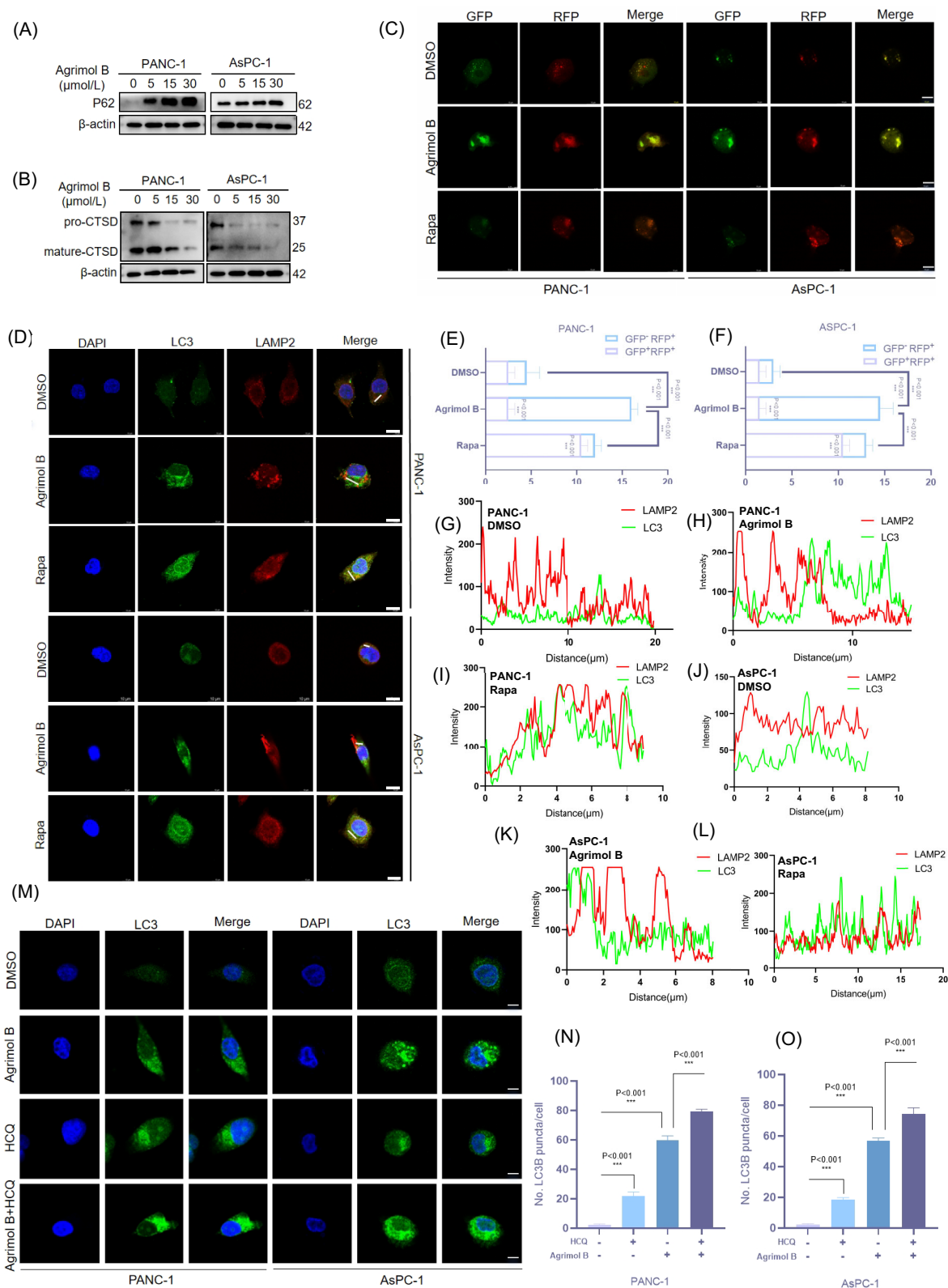


Figure 4 Agrimol B blocks autophagic flux in PDAC cells. (A, B) Western blot analysis of P62 and CTSD in PANC-1 and AsPC-1 cells treated with Agrimol B for 24 h. (C, E, F) Immunofluorescence analysis of RFP-GFP-LC3 after PANC-1 and AsPC-1 cells were transfected with RFP-GFP-LC3 for 48 h, followed by treatment with or without Agrimol B for another 24 h. Scale bars, 10 μm . (D, G-L) Immunofluorescence analysis of the colocalization of endogenous LC3 with LAMP2 after treatment with Agrimol B or rapamycin for 24 h in PANC-1 and AsPC-1 cells. Scale bars, 10 μm . (M-O) Immunofluorescence analysis of LC3 in PDAC cells treated with or without Agrimol B in the presence or absence of HCQ. Scale bars, 10 μm .

getting different autophagic phases. CCK-8 assays revealed that the growth of Agrimol B-treated PDAC cells was markedly restored when autophagy initiation was inhibited using wortmannin (Fig. 5A and B). In addition to pharmacological inhibition, similar observations were also found by genetic silencing of key autophagy initiation-related genes (ATG7), highlighting the necessary role of autophagic induction in Agrimol B-triggered cytotoxicity (supplementary Fig. 4A and B, see online supplementary material). In contrast, when combined with HCQ, causing the accumulation of autophagosomes, the antitumour activity of Agrimol B in PDAC cells was significantly exacerbated (Fig. 5C and D). Besides, after silencing PINK1 and Parkin with siRNA, Agrimol B could inhibit the proliferation of PDAC cells (Fig. 5E-H), which was confirmed by EdU experiments (supplementary Fig. 4G-L). Similarly, combined treatment with the mitophagy inhibitor Mdivi-1 and the ROS inhibitor *N*-acetylcysteine attenuated Agrimol B-mediated inhibition of PDAC cell proliferation (supplementary Fig. 4C-F). These findings confirmed that Agrimol B inhibits the proliferation of PDAC cells by impaired mitophagy flux. In addition, the results from flow cytometry revealed that wortmannin, Mdivi-1, and *N*-acetylcysteine weakened the ability of Agrimol B to cause apoptosis in PDAC cells (Fig. 5I), while HCQ aggravated Agrimol B's ability to induce apoptosis in PDAC cells (Fig. 5J). Actually, this massive increase in autophagosomes induced by Agrimol B goes far beyond the scope of what PDAC cells could withstand, possibly moving the controlled, pro-survival autophagy into an uncontrolled, pro-death mechanism in which lysosomal scavenging pathways were blocked to varying degrees. These results suggest that the obstructed mitophagy flux contributed to the anti-PDAC effect of Agrimol B.

Agrimol B regulates mitophagy by downregulating MTERF3 expression

Next, to investigate the mechanism underlying Agrimol B-mediated autophagy, label-free protein sequencing was performed in two PDAC cell lines incubated with or without Agrimol B. This analysis identified 91 overlapping differentially expressed genes (DEGs) with statistical significance ($P < 0.05$, log2 fold-change $> \pm 1$) (Fig. 6A-C). These DEGs were grouped by Kyoto Encyclopedia of Genes and Genomes analysis using the DAVID bioinformatics tool, deciphering multiple putative downstream molecules of Agrimol B treatment. Through a literature review and western blot analysis to verify the changes in differentially expressed proteins, we identified MTERF3 as a candidate, supported by the sequencing results. Western blot analysis revealed that the expression of MTERF3 was decreased with increasing Agrimol B concentration (Fig. 6D). The role of MTERF3 as a promoter in PDAC was preliminarily confirmed by employing the UCSC Xena database (Fig. 6E), echoing its effect in pan-cancer. To further explore the prognostic value of MTERF3 in PDAC, we analyzed the correlation between MTERF3 expression and patient survival in 64 PDAC patient samples. Consistently, increased MTERF3 expression was correlated with worse overall survival in PDAC patients (Fig. 6F). In addition, the expression of MTERF3 in early-stage PDAC tissues, lymph node-negative, and moderately and highly differentiated PDAC tissues was lower (supplementary Fig. 5A-D, see online supplementary material). Taken together, these data indicate that MTERF3 may serve as a prognostic indicator in PDAC.

Considering the involvement of MTERF3 in Agrimol B-induced mitophagy, we next constructed a plasmid overexpressing MTERF3 to determine how MTERF3 functions in this process. CCK-8 assays revealed that MTERF3 overexpression attenuated the Agrimol B-induced inhibition of PDAC cell proliferation (Fig. 6G and H), which was also demonstrated by the results of the EdU experiments (supplementary Fig. 5E-G). Meanwhile, exogenous MTERF3 expression led to lower expression of LC3 (Fig. 6I). Following transient transfection with the MTERF3-expressing plasmid, mitochondrial proteins were isolated, and western blot analysis showed that MTERF3 overexpression reversed the Agrimol B-induced increases in PINK1 and Parkin (Fig. 6J). To assess the correlation between MTERF3 and PINK1, we examined the expression of MTERF3 and PINK1 in 20 pairs of human PDAC tissues and found that while PINK1 expression was decreased in high MTERF3-expressing PDAC tissues, PINK1 was highly expressed in PDAC tissues with low MTERF3 expression (Fig. 6K). Statistical correlation analysis revealed that the expression of MTERF3 was negatively correlated with the expression of PINK1 in PDAC tissues (Fig. 6L). Interestingly, exogenous MTERF3 expression reversed the Agrimol B-induced decrease in the levels of mitochondrial proteins (HADHA, TIM23, and SOD2) (Fig. 6M) and ameliorated the ROS-induced mitophagy phenotype (supplementary Fig. 5H). However, the ROS inhibitor *N*-acetylcysteine attenuated the proliferation inhibition caused by Agrimol B (supplementary Fig. 5I). Broadly speaking, these data indicate that the MTERF3 is involved in Agrimol B-induced PINK1/Parkin-dependent mitophagy.

Molecular docking is a computational technique that predicts the binding modes, affinities, and interaction forces between small-molecule ligands and biological macromolecular receptors, and is widely applied in structure-based drug design and screening [26]. Molecular docking analysis revealed that Agrimol B binds to MTERF3 with a binding energy of -6.085 kcal/mol (Fig. 6N). To further verify how Agrimol B interacts with MTERF3, we first assessed the expression of MTERF3 mRNA by qPCR and found no significant change in MTERF3 mRNA in PDAC cells after Agrimol B treatment (supplementary Fig. 5J and K). These findings indicate that Agrimol B may regulate MTERF3 at the posttranscriptional level. We wondered whether Agrimol B could reduce the expression level of MTERF3 by influencing the protein stability of MTERF3. MG132 is a protease inhibitor that effectively inhibits protein ubiquitination and degradation. Therefore, we cotreated PDAC cells with MG132 and Agrimol B and found that MG132 stabilized the level of MTERF3 in PANC-1 and AsPC-1 cells under Agrimol B treatment. The results showed that Agrimol B may reduce the expression of MTERF3 by influencing the protein stability of MTERF3 (Fig. 6O). These results suggest that Agrimol B promoted mitophagy in PDAC cells by increasing PINK1 expression through the degradation of MTERF3.

Agrimol B sensitizes PDAC cells to first-line chemotherapy drugs

To investigate the clinical translational potential of Agrimol B, we generated a PDO from surgically resected samples obtained from patients with pathologically confirmed PDAC, following ethics committee approval (Fig. 7A). Notably, as the duration of drug exposure increased, PDOs under Agrimol B treatment exhibited

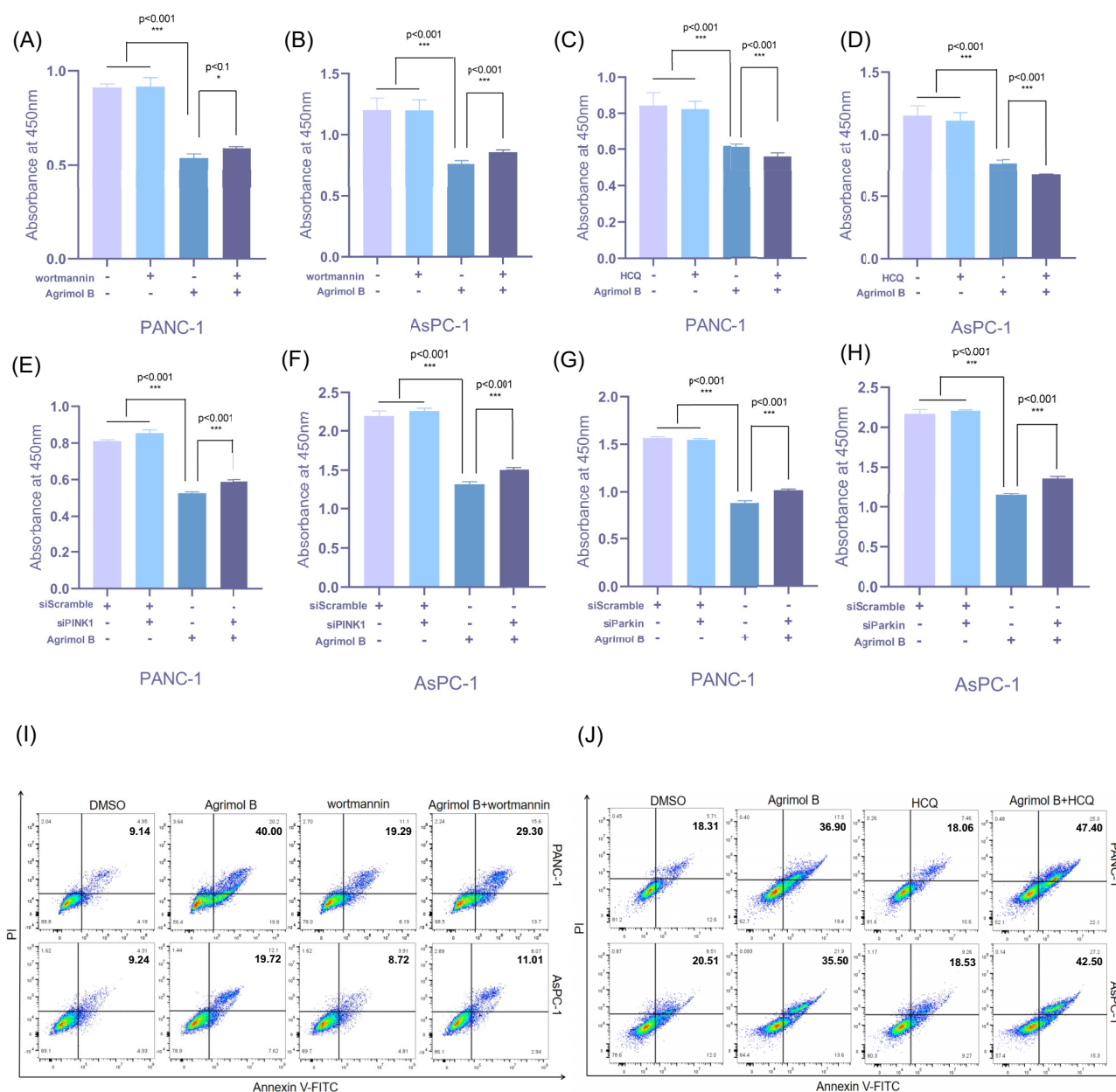


Figure 5 Accumulation of mitophagosomes contributes to the anti-PDAC effects of Agrimol B. (A–D) CCK-8 assay of PDAC cells incubated with or without Agrimol B in the presence or absence of wortmannin and HCQ. (E–H) CCK-8 assay in PDAC cells transfected with siScramble, siPINK1, or siParkin following treatment with or without Agrimol B. (I, J) Flow cytometric analysis of PDAC cells incubated with or without Agrimol B in the presence or absence of wortmannin, HCQ.

dramatic morphological alterations via 3-day serial monitoring, which was characterized by the sharpest decline in overall size among all the candidates measured, and apoptotic morphology was observed at a drug concentration of 5 $\mu\text{mol/l}$ on the first day (Fig. 7B–C). The AG regimen (albumin-bound paclitaxel + gemcitabine) with FOLFIRINOX (irinotecan + 5-fluorouracil + oxaliplatin) has become the first-line treatment regimen for PDAC [27, 28] (Fig. 7D). However, patients are prone to developing resistance to chemotherapy during treatment. A recent study revealed that the use of natural biological products in combination with chemical drugs can enhance the anticancer effect of these drugs [29–32]. The natural polyphenol Agrimol B, which in-

hibits the growth and proliferation of PDAC cells, can be used to study the sensitivity of PDAC to chemotherapy. Therefore, we combined Agrimol B with albumin-bound paclitaxel, gemcitabine, irinotecan, 5-fluorouracil, and oxaliplatin respectively in an organoid model. Compared with the Agrimol B group and the chemotherapy drug treatment group alone, the organoids in the combination group exhibited a more complete apoptotic state, and the combination of albumin-bound paclitaxel and gemcitabine was more pronounced, evidenced by obvious apoptotic morphology appearing in the organoids on the first day (Fig. 7E–H), hinting at its potential clinical prospects in combined pharmacotherapy.

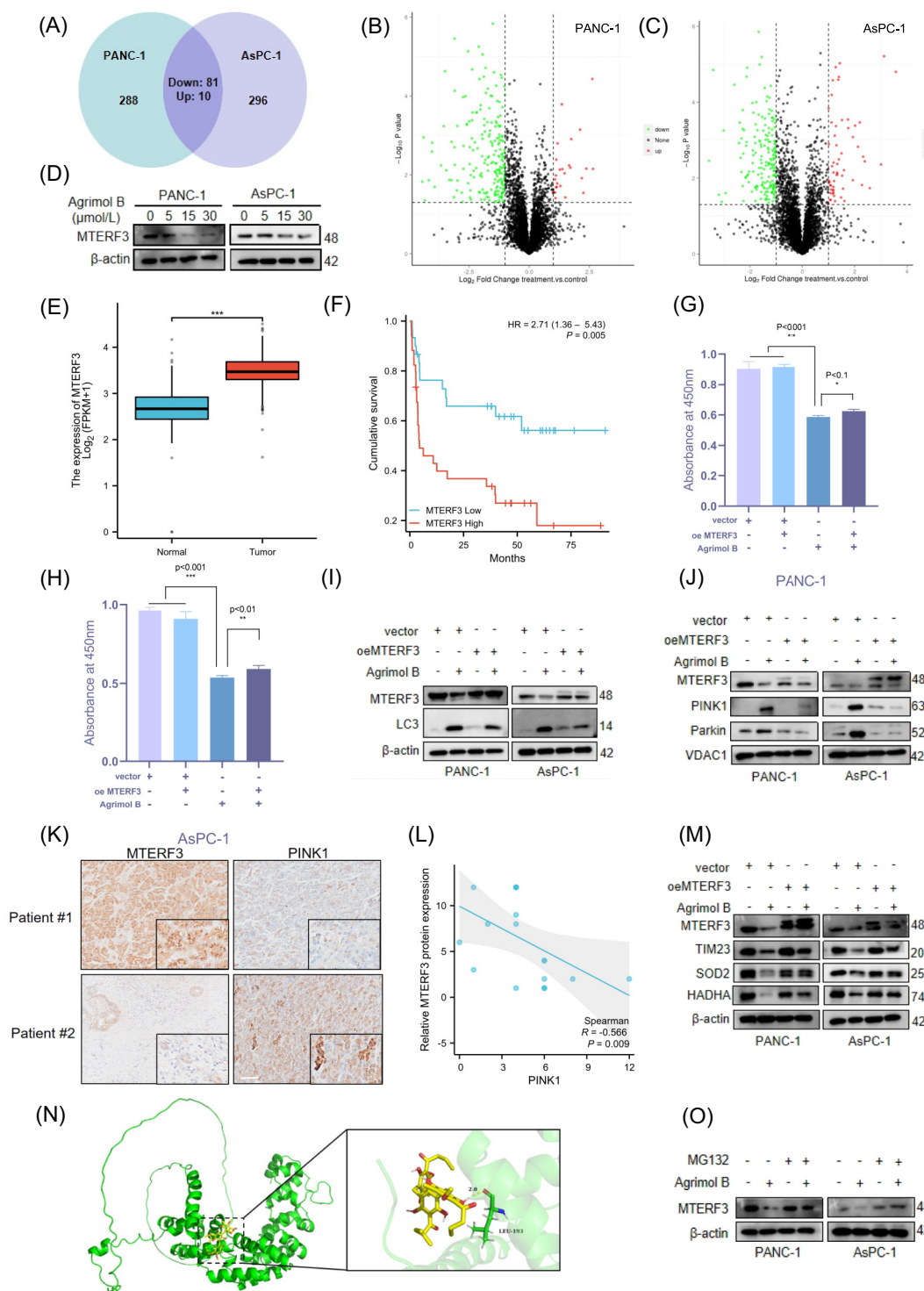


Figure 6 Agrimol B regulates mitophagy by downregulating MTERF3 expression. (A) Venn diagram showing the overlap of differentially expressed proteins (fold-change ≥ 1.3 or ≤ 0.76) between PANC-1 and AsPC-1 cells. (B, C) Volcano plots of DEGs identified via label-free quantitative proteomics in PANC-1 and AsPC-1 cells. (D) Western blot analysis of MTERF3 in PANC-1 and AsPC-1 cells treated with Agrimol B for 24 h. (E) Differences in MTERF3 expression between normal tissues and cancer tissues in the UCSC Xena database. (F) Kaplan-Meier analysis of MTERF3 expression and overall survival in 64 patients with PDAC. (G, H) CCK-8 assay in PDAC cells transfected with vector or oeMTERF3 following treatment with or without Agrimol B. (I) Western blot analysis of LC3 in PDAC cells transfected with vector or oeMTERF3 following treatment with or without Agrimol B. (J) Western blot analysis of PINK1 and Parkin in PDAC cells transfected with vector or oeMTERF3 following treatment with or without Agrimol B. (K) Immunohistochemical analyses of PINK1 and MTERF3 expression in PDAC tissues. Scale bars, 100 μm . (L) Correlation of the immunostaining intensities of PINK1 and MTERF3. (M) Western blot analysis of TIM23, SOD2, and HADHA in PDAC cells transfected with vector or oeMTERF3 following treatment with or without Agrimol B. (N) Molecular docking suggests that Agrimol B can bind to MTERF3 with a binding energy of -6.085 kcal/mol. (O) Western blot analysis of MTERF3 in cells treated with or without Agrimol B in the presence or absence of MG132.

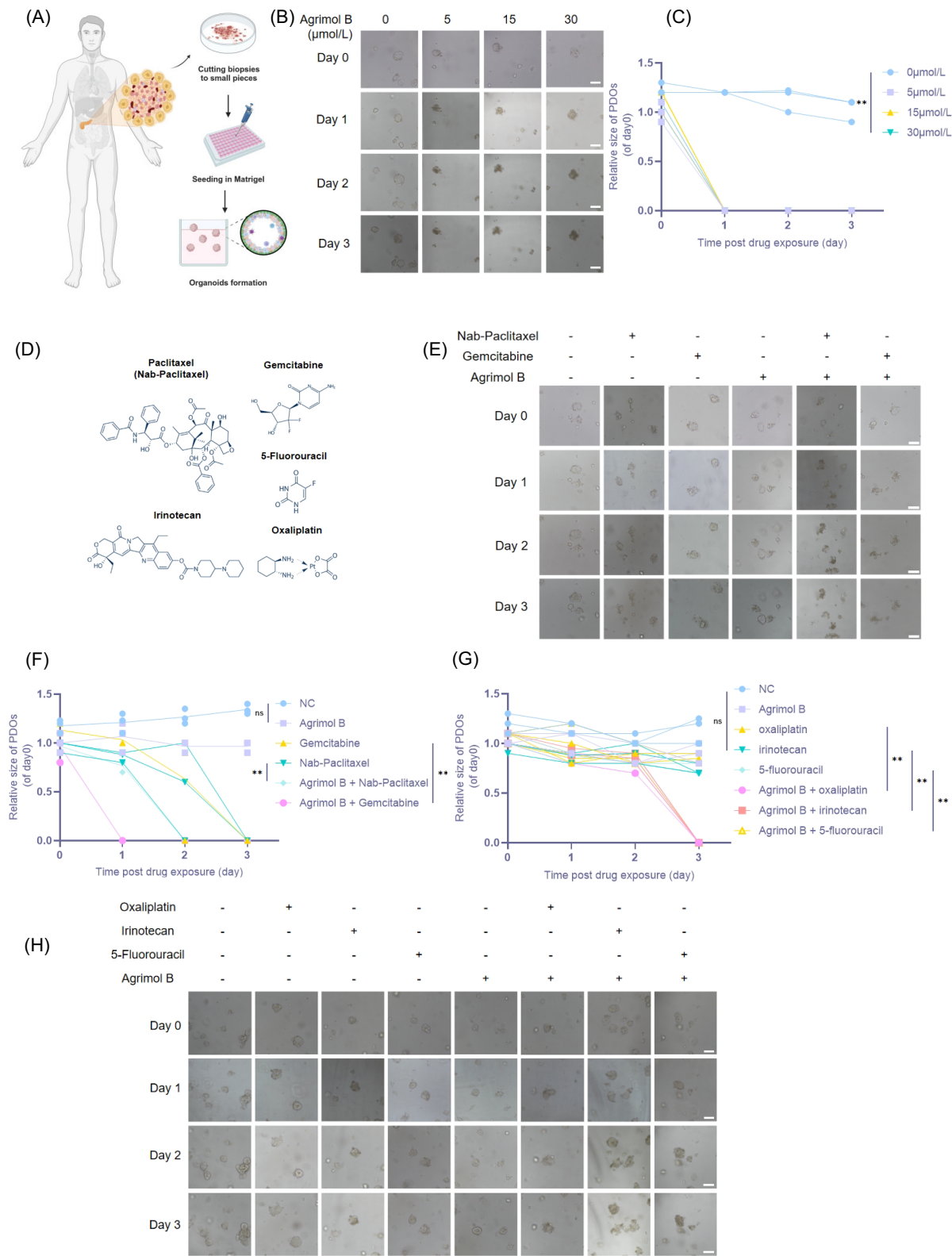


Figure 7 Agrimol B sensitizes PDAC cells to first-line chemotherapy drugs. (A) Schematic overview of PDAC PDO establishment and drug assessment. (B, C) Brightfield images of organoids treated with the indicated concentrations of Agrimol B. Scale bars, 10 μ m. (D) Chemical structures of nab-paclitaxel (Paclitaxel), irinotecan, 5-fluorouracil, oxaliplatin, and gemcitabine. (E–H) Brightfield images of organoids treated with or without Agrimol B in the presence or absence of nab-paclitaxel, irinotecan, 5-fluorouracil, oxaliplatin, or gemcitabine. Scale bars, 10 μ m.

Discussion

In this study, we investigated the regulatory role and mechanism of mitophagy in PDAC cells under Agrimol B treatment. Our data indicated that Agrimol B promoted apoptosis by inducing excessive mitophagy and the accumulation of autophagosomes. We found that Agrimol B may reduce MTERF3 expression, resulting in excessive PINK1 accumulation in mitochondria, Parkin mitochondrial translocation, and mitophagy. In addition, Agrimol B-induced blockade of the fusion of autophagosomes and lysosomes resulted in obstructed autophagic flow. All of these factors contribute to the apoptosis and suppression of PDAC cells. In addition, Agrimol B exerted a powerful anticancer effect in combination with first-line chemotherapy drugs in PDAC PDOs. We propose that the anti-PDAC effect of Agrimol B originates from a self-amplifying lethal cycle, rather than a single linear pathway. On one hand, Agrimol B directly induces mitochondrial damage and rapidly initiates the apoptotic programme. On the other hand, it downregulates MTERF3 to hyperactivate PINK1/Parkin-mediated mitophagy, while simultaneously impairing lysosomal function and blocking autophagic flux. Crucially, these two pathways act in synergy: abnormal accumulation of autophagosomes exacerbates organelle stress, which in turn feedback-amplifies the initial apoptotic signaling; meanwhile, sustained mitochondrial damage further increases the autophagic burden. This model is strongly supported by pharmacological inhibition experiments—blocking autophagy initiation partially rescues cell death, whereas aggravating autophagic flux blockade significantly enhances apoptosis. Therefore, Agrimol B-induced cell death is an integrated outcome resulting from both direct pro-apoptotic effects and dysfunctional autophagy, which are inseparable in this process.

MTERF3 is an important molecule for regulating mitochondrial transcription. A study by Park *et al.* revealed that MTERF3 is a mitochondrial protein that interacts with mtDNA promoter regions and reduces transcription initiation in mammalian mitochondria; this negative regulation may be important for fine-tuning mitochondrial transcription in response to physiological demands [33]. In addition, Zheng *et al.* confirmed that MTERF3 was overexpressed in liver cancer and that high MTERF3 expression was positively correlated with poor overall survival in liver cancer patients [34]. MTERF3 knockdown induces mitochondrial dysfunction, promotes ROS accumulation, activates the p38 mitogen-activated protein kinase signalling pathway, and inhibits liver cancer cell proliferation [34]. The normal function of mitochondria is dependent on the regulation of MTERF3. Some studies have reported that MTERF3 may be a potential regulatory molecule of mitophagy [35, 36]. Mitophagy plays an important role in clearing senescent, damaged, or excess mitochondria, but how MTERF3 regulates mitophagy is still unknown. Our study is the first to report the mechanism by which MTERF3 regulates mitophagy. Loss of MTERF3 leads to PINK1 accumulation in mitochondria and Parkin mitochondrial translocation to activate mitophagy. Our study also suggests for the first time that MTERF3 is an oncogenic factor in PDAC and that the expression of MTERF3 is associated with a poor prognosis in patients with PDAC. These findings were validated in clinical patient samples, suggesting that MTERF3 can be used as a potential therapeutic target for patients with PDAC. Our study revealed that Agrimol B exerts a powerful anticancer effect by regulating MTERF3-induced mitophagy and obstructing autophagic

flux, which provides a feasible basis for the development of drugs that target MTERF3. However, the specific mechanism by which MTERF3 regulates PINK1 needs to be further explored.

Polyphenols, as a source of popular new antitumour active substances, present significant market and scientific research prospects [37]. However, the excellent anticancer effect of polyphenols is not due to a single factor but rather to the superposition or interaction of multiple mechanisms. Our study demonstrated that Agrimol B, a polyphenol from agrimol, has potent anti-PDAC effects and that Agrimol B sensitizes PDOs to current first-line regimens, providing detailed evidence to support the clinical application of Agrimol B. It is noteworthy that Agrimol B exhibits diverse mechanisms of action across different pathological contexts, consistent with its multi-target nature and context-dependent effects. For example, in an acute kidney injury model, Agrimol B acts as a SIRT1 activator to exert protective effects [38]; in colon cancer, it impairs mitochondrial biogenesis by inhibiting the PGC-1 α /NRF1/TFAM pathway [22]; and studies have also reported that it promotes mitochondrial ROS accumulation by degrading NDUFS1 [24]. Collectively, these studies suggest that the core targets of Agrimol B converge on pathways related to mitochondrial function and cellular energy metabolism. In this study, we found that Agrimol B induces MTERF3 degradation, hyperactivates PINK1/Parkin-mediated mitophagy, blocks autophagic flux, and thereby triggers cell death. Although the specific targets differ across the aforementioned studies (e.g. SIRT1, PGC-1 α , NDUFS1, and MTERF3), they all point to the common theme of regulating mitochondrial homeostasis. We speculate that such mechanistic differences may stem from cell-type specificity, tumour microenvironment, or disease status. Particularly in PDAC, a tumour characterized by unique metabolic features and high drug resistance, Agrimol B may exert a more selective killing advantage through the MTERF3-regulated crosstalk between mitophagy and apoptosis. Moreover, our finding that Agrimol B synergizes with chemotherapeutic agents in PDAC patient-derived organoids highlights its translational potential distinct from other mechanistic contexts. Thus, our study not only reveals a novel target of Agrimol B, MTERF3, in PDAC but also enriches the understanding of its multi-mechanistic and multi-target pharmacological profile, suggesting its context-dependent application prospects in precision oncology.

However, several limitations remain in the research on polyphenols. First, most existing studies have focused on single polyphenols. Traditional Chinese Medicine usually exerts its therapeutic effect through the combination of multiple components of the same type. Therefore, research on the integration mechanism of Chinese medicinal polyphenols should be carried out for monomers, structure–activity relationships should be explored, and the common mechanism of different structural polyphenols against tumours should be further investigated, with the goal of identifying a combination of drugs with synergistic effects. Second, most studies on the mechanisms of polyphenols have been conducted on a single tumour type. In the future, a variety of tumour cells and tissues should be used as carriers to explore the common intervention mechanisms among different diseases. Finally, the poor water solubility and lack of stability of polyphenolic compounds limit their clinical application. Modern technologies should be combined to improve drug preparation to provide strong support for further in-depth development and clinical transformation.

Conclusion

Guided by the traditional Chinese medicine monomer indication expansion strategy, we demonstrated that Agrimol B, a natural polyphenol, elicited a potent therapeutic response in patient-derived PDAC organoids. More importantly, Agrimol B sensitized PDAC to chemotherapy. The cytotoxicity of Agrimol B in PDAC cells is dependent on the obstruction of autophagic flux. Specifically, Agrimol B may degrade MTERF3, leading to increased PINK1 levels and inducing excessive mitophagy. Additionally, Agrimol B impedes the fusion of mitophagosomes and lysosomes, resulting in the excessive accumulation of autophagosomes. In conclusion, our study provides direct preclinical and experimental evidence for the efficacy of Agrimol B in the treatment of PDAC, reveals the mechanism by which Agrimol B inhibits the growth of PDAC, lays the foundation for potential therapeutic opportunities, and holds translational significance in clinical practice.

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Author contributions

Yifei Ma (Formal Analysis, Methodology, Writing—original draft, Writing—review & editing), Ying Zheng (Investigation, Methodology, Validation), Ying Zhou (Supervision), Yang Yang (Methodology, Software, Supervision), Jinlu Liu (Investigation, Resources), Ningna Weng (Data curation, Validation, Visualization, Writing—review & editing), Junhong Han (Funding acquisition, Writing—review & editing), and Qing Zhu (Resources, Validation, Visualization).

Supplementary material

Supplementary material is available at *PCMDI* Journal online.

Conflicts of interest

None declared.

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