

Original Article

MerTK inhibition by UNC569 triggers DNA damage and JNK/p38 MAPK cascade-driven apoptosis in pancreatic cancer

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a malignancy with increasing mortality rates and remains a major clinical challenge due to its aggressive progression and limited therapeutic options. Therefore, the identification of early biomarkers and the development of effective targeted therapies are critically needed. MerTK, a receptor tyrosine kinase aberrantly expressed in various cancers, can be selectively inhibited by UNC569, a small-molecule antagonist with demonstrated efficacy in hematologic malignancies. This study shows that UNC569 potently suppresses PDAC cell proliferation and clonogenic growth, inhibits migration and invasion by attenuating epithelial-mesenchymal transition, and enhances the sensitivity of PDAC cells to Gemcitabine while promoting apoptosis. Mechanistically, UNC569 induces DNA damage-mediated G2/M phase arrest and activates JNK/p38 mitogen-activated protein kinase-dependent apoptotic signaling. Collectively, these results establish MerTK as a promising therapeutic target in PDAC and highlight the translational potential of UNC569 as a dual-pathway inhibitor for PDAC treatment.

Key words pancreatic cancer, UNC569, MerTK, JNK/p38 MAPK, DNA damage

Introduction

Pancreatic ductal adenocarcinoma (PDAC), a highly lethal malignancy, ranked as the seventh leading cause of global cancer mortality in 2020 [1]. Both its incidence and mortality rates continue to rise, and projections suggest that PDAC may become the second leading cause of cancer-related deaths by 2030 [2]. In China, clinical outcomes are particularly dismal: the majority of patients are diagnosed at an advanced-stage, with a median overall survival of less than 7.8 months and 5-year survival rates barely exceeding 10% [3]. Given the substantial toxicity and limited efficacy of current chemotherapeutics regimens, surgical resection remains the

primary curative option. Consequently, the identification of novel therapeutic targets and the development of safe and effective agents are urgently required to improve PDAC prognosis.

The receptor tyrosine kinase MerTK plays a pivotal role in maintaining tissue homeostasis and innate immunity. It is over-expressed in a wide range of malignancies including melanoma [4], lung cancer [5], glioblastoma [6,7], hepatic [8], gastric carcinomas [9], as well as hematologic tumors. Emerging evidence highlights a critical role for MerTK and other members of the TAM family receptor in driving the initiation and progression of PDAC [10]. MerTK-targeting monoclonal antibodies have been shown to induce

apoptosis in triple-negative breast cancer [11], establishing MerTK as a promising therapeutic target. Several small-molecule MerTK inhibitors (e.g., UNC569, UNC1062, UNC1666, UNC2025, and UNC2250) have demonstrated potent anti-tumor efficacy in preclinical models [12–16]. UNC569, a highly selective MerTK inhibitor, binds to the adenine pocket of MerTK to prevent its phosphorylation [12], exhibiting robust activity against acute lymphoblastic leukemia *in vitro* and *in vivo*. Furthermore, MerTK contributes to chemotherapy resistance by enhancing pro-survival signaling pathways and facilitating tumor immune evasion [17]. For example, the MerTK ligand Gas6 activates oncogenic signaling in Osimertinib-treated EGFR mutation non-small cell lung cancer (NSCLC) [18], whereas in PD-1/PD-L1-resistant hepatocellular carcinoma (HCC), MerTK upregulates SLC7A11 via the ERK/SP1 pathway to inhibit ferroptosis and concurrently recruits myeloid-derived suppressor cells (MDSCs), thereby fostering an immunosuppressive tumor microenvironment (TME) [19]. Despite these advances, the molecular mechanisms underlying resistance to MerTK-targeted small-molecule inhibitors remain poorly understood, thereby limiting their clinical translation.

Cellular genomic integrity is essential for cell survival and is safeguarded by the DNA damage response (DDR) [20]. In malignancies, deletions of key DDR-related genes lead to the accumulation of DNA damage, thereby accelerating tumor progression [21]. For instance, DNA damage in the kidney has been correlated with the progression of renal cell carcinoma [22]. Exploiting tumor-specific genomic instability has allowed DNA-damaging radio/chemotherapies to emerge as effective anti-cancer strategies [23]. The AXL receptor tyrosine kinase (RTK) inhibitor BGB324 induces replication stress and DNA breaks, suppressing the proliferation of NSCLC cells [24]. Similarly, Gemcitabine (GEM) promotes apoptosis in pancreatic cancer cells through DNA break accumulation [25]. Combined L-Arg/5-FU treatment enhances iNOS/NO-driven reactive oxygen species (ROS) generation, inducing DNA damage that ultimately triggers cell cycle arrest and apoptosis in HCC [26]. However, activation of the MerTK signaling pathway has been implicated in chemoresistance by enhancing pro-survival signaling and DNA repair mechanisms [17], which may partially account for the limited therapeutic efficacy of GEM in cancers. Indeed, pharmacologic inhibition of MerTK, such as with the selective inhibitor UNC2250, has been shown to sensitize lymphoma cells to conventional chemotherapeutic agents [27]. Importantly, accumulating evidence suggests crosstalk between the DDR and mitogen-activated protein kinases (MAPK) pathways [28]. For example, inhibition of aldehyde dehydrogenase 2 family member (ALDH2) activates JNK/p38 signaling and induces genomic lesions in colon cancer [29], while Cisplatin-induced apoptosis depends on DNA damage-mediated activation of JNK/p38 [30,31]. However, whether UNC569 inhibits pancreatic cancer proliferation, migration, and invasion through DNA damage and JNK/p38 signaling remains to be determined.

In this study, UNC569 is shown to trigger DNA damage, activate JNK/p38 MAPK signaling, and increase chemosensitivity, leading to enhanced apoptosis and suppressed tumor growth in PDAC. These results establish UNC569 as a promising therapeutic candidate for this malignancy.

Materials and Methods

Cell lines

H6c7 human normal pancreatic ductal epithelial cells and pancrea-

tic cancer cell lines (PANC-1, PATU8988 and SW1990) were obtained from the Shanghai Cancer Institute (Shanghai, China) and cultured in DMEM (Servicebio, Wuhan, China) supplemented with 10% FBS (ExCell Bio, Shanghai, China) and 1% penicillin-streptomycin under a humidified atmosphere of 5% CO₂ at 37°C. All cell lines were routinely tested and confirmed to be free of mycoplasma contamination throughout the study.

Reagents and antibodies

Reagents were obtained from the following sources: UNC569 (TargetMol, Boston, USA); GEM and Protease inhibitor cocktail (APEXBio, Houston, USA); Annexin V-FITC/PI apoptosis kit and PI/RNase staining solution (BD Biosciences, San Jose, USA); Pierce BCATM protein assay kit, PierceTM ECL Western Blotting Substrate, and TRIzol reagent (Thermo Fisher Scientific, Waltham, USA); *N*-acetylcysteine (NAC) and total ROS detection kit (Beyotime Biotechnology, Shanghai, China); and Cell Counting Kit-8 (CCK-8; abs50003; Absin, Shanghai, China).

Cell viability and proliferation assay

To evaluate the half maximal inhibitory concentration (IC₅₀) of UNC569, H6c7, PANC-1, PATU8988 and SW1990 cells (2×10^3 cells/well) were seeded in 96-well plates and incubated for 24 h at 37°C in a humidified incubator at 37°C with 5% CO₂. Cells were subsequently exposed to varying concentrations of UNC569 (2, 4, 8, 16, and 32 μ M) or vehicle (DMSO) for 36 h. Cell viability was then assessed using the CCK-8 assay kit following the manufacturer's instructions. For proliferation analysis, PANC-1, PATU8988 and SW1990 cells (2×10^3 cells/well) were seeded in 96-well plates and incubated for 24 h. The next day, cells were treated with UNC569 (2, 4, and 8 μ M) or DMSO for 24, 36, 48 or 60 h, and proliferation rates were quantified using the CCK-8 assay kit.

Colony formation assay

PANC-1, PATU8988, and SW1990 cells (1×10^3 cells/well) were seeded in 6-well plates and maintained at 37°C in a humidified incubator with 5% CO₂ for 7 days to allow colony formation. Cells were then exposed to UNC569 (4 or 8 μ M) or DMSO for an additional 7 days. Subsequently, the cells were rinsed three times with pre-warmed PBS, fixed in methanol, and stained with 0.5% crystal violet for 20 min. Colonies were quantified from three independent wells.

Cell migration and invasion assays

For the migration assay, PANC-1, PATU8988 and SW1990 cells (4×10^4 cells) suspended in serum-free DMEM were added into the upper chambers of Transwell inserts, while 600 μ L of DMEM supplemented with 15% FBS was placed in the lower chambers. After a 12-h pre-incubation in a humidified incubator, the medium in the upper chamber was replaced by serum-free DMEM containing UNC569 (4 or 8 μ M) or DMSO. Following 36 h of incubation, migrated cells on the lower membrane surface were fixed with methanol for 30 min and stained with 0.5% crystal violet for 15 min. Images from three randomly selected fields per well were captured using an inverted microscope. The number of migrated cells was quantified with ImageJ Plus software.

For the invasion assay, Matrigel was thawed at 4°C and diluted 1:9 with pre-cooled F-12 medium. A total of 40 μ L of the Matrigel mixture was added to the upper chamber of each Transwell insert

and incubated at 37°C for 12 h to allow gelation. Subsequently, 50 μ L of pre-warmed, serum-free F-12 medium was added to rehydrate the Matrigel layer, and plates were incubated at 37°C with 5% CO₂ for 30 min. Cell seeding, treatment, and analysis were performed following the same procedures described for the migration assay.

Intracellular ROS assay

PANC-1, PATU8988 and SW1990 cells (5×10^5 cells) were seeded into each well of 6-cm dishes and cultured for 12 h, followed by treatment with UNC569 (4 or 8 μ M) or DMSO for 36 h. Intracellular ROS levels were then measured using the 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescent probe according to the manufacturer's instructions.

Cell cycle distribution and apoptosis assay

To assess the cell cycle distribution, PANC-1, PATU8988 and SW1990 cells (5×10^5 cells) were seeded into each well of 6-cm dishes and cultured for 12 h. The following day, the cells were treated with UNC569 (4 or 8 μ M) or DMSO for 36 h. Then, the cells were collected and fixed in 70% ethanol at 4°C for 24 h. After RNase treatment, the cells were stained with propidium iodide (PI) for 25 min in the dark, and the cell cycle distribution was assessed using a flow cytometer (BD Accuri™ C6 Plus, Franklin Lakes, USA).

For apoptosis measurement, the same cell lines were plated and treated under identical conditions. After 36 h of exposure to UNC569 or DMSO, cells were harvested and stained with Annexin V-FITC/PI (BD Biosciences, San Jose, USA) for 20 min. Apoptotic cells were quantified with a flow cytometer.

Mouse xenograft study

Male BALB/c nude mice (5-week-old; GemPharmatech, Nanjing, China) were housed under specific pathogen-free (SPF) conditions. PANC-1, PATU8988 and SW1990 cells (1×10^7 cells per mouse) were suspended in 100 μ L culture medium and subcutaneously injected into the right-flank of each mouse. When the average tumor volume reached approximately 100 mm³, the mice were randomly divided into two groups ($n = 6$ per group), and administered once daily by intraperitoneal injections with UNC569 (30 or 60 mg/kg) or 0.9% saline for 6 consecutive days. The tumor dimensions were measured using a digital caliper every other day, and the tumor volumes (V) were calculated as follows: $V = 1/2 \times (L \times W^2)$, where L and W represent the tumor length and width, respectively. After 6 days of treatment, all mice were euthanized, and tumors were dissected, weighed and photographed. All animal experiment procedures were approved by the Ethics Committee of the Jinhua Institute for Food and Drug Control (approval number: AL-JSYJ202434).

Western blot analysis

Western blot analysis was performed as previously described [32]. The following primary antibodies were used: p-Rb (8516), p21 (2947), Snail (3879), cleaved caspase3 (9669), PARP (9542), JNK (9252), p-p38 (4511), γ -H2A.X (9718), p-JNK (4668) and p38 (8690) from Cell Signaling Technology (Danvers, USA); p-MerTK (ab14921) from Abcam (Cambridge, UK); Rb (A3618) and p-CDK6 (AP0326) from AbClonal (Wuhan, China); β -actin (P30002) from Abmart (Shanghai, China); N-cadherin (22018-1-AP) and E-

cadherin (20874-1-AP) from Proteintech (Wuhan, China); Cyclin B1 (sc-245) and c-Myc (sc-42) from Santa Cruz Biotechnology (Dallas, USA); CDK6 (VWA00022) and p27 (AHP2662) from Bio-Rad (Hercules, USA).

Statistical analysis

All data are expressed as the mean $\pm$ SD from three independent experiments. Statistical differences between two groups were analyzed by Student's t test, and comparisons among multiple groups were performed by one-way ANOVA using GraphPad Prism 8.0 (GraphPad Software, La Jolla, USA). $P < 0.05$ was considered statistically significant.

Results

UNC569 inhibits PDAC cell proliferation

Accumulating evidence has highlighted the critical role of tyrosine kinase receptors in cancer pathogenesis, as their dysregulated expression drives tumor cell proliferation, invasion, and immune escape. In our prior work, we identified MerTK as one such receptor, showing high expression in breast cancer [33]. Furthermore, data from the Human Protein Atlas indicate elevated MerTK protein in a subset of PDAC patient samples (3 out of 10 cases) (<https://www.proteinatlas.org/ENSG00000153208-MERTK/cancer>). This led us to hypothesize that MerTK contributes to PDAC progression and may be a therapeutic target. To evaluate this, we examined the effects of the MerTK inhibitor UNC569 on PDAC cells.

This study first examined the antitumor efficacy of UNC569 on cell proliferation and viability. UNC569 exhibited potent inhibitory activity against PDAC cells, with IC₅₀ values of 7.9 μ M (PANC-1), 6.66 μ M (PATU8988), and 3.35 μ M (SW1990), and suppressed cell viability in a dose-dependent manner (Figure 1A,B). Consistent with these anti-proliferative effects, colony formation assays demonstrated that treatment with 4 or 8 μ M UNC569 significantly reduced the clonogenic capacity of PDAC cells (Figure 1C,D). Cell cycle analysis revealed that UNC569 induced significant G2/M phase arrest (Figure 1E). Mechanistically, UNC569 treatment markedly decreased the levels of Cyclin B1 and phosphorylated CDK6 (p-CDK6), reduced the Rb phosphorylation ratio (p-Rb/Rb), and upregulated the expression of p21 and p27 (Figure 1F). Collectively, these findings indicate that UNC569 exerts antitumor effects in PDAC, at least partially, by inducing G2/M phase cell cycle arrest through the modulation of key cell cycle regulatory proteins.

UNC569 suppresses PDAC metastatic capacity

Given that metastasis is the leading cause of death in pancreatic cancer, we next investigated the effect of UNC569 on PDAC cell motility. Our results show that UNC569 significantly and dose-dependently inhibits both the migratory (Figure 2A,B) and invasive (Figure 2C,D) capacities of PDAC cells. To explore the mechanism behind this anti-migratory effect, we analyzed key epithelial-mesenchymal transition (EMT) markers. UNC569 treatment upregulated E-cadherin (an epithelial marker) and downregulated N-cadherin (a mesenchymal marker), concomitantly reducing the expression of the EMT transcription factors c-Myc and Snail (Figure 2E). In summary, these results demonstrate that UNC569 inhibits the metastatic potential of PDAC cells, at least partially, through modulation of the EMT program.

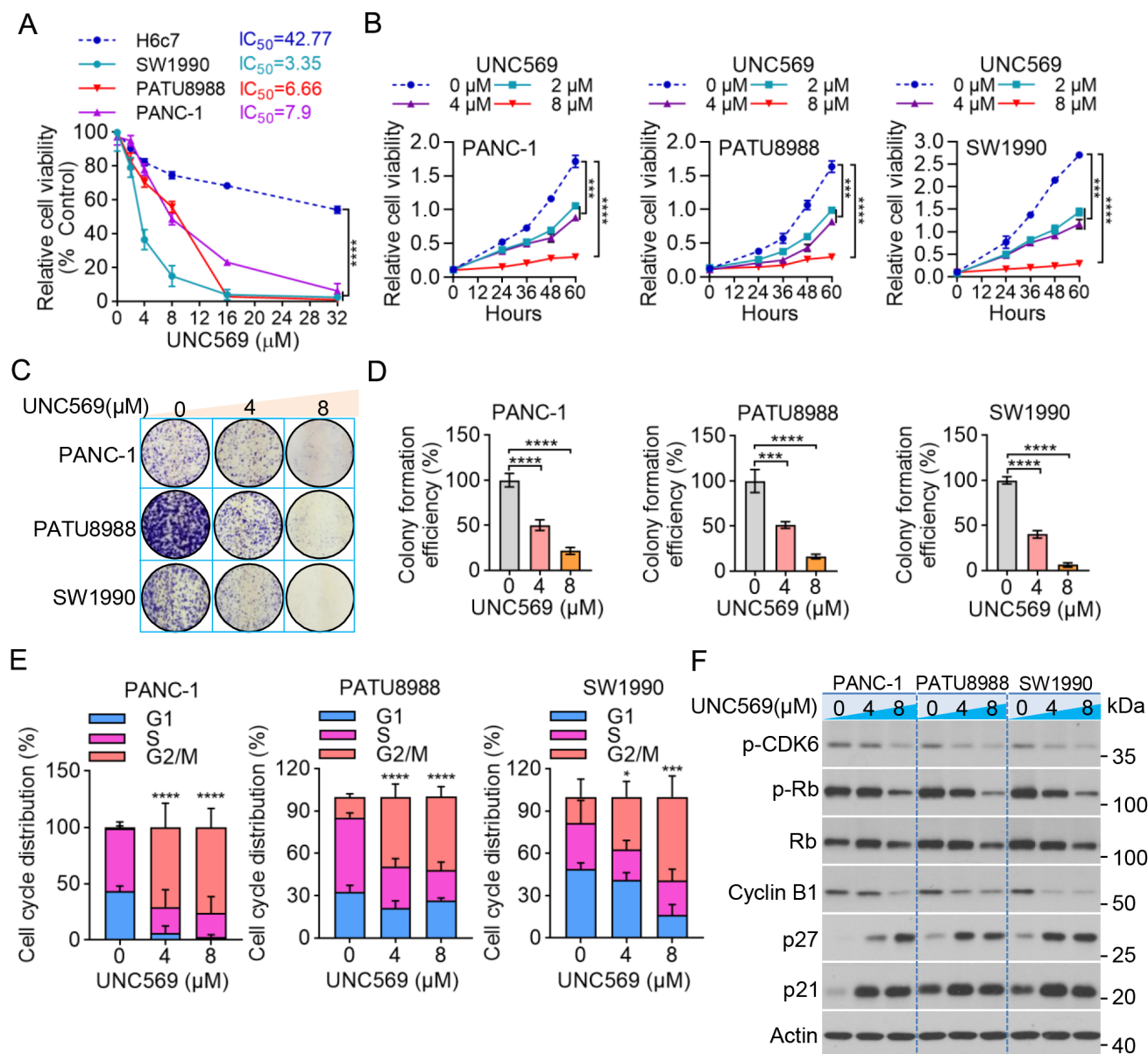


Figure 1. UNC569 inhibits pancreatic cancer cell proliferation (A) The viability of PDAC cells and normal ductal epithelial cells (H6c7) was assessed using the CCK-8 assay, and the corresponding IC_{50} values were determined. (B) Proliferation of PDAC cells treated with the indicated concentrations of UNC569 or DMSO was measured by the CCK-8 assay. (C,D) Representative images (C) and quantitative analysis (D) of colony formation in PDAC cells treated with UNC569 or DMSO. (E) Flow cytometry analysis showing that UNC569 treatment induced G2/M phase arrest in PDAC cells compared with the DMSO control. (F) Western blot analysis of cell cycle-related proteins in PDAC cells treated with the indicated concentrations of UNC569 or DMSO. Data are presented as the mean $\pm$ SD ($n=3$). * $P<0.05$, *** $P<0.001$, **** $P<0.0001$, one-way ANOVA (A,B,D,E).

UNC569 promotes apoptosis in PDAC cells via ROS accumulation

To assess the pro-apoptotic effect of UNC569, we performed flow cytometry analysis on PDAC cells. UNC569 induced dose-dependent apoptosis across all tested cell lines. For example, in PANC-1 cells treated with 8 μM UNC569, the proportions of early and late apoptotic cells increased to 15.8% and 6.26%, respectively, versus 2.43% and 1.31% in controls (Figure 3A and Supplementary Figure S1A). Similar responses were observed in PATU8988 and SW1990 cells (Figure 3A and Supplementary Figure S1A). Consistent with this, UNC569 treatment markedly enhanced the cleavage of caspase-3 and PARP, key executioners of apoptosis (Figure 3B).

We next explored the underlying mechanism. Given that moderate ROS levels can act as apoptotic signaling mediators [34], we measured intracellular ROS and found that UNC569 significantly increased their production (Figure 3C). To determine whether ROS accumulation is required for UNC569-induced apoptosis, we pretreated cells with the ROS scavenger NAC. NAC pretreatment effectively eliminated UNC569-induced ROS (Figure 3D) and concomitantly reduced the rates of apoptosis, along with diminished cleavage of PARP and caspase-3 (Figure 3E,F and Supplementary Figure S1B). These data collectively indicate that UNC569 exerts its anti-tumor effect in PDAC primarily by activating an ROS-dependent apoptotic pathway.

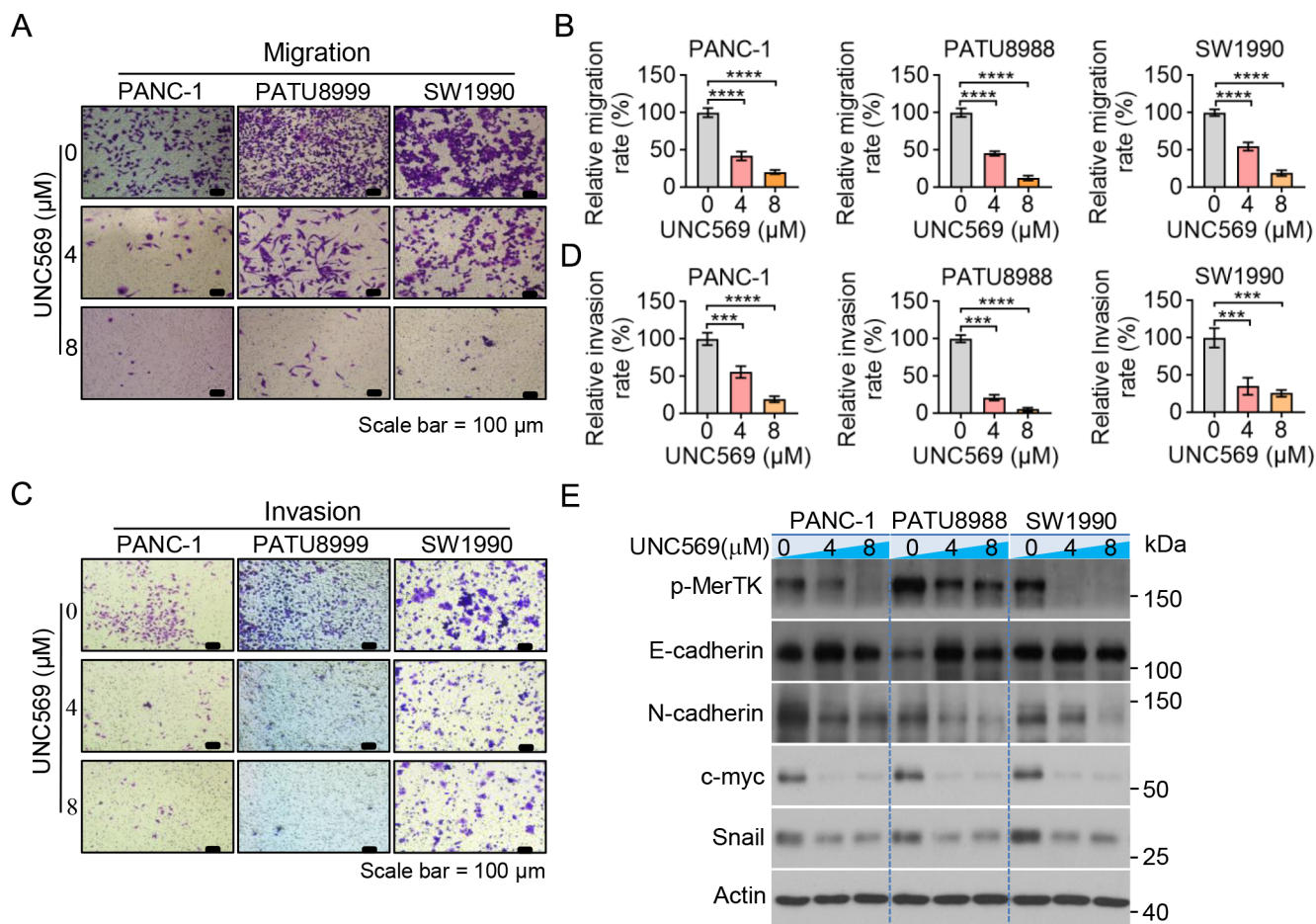


Figure 2. UNC569 suppresses migration and invasion in PDAC cells (A–D) Transwell assays assessing the effect of UNC569 on PDAC cell motility. Representative images (A,C) and corresponding quantitative analysis (B,D) of cell migration and invasion are shown. (E) Western blot analysis of migration- and invasion-associated proteins in PDAC cells treated with UNC569 or DMSO for 36 h. Data are presented as the mean $\pm$ SD ($n = 3$). *** $P < 0.001$, **** $P < 0.0001$, one-way ANOVA (B,D).

UNC569 inhibits tumor growth *in vivo*

The antitumor activity of UNC569 was further validated *in vivo* using a mouse xenograft model. In agreement with the *in vitro* data, administration of UNC569 resulted in significant inhibition of PANC-1 tumor growth and a reduction in tumor weight (Figure 4A–C). Notably, the treatment was well-tolerated, with no significant body weight loss observed in mice (Figure 4D). Comparable tumor growth inhibition was also observed in xenograft models established from PATU8998 and SW1990 cell lines (Figure 4E–L). To investigate the mechanism underlying this *in vivo* efficacy, we analyzed the expression of key cell cycle regulators in tumor tissues. UNC569 treatment downregulated Cyclin B1, CDK6, and p-Rb, while upregulating p21 and p27 (Figure 4M,N), suggesting that its antitumor activity is associated with the induction of cell cycle arrest.

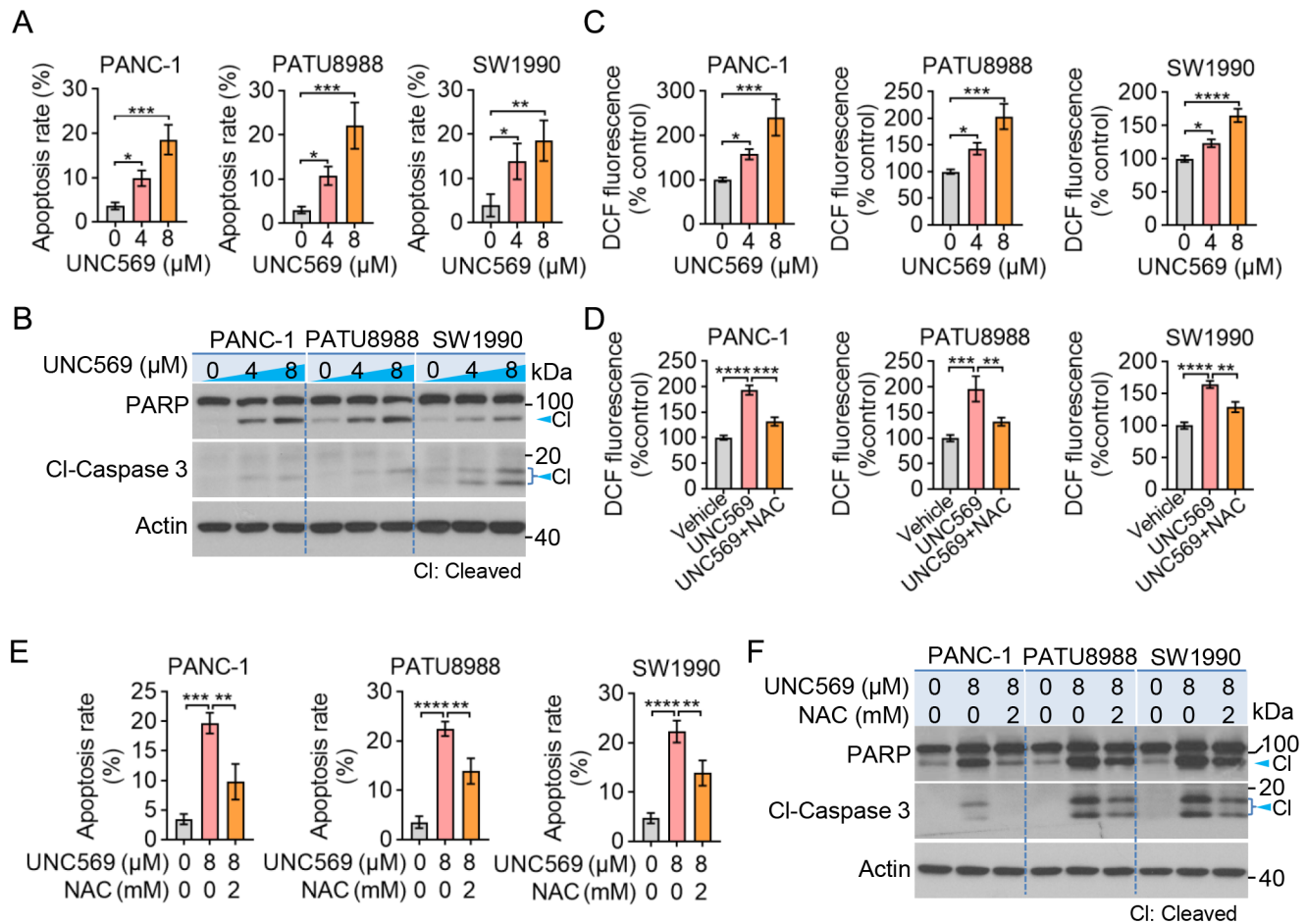
UNC569 enhances the chemosensitivity of PDAC cells to Gemcitabine

MerTK promotes tumor cell proliferation and may contribute to chemoresistance [17]. Since our previous results showed that the MerTK inhibitor UNC569 inhibits PDAC growth and induces apoptosis, we reasoned that pharmacologically inhibiting MerTK

might overcome chemoresistance and enhance the efficacy of standard therapies. We therefore evaluated its combination with Gemcitabine, a frontline chemotherapeutic for PDAC. In PDAC cells, the combination of UNC569 (8 μ M) and GEM (20 μ M) for 36 h produced superior antitumor effects compared to monotherapies, including greater suppression of proliferation, clonogenicity, migration, and invasion (Figure 5A–E). This enhanced efficacy was associated with a significant increase in intracellular ROS (Figure 5F) and a marked elevation in apoptosis (Figure 5G and Supplementary Figure S2). Collectively, these findings indicate that UNC569 effectively sensitizes PDAC cells to GEM, likely through amplifying ROS-mediated apoptotic signaling.

UNC569 inhibits PDAC cell growth by inducing DNA damage and activating the JNK/p38 pathways.

Maintaining genomic integrity is critical, given the severe consequences of DNA damage. In eukaryotic cells, the histone variant H2A.X functions as a primary sensor that initiates the DDR within chromatin [35]. To determine whether DNA damage contributes to the growth-inhibitory effect of UNC569, we assessed H2A.X status in treated PDAC cells. We found that UNC569 treatment markedly enhanced H2A.X phosphorylation (Figure 6A). Since the phos-



phorylated form, γ -H2A.X, is a well-established biomarker of DNA double-strand breaks, these results indicate that UNC569 induces DNA damage.

The JNK/p38 MAPK cascade orchestrates essential cellular functions, including apoptosis and cell cycle regulation. To examine whether UNC569 modulates cell cycle progression through this pathway, we assessed the phosphorylation status of JNK and p38 by western blot analysis. The results showed that UNC569 treatment markedly increased the phosphorylation of both JNK and p38, indicating activation of the JNK/p38 MAPK signaling pathway (Figure 6B). Taken together, these findings suggest a coordinated mechanism wherein UNC569 inhibits MerTK phosphorylation, promotes ROS generation, induces DNA damage (marked by H2A.X phosphorylation), and activates the JNK/p38 MAPK pathway. Through these integrated actions, UNC569 drives cell cycle arrest and apoptosis, thereby restraining pancreatic tumor progression (Figure 6C).

Discussion

This study identifies UNC569 as a therapeutic agent with dual mechanisms of action in pancreatic cancer. It suppresses tumor progression by inducing γ -H2A.X-mediated DNA damage and

activating the JNK/p38 pathway, leading to cell-cycle arrest, apoptosis, and inhibition of proliferation and metastasis. These *in vitro* findings are corroborated *in vivo*, where UNC569 significantly inhibits tumor growth in xenograft models. Moreover, UNC569 significantly increases the sensitivity of PDAC cells to Gemcitabine. Collectively, UNC569 overcomes therapeutic resistance through these complementary mechanisms, offering a promising translational approach for PDAC.

PDAC is characterized by dysregulated cell cycle progression and aberrant proliferation [36,37], which drive rapid disease onset and progression. Mechanistically, uncontrolled traversal through the G1-S-G2-M phases is a major driver of tumor growth. This process is tightly regulated by key proteins such as Cyclin B1 and p21; specifically, p21 enforces G2/M arrest by inhibiting CDK1-Cyclin B1 kinase activity [38–40]. Therefore, we investigated whether UNC569 affects this regulatory axis. Our results show that UNC569 alters the expression of both Cyclin B1 and p21. This finding is consistent with flow cytometry analysis indicating G2/M phase blockade in PDAC cells. Importantly, although compensatory mechanisms may allow cells to evade G1/S arrest despite Rb/CDK6 inhibition [41], UNC569 reliably induces G2/M arrest both *in vitro*

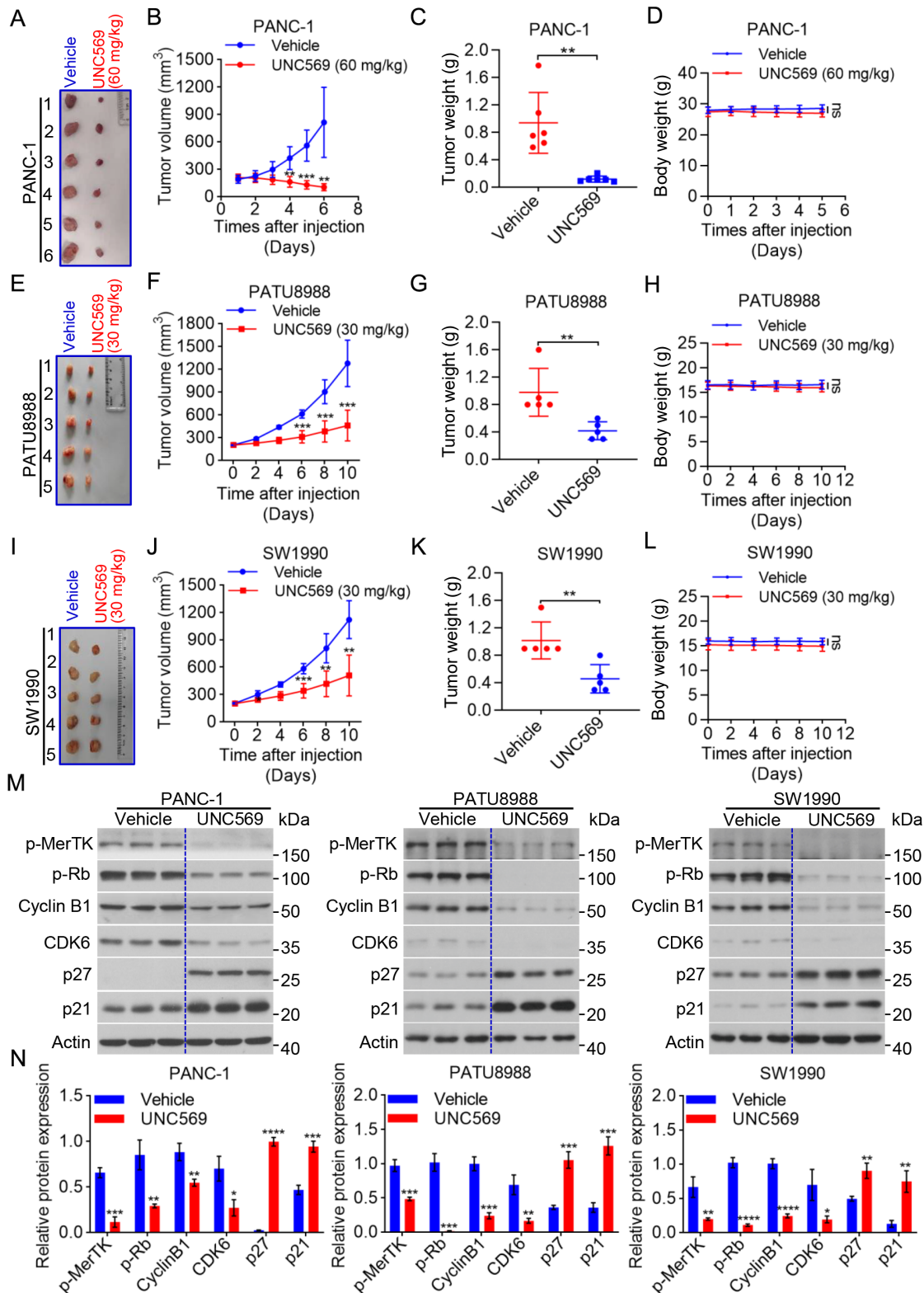


Figure 4. UNC569 suppresses tumor growth in a mouse PDAC xenograft model (A–L) Nude mice bearing PANC-1, PATU8988, or SW1990 xenografts were treated by intraperitoneal injection of 0.9% NaCl (control) or UNC569 (30 or 60 mg/kg). (A,E,I) Representative images of dissected tumors from PANC-1 ($n = 6$), PATU8988 ($n = 5$), and SW1990 ($n = 5$) xenografts. (B,F,J) Tumor growth curves recorded during the treatment period. (C,G,K) Final tumor weights measured at the endpoint. (D,H,L) Body weight changes monitored throughout the study to evaluate systemic toxicity. (M,N) Western blot images (M) and corresponding quantification (N) of cell cycle-related proteins in tumor tissues harvested from xenografted mice treated with UNC569 or vehicle. Data are presented as the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. ns, not significant, unpaired Student's t test (C,G,K,N), two-way ANOVA (B,D,F,H,J,L).

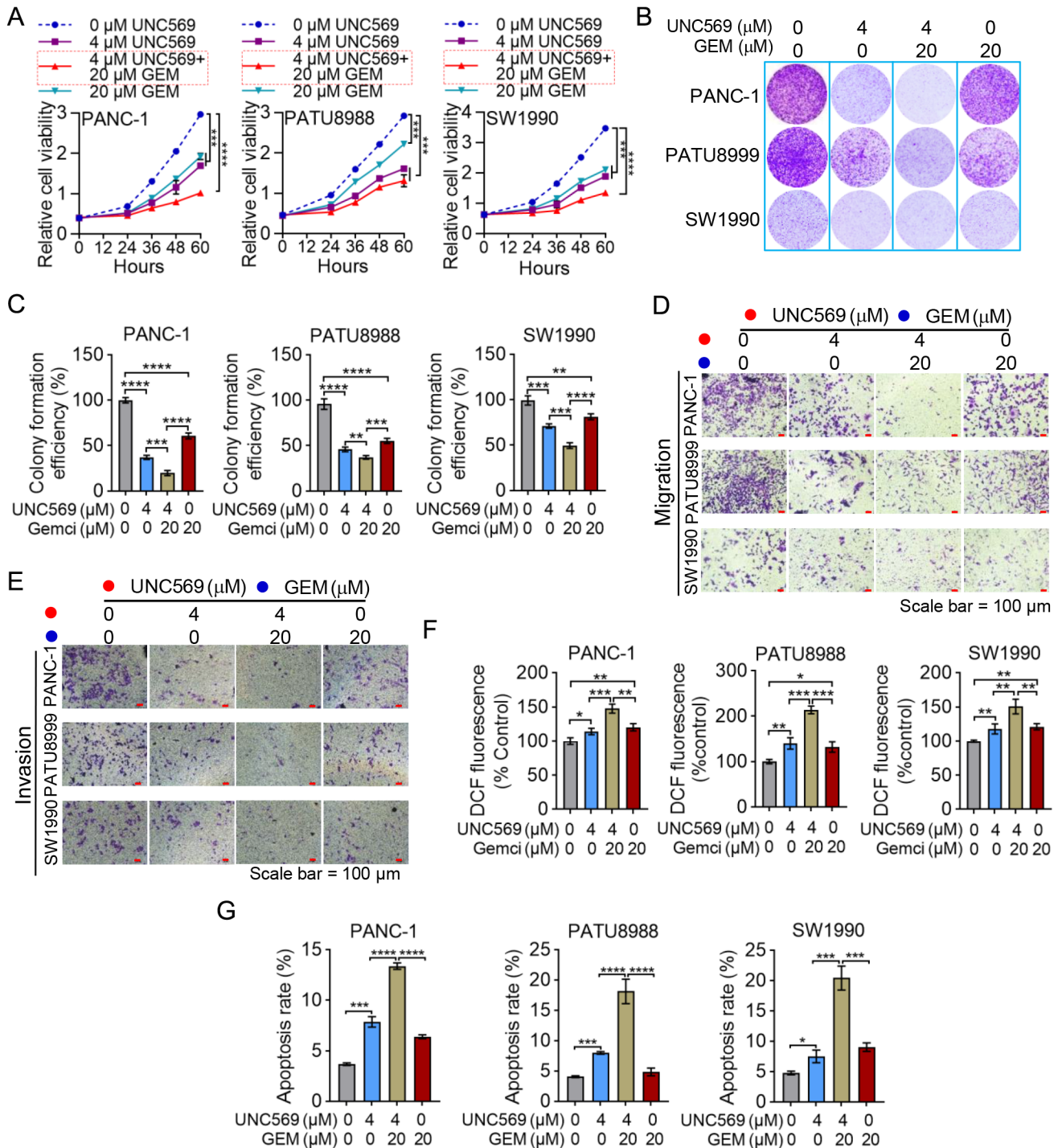


Figure 5. UNC569 enhances the chemosensitivity of PDAC cells to GEM *in vitro* (A–G) PDAC cells were treated for 36 h with DMSO, UNC569 (4 μ M), UNC569 (4 μ M) combined with gemcitabine (20 μ M), or GEM (20 μ M) alone. (A) Cell proliferation was assessed by CCK-8 assay. (B,C) Representative images (B) and quantitative results (C) of colony formation assays. (D,E) Representative images of migration (D) and invasion (E) in PDAC cells. (F) Total intracellular ROS levels were measured using DCFH-DA staining and flow cytometry. (G) Apoptosis was quantified by flow cytometry. Data are presented as the mean $\pm$ SD. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001. Two-way ANOVA (A,C,F,G).

and *in vivo*, effectively suppressing PDAC proliferation.

The poor prognosis of PDAC, often termed the “king of cancers”, is largely due to its high metastatic potential [42], most commonly to the liver [43]. Mechanistically, EMT is central to driving invasion and metastasis in PDAC and other malignancies [44,45]. During

EMT, the transcription factor Snail facilitates progression by repressing E-cadherin and upregulating N-cadherin. Additionally, the oncoprotein c-Myc contributes to tumorigenesis [46] and induces Snail expression [47], collectively regulating EMT in cancer [48–50]. In our study, UNC569 treatment significantly reduced the

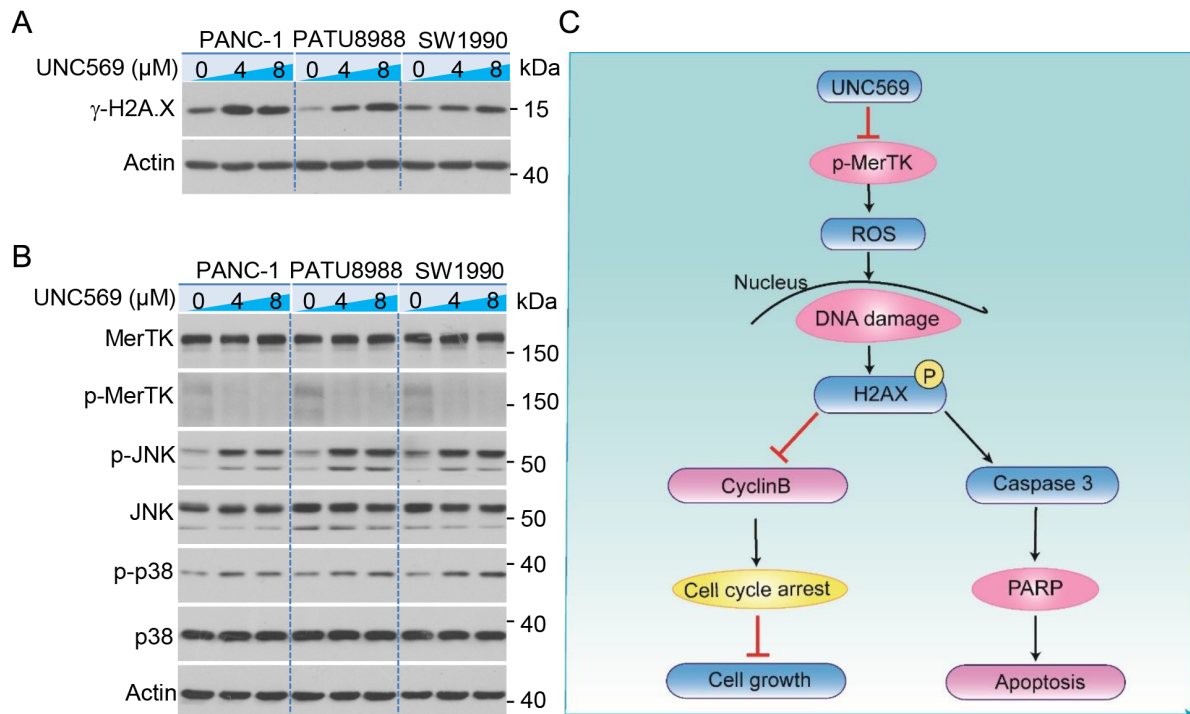


Figure 6. UNC569 suppresses PDAC cell growth by inducing DNA damage and activating the JNK/p38 MAPK pathway (A) Western blot analysis of γ -H2A.X expression in PDAC cells treated with the indicated concentrations of UNC569 or DMSO. (B) Western blot analysis of JNK/p38 signaling pathway-related proteins in PDAC cells following UNC569 treatment. (C) Schematic diagram illustrating the proposed mechanism through which UNC569 inhibits PDAC cell growth via induction of DNA damage and activation of the JNK/p38 MAPK signaling pathway.

protein levels of both Snail and c-Myc in PDAC cells. In line with this, Transwell assays demonstrated a concurrent decrease in cell migration and invasion.

ROS, generated as byproducts of mitochondrial oxidative phosphorylation, must be tightly controlled to maintain cellular homeostasis. While moderate ROS levels support tumor growth, excessive accumulation induces oxidative stress and triggers programmed cell death, a process that often preferentially targets malignant cells [51,52]. Accordingly, the antioxidant NAC can alleviate ROS overproduction and reduce subsequent apoptosis. Here, we demonstrate that UNC569-induced apoptosis in PDAC cells is mediated, at least partially, by ROS accumulation. Importantly, MerTK has been implicated in chemoresistance through various mechanisms, including pro-survival signaling, DNA repair, and EMT induction [17,53]. Consistent with this role, we observed that the combination of UNC569 and GEM significantly elevated ROS levels and augmented apoptosis compared to single-agent treatments, demonstrating that UNC569 can potentiate chemosensitivity in PDAC cells.

To counteract DNA damage caused by ionizing radiation, chemotherapy, or mutagens, tumor cells intrinsically coordinate DNA repair with cell cycle checkpoint activation [54]. Key molecular responses involve the phosphorylation of the histone variant H2A.X at Ser139 (γ -H2A.X) [55–59] and the downregulation of CyclinB1/CDK1, both of which are established hallmarks of DNA damage [60]. In this study, UNC569 treatment led to CyclinB1 suppression and γ -H2A.X elevation in PDAC cells, indicative of accumulated DNA damage and compromised repair capacity, ultimately resulting in cell death. Notably, GEM—an agent known to inhibit DNA synthesis and induce DNA damage—may synergize

with UNC569-mediated DNA damage to further enhance apoptosis in PDAC cells. MAPK pathways are broadly implicated in the regulation of cellular proliferation [61–63]. Specifically, JNK signaling modulates H2A.X phosphorylation to facilitate the repair of radiation-induced DNA breaks [64], whereas ROS accumulation activates JNK/p38 pathways [65,66], thereby promoting apoptosis [66–69]. In line with these mechanisms, UNC569 treatment upregulated γ -H2A.X, induced caspase-3 cleavage and increased phospho-JNK levels, confirming the activation of DNA damage-driven apoptosis in PDAC cells. Collectively, these results demonstrate that γ -H2A.X-mediated DNA damage and ROS-dependent JNK/p38 MAPK signaling play crucial roles in UNC569-induced apoptosis in PDAC cells.

Taken together, our findings implicate UNC569 in suppressing PDAC progression via two convergent mechanisms: the induction of DNA damage and the activation of ROS-dependent JNK/p38 MAPK signaling, both of which lead to apoptosis. UNC569 also exhibits a significant chemo-sensitizing effect when combined with GEM. These results, validated across experimental models, reveal a novel therapeutic axis and highlight the translational relevance of MerTK inhibition for PDAC treatment.

Supplementary Data

Supplementary data are available at *Acta Biochimica et Biophysica Sinica* online.

Data Availability

All data supporting the findings of this study, including raw data and original images, are available from the corresponding author upon reasonable request.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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