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ARTICLE

SLFN11 Deficiency-Induced Gemcitabine Resistance Is Overcome by Agents Targeting the DNA Damage Response in Pancreatic Cancer Cells

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ABSTRACT: Objectives: *SLFN11* (Schlafen-11) enhances sensitivity to DNA-damaging agents (DDAs) and DNA damage response (DDR) inhibitors in various cancer types. However, its function in pancreatic cancer (PC) remains largely unknown. This research aims to investigate the expression patterns of *SLFN11* and other *SLFN* family members in PC and their correlation with drug sensitivity. **Methods:** *SLFN11* expression and genetic alterations were analyzed using publicly available datasets (TCGA and GTEx). Functional studies, including cell cycle, apoptosis assays, and proliferation assays, were performed in *SLFN11*-knockdown and *SLFN11*-knockout (KO) PC cells. The relationship between *SLFN11* expression and drug responsiveness was assessed via the CellMiner Cross-Database. **Results:** Analysis of multiple public datasets demonstrated that elevated *SLFN11* expression is significantly linked with poor survival outcomes in PC, supporting its function as a predictive marker. Functional assays in PC cell lines demonstrated that *SLFN11* knockdown disrupted G1 phase progression and increased apoptosis, indicating its involvement in tumor cell survival. Moreover, while elevated *SLFN11* expression correlated with improved sensitivity to gemcitabine in some cell lines, CRISPR/Cas9-mediated *SLFN11* knockout resulted in notable gemcitabine resistance. Importantly, this resistance was partially reversed when gemcitabine was combined with cisplatin and DDR inhibitors (Poly (ADP-ribose) polymerase (PARP), ataxia telangiectasia and Rad3 related (ATR), and Wee1 inhibitors), suggesting that *SLFN11* modulates the reaction to both DNA-damaging agents and DDR-targeted therapies. **Conclusion:** Our findings indicate that *SLFN11* plays a dual role in PC: as a prognostic marker, with high expression linked to poor clinical outcomes, and as a predictor of drug sensitivity, where its presence is associated with increased gemcitabine efficacy. However, the development of chemoresistance upon *SLFN11* loss (and its partial reversal by DDR inhibitors) highlights the complexity of its function. These results underscore that *SLFN11* expression alone may not fully determine gemcitabine response, and additional factors are likely involved. Further clinical validation is therefore essential to establish *SLFN11* as a reliable biomarker for guiding DDR-targeted therapeutic strategies in PC.

KEYWORDS: *SLFN11*; DNA-damaging agents; DNA damage response; pancreatic cancer

1 Introduction

Pancreatic cancer (PC) is one of the most critical causes of cancer-associated mortality globally, with an approximate survival rate over five-years of 13% [1]. A global analysis of PC burden from 1990 to 2021 revealed a continuous rise in both incidence and mortality rates, suggesting that the disease will continue to pose a significant health challenge in the future [2]. Despite ongoing advancements in research and treatment, PC continues to have a poor prognosis, with only 15%–20% of cases being eligible for surgical resection at

diagnosis. Furthermore, its inherent resistance to chemotherapy makes clinical management particularly challenging [3,4].

DNA-damaging agents (DDAs), such as gemcitabine and cisplatin, are chemotherapeutic agents widely used to treat solid cancers. Gemcitabine, originally introduced as an effective treatment for PC, remains a key component of therapy and is also widely utilized for various refractory cancers, such as breast, bladder, and ovarian cancers [5,6]. The cytotoxic mechanism of gemcitabine involves disrupting DNA synthesis, inducing cell cycle interruption in the S phase. At higher concentrations, it can also induce apoptotic cell death during the G1 and G2/M phases [7]. Although gemcitabine has been the cornerstone of PC chemotherapy for over two decades, its efficacy remains limited. Consequently, researchers have explored combination therapies incorporating agents such as cisplatin, capecitabine, checkpoint kinase 1 (Chk1) inhibitors, and autophagy inhibitors to enhance therapeutic outcomes and develop effective strategies for targeted PC treatment [8]. Cisplatin, a widely used chemotherapeutic agent, has shown efficacy in treating multiple cancers, including ovarian, lung, head and neck, bladder, head, and testicular cancers. Its mechanism of action involves the formation of platinum-DNA adducts, which generate inter- and intra-strand linkages, which interrupt DNA synthesis and transcription. This ultimately triggers the DNA damage response, leading to the apoptosis in cancer cells [9]. Moreover, the combination of gemcitabine and cisplatin has shown promising therapeutic potential in PC treatment [10]. DNA damage response (DDR) plays an essential role in maintaining genome stability. Poly (ADP-ribose) polymerase (PARP), ataxia telangiectasia and Rad3 related (ATR), ataxia telangiectasia mutated (ATM), checkpoint kinase 1/2 (Chk1/2), and Wee1 inhibitors are being developed as targeted inhibitors of key regulators involved in the DDR. Notably, PARP inhibitors target PARP, which is crucial in the repair of single-strand DNA breaks. Rather than functioning as kinase inhibitors, these agents exploit synthetic lethality in specific genetic contexts, demonstrating great potential in cancer therapy [11,12].

In humans, the *SLFN* gene family includes five distinct members: *SLFN5*, *SLFN11*, *SLFN12*, *SLFN13*, and *SLFN14* [13]. These proteins were initially recognized for their functions in regulating cell proliferation, transformation, and growth [14]. Recent research has emphasized the significant function of the *SLFN* family in cancer progression and drug resistance; notably, all *SLFN* family members are downregulated in cancers such as lung squamous carcinoma, breast cancer, rectal carcinoma, and prostate cancer [15,16]. In contrast, *SLFN* expression is elevated in renal cell carcinoma and PC [14]. Among these, *SLFN11* has emerged as a potential prognostic marker for multiple anticancer drugs, including topoisomerase inhibitors [17–19], platinum-based agents such as cisplatin and carboplatin [20–22], and PARP inhibitors [23–25] based on bioinformatics analyses of cancer cell databases [26] and multiple experimental studies. In gastric cancer, *SLFN11* methylation is observed in approximately 29.9% of cases, correlating with larger tumor size, accelerated tumor growth, and increased resistance to cisplatin [27]. Additionally, *SLFN11* knockout has been identified to induce resistance to platinum-containing chemotherapeutics, including oxaliplatin, cisplatin, and irinotecan [21]. In colorectal cancer, *SLFN11* expression varies among cell lines, with high levels enhancing SN-38-mediated cell cycle arrest and apoptosis, leading to increased drug sensitivity [19]. Moreover, studies have demonstrated that expression *of SLFN11* is modulated by DNA methylation in esophageal cancer and could function as a predictive marker for responsiveness to ATM inhibitors [28]. In PC, *SLFN5* is highly expressed, and its upregulation is associated with poorer overall survival, whereas its downregulation reduces PC cell viability [29].

Despite these findings, the role of *SLFN11* in drug resistance in PC remains largely unexplored. To address this gap, we analyzed *SLFN11* expression levels using The Cancer Genome Atlas (TCGA) database and assessed its impact on cell cycle regulation and apoptosis. Additionally, we examined the influence of

SLFN11 expression variations on PC cell sensitivity to DDAs and DDR inhibitors using the CellMiner Cross-Database and drug sensitivity analyses. Finally, to more comprehensively the predictive function of *SLFN11* in drug response, we created *SLFN11*-KO PC cells and tested their response to DDAs and DDR inhibitors, offering potential insights into novel therapeutic strategies for PC.

2 Materials and Methods

2.1 Database Analysis

SLFN family genetic information was analyzed using publicly available datasets, including the International Cancer Genome Consortium (ICGC, Nature 2012), Queensland Centre for Medical Genomics (QCMG, Nature 2016), Clinical Proteomic Tumor Analysis Consortium (CPTAC, Cell 2021), The Cancer Genome Atlas (TCGA, PanCancer Atlas), and the University of Texas Southwestern (UTSW, Nat Commun 2015). These datasets were accessed via the cBio Cancer Genomics Portal (cBioPortal, https://www.cbioportal.org/ (accessed on 1 January 2025), Memorial Sloan Kettering Cancer Center, New York, NY, USA) [30]. The expression patterns of *SLFN* family members were evaluated in 179 PC tissues and 171 normal pancreatic tissues using the Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/ (accessed on 1 January 2025), Peking University, Beijing, China) [31]. This analysis was conducted using patient data obtained from TCGA (https://www.cancer.gov/tcga (accessed on 1 January 2025)) [32] and from the Genotype-Tissue Expression (GTEx, https://gtexportal.org/home/ (accessed on 1 January 2025)) database [33–35], normal tissue samples were acquired. The TCGA dataset includes RNA sequencing data from 179 PC tissue samples collected from 178 patients, comprising 178 primary tumor samples and one metastatic sample. Gene expression levels were quantified in transcripts per million (TPM) and log-transformed using a $\log_2(\text{TPM} + 1)$ scale for comparative analysis. Expression thresholds were defined at $|\log_2$ fold change (FC)$| \geq 1$ with a significance level of p-value < 0.01. Additionally, overall survival (OS) data for PC patients were retrieved from TCGA and analyzed about the expression of *SLFN* family genes. *SLFN11* mRNA expression profiling and drug sensitivity analyses were performed using public datasets from the CellMiner Cross-Database (https://discover.nci.nih.gov/cellminercdb/ (accessed on 1 January 2025)), including National Cancer Institute 60 (NCI-60) (60 cell lines × 237 drugs), Cancer Therapeutics Response Portal (CTRP) (860 cell lines × 481 drugs), CTRP (860 cell lines × 481 drugs), and Cancer Cell Line Encyclopedia (CCLE) (860 cell lines × 481 drugs).

2.2 Cell Culture and Cell Cycle Synchronization

The human PC cell lines AsPC-1 [CRL-1682] and BxPC-3 [CRL-1687] were cultured in RPMI1640 medium (Gibco, 11875-093, Grand Island, NY, USA). MIA PaCa-2 [CRL-1420] and PANC-1 [CRL-1469] were cultured in DMEM (Gibco, 11965-092). HPAF-II [CRL-1997] was cultured in MEM (Gibco, 11095-080). Capan-2 [HTB-80] was cultured in McCoy's 5A medium (Gibco, 16600-082). These cell culture media contained 10% fetal bovine serum (FBS) (Gibco, 16000-044) and 1% penicillin-streptomycin (pen-strep) (Gibco, 15140-122). Capan-1 [HTB-79] was cultured in IMDM (Gibco, 12440-053) supplemented with 20% FBS and 1% pen-strep. Panc10.05 [CRL-2547] was cultured in RPMI1640 (Gibco, 11875-093) supplemented with 10 units/mL insulin (Sigma-Aldrich, I9278, St. Louis, MO, USA), 15% FBS, and 1% pen-strep. All cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in a humidified incubator set at 37°C with 5% CO_2. Mycoplasma contamination was regularly monitored using the MycoAlert mycoplasma assay kit (Lonza, LT07-318, Basel, Switzerland), and all cell lines were confirmed to be mycoplasma-free. Normal human pancreatic ductal epithelial (HPDE) cells were obtained from Joo Kyung Park, MD (Samsung Medical Center, Seoul, Republic of Korea) and cultured in K-SFM (Gibco, 17005-042) added with 10% FBS and 1% pen-strep. For double thymidine block

(DTB) experiments, cells were synchronized near the G1/S boundary using 2 mM thymidine (Sigma-Aldrich, T9250). After the second thymidine treatment, a fresh culture medium was changed, and cells were collected at each measurement time [36].

2.3 Cell Cytotoxicity Assay

To measure cytotoxicity, the survival of PC cells was evaluated using the Cell Counting Kit-8 (CCK-8) assay (Dojindo, CK04, Fukuoka, Japan), according to the manufacturer's protocol. PC cells were plated in 96-well dishes at a concentration of 1×10^4 cells per well. After treating each well with 10 μL of CCK-8 reagent per well in 100 μL of medium, the cells were maintained at 37°C for 4 h. The negative control consisted of wells with only the culture medium and CCK-8 solution but without cells. Absorbance at 450 nm was measured with an Epoch 2 microplate spectrophotometer (BioTek, Santa Clara, CA, USA). All tests were carried out in triplicates, and the results provided are the averages from multiple biological replicates.

2.4 Gene Silencing Using Small Interfering RNA (siRNA) and Genome Editing through CRISPR-Cas9

SLFN11 siRNA was synthesized as *SLFN11*_1: 5′-CAG GGA ACC UUA CGA AUU A-3′ and 5′-UAA UUC GUA AGG UUC CCU G-3′, *SLFN11*_2 siRNA: 5′-GGU AUU UCC UGA AGC CGA A-3′ and 5′-UUC GGC UUC AGG AAA UAC C-3′, and *SLFN11*_3 siRNA: 5′-CCA GGA UAU UUG CGA UAU A-3′ and 5′-UAU AUC GCA AAU AUC CUG G-3′ [27,37]. Control and *SLFN11* siRNAs were obtained from Cosmo Bio Co., Ltd. (Cosmo Genetech, Seoul, Republic of Korea). Transfection of cells with control and *SLFN11* siRNAs was carried out using the Lipofectamine RNAiMAX reagent (Invitrogen, 13778075, Carlsbad, CA, USA) as per the manufacturer's protocol. WT CRISPR/Cas9 (sc-418922) and *SLFN11* CRISPR/Cas9 KO (sc-401137-KO-2) plasmids were acquired from Santa Cruz Biotechnology (Dallas, TX, USA). Transfection of WT and *SLFN11*-KO cells was performed using Santa Cruz Biotechnology's UltraCruz transfection reagent (sc-395739) and plasmid transfection medium (sc-108062), then subjected to puromycin selection following the manufacturer's protocol.

2.5 Fluorescence-Assisted Cell Sorting (FACS) Analysis

Cells were collected, rinsed with 1× phosphate-buffered saline (PBS), stored in 70% ethanol at 4°C overnight, and labeled with Propidium iodide (PI, Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 50 μg/mL, along with 100 U RNase (Ribonuclease A from bovine pancreas, Sigma-Aldrich, St. Louis, MO, USA) to evaluate cell cycle and DNA content. The experiment was conducted at a rate of 150–300 cells/sec [38]. Cell cycle analysis was conducted in triplicate, and representative results were displayed because the findings were consistent across all repeats. The data were analyzed using an FACS Aria Calibur flow cytometer (BD Biosciences, San Diego, CA, USA) following standard protocols.

2.6 Western Blot and Antibodies

Whole PC cells were collected and washed once with ice-cold 1× PBS. Then, cells were lysed using 1× RIPA lysis buffer (Cell Signaling Technology, 9806, Danvers, MA, USA), and protein content in the lysates was quantified using a BCA protein assay kit (Pierce, 23225, Rockford, IL, USA). The extracted proteins were then resuspended with 4× sample buffer including 10% 2-Mercaptoethanol and denatured by boiling for 5 min. For protein separation, 8%–10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed, then electrotransfer onto a Trans-blot nitrocellulose membrane (Whatman International Ltd., 10401196, Maidstone, UK). Membranes were pretreated with 5% skim milk in TBST buffer (10 mM Tris [pH 8.0], 150 mM NaCl, 0.05% Tween 20). The blocked membranes were subsequently exposed to primary antibodies overnight at 4°C [39]. Primary antibodies were sourced from various commercial

suppliers: anti-SLFN11 (Novus Biologicals, NBP1-92368, Littleton, CO, USA), anti-cell division cycle 6 (CDC6, Novus Biologicals, NBP2-47514), anti-cyclin A2 (Cell Signaling Technology, 4656), and anti-β-actin (Sigma-Aldrich, A5441, St. Louis, MO, USA). The antibodies were diluted in different blocking buffers as follows: anti-SLFN11 was diluted to 1:1000 in 3% skim milk in 1× PBS, anti-CDC6 at 1:200 in 3% BSA in 1× PBS, anti-cyclin A2 at 1:2000 in 5% skim milk in 1× PBS, and anti-β-actin at 1:5000 in 5% skim milk in 1× PBS. Protein expression levels were analyzed using chemiluminescence detection with the SuperSignal West Pico PLUS Chemiluminescent Substrate (Pierce, 34580). Horseradish peroxidase-conjugated secondary antibodies (Jackson Immunoresearch Laboratories, 315-035-045, 111-005-045, West Grove, PA, USA) was diluted at 1:5000 for detection.

2.7 Chemicals

Gemcitabine (LY-188011, S1714) and cisplatin (NSC119875) were obtained from Selleckchem (Selleck Chemicals, Houston, TX, USA). Olaparib (AZD2281), ceralasertib (AZD6738), and adavosertib (AZD1775) were supplied by AstraZeneca (Cambridge, UK).

2.8 Statistical Analysis

All data are represented as the central tendency, derived from two or three independent experimental replicates. Statistical analyses were performed using GraphPad Prism (version 5.0, GraphPad Software, San Diego, CA, USA), and the results are shown as means ± standard errors of the means (SEMs). Group comparisons were made using a two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test for multiple comparisons. A statistically significant difference was defined as $p < 0.05$ unless otherwise stated, and specific p-values are provided in the figure legends or results section.

3 Results

3.1 Increased SLFN11 Expression Is Associated with Poor Survival Outcomes

We initially analyzed genetic alterations, including mutations and gene amplification, in the *SLFN* family and *SLFN11* across five publicly available PC datasets using the cBioPortal platform (http://cbioportal.org (accessed on 1 January 2025)). In the ICGC ($n = 99$), QCMG ($n = 456$), CPTAC ($n = 140$), UTSW ($n = 109$), and TCGA ($n = 186$) datasets that analyzing patients with PC, the genetic variation of the *SLFN* family (including *SLFN11*) was 3.67% in UTSW, 3.24% in TCGA, and 1.31% in QCMG. Low amplification rates were typically found, and no amplifications were detected in the ICGC and CPTAC datasets. When analyzing *SLFN11* alone, amplification was observed: of 2.16% and 1.83% in the TCGA and UTSW datasets, respectively; however, no amplification was observed in the ICGC, QCMG, and CPTAC datasets (Fig. 1A). Analysis of all five datasets (ICGC, QCMG, CPTAC, UTSW, and TCGA) showed variation in the *SLFN* family ranging from 0.7% to 1.1%. Genetic alterations were observed in 0.9% of cases for *SLFN5*, 0.7% for *SLFN11*, 0.8% for *SLFN12*, 0.7% for *SLFN13*, and 1.1% for *SLFN14*. In addition, *SLFN5*, *SLFN12*, and *SLFN14* showed amplification, missense mutations, and truncating mutations, while only amplifications were observed in *SLFN11* (Fig. 1B).

Next, we analyzed the mRNA expression levels of *SLFN11* and other *SLFN* family genes in PC using TCGA data ($n = 179$) and compared them with normal tissue expression from GTEx ($n = 171$). Survival analysis was conducted based on publicly available data via the GEPIA web portal (http://gepia.cancer-pku.cn/ (accessed on 1 January 2025)). Analysis of TCGA data revealed that *SLFN11* expression patterns were elevated in PC tissues relative to normal tissues (Fig. 1C). Then, we assessed the association between *SLFN11* mRNA expression and clinical-pathological parameters using overall survival analysis (Kaplan-Meier curves). Although high *SLFN11* expression appeared to correlate with lower survival rates after 20

months, the overall survival difference was not statistically significant (Fig. 1D). Further investigations with expanded cohorts and additional prognostic factors are necessary to clarify the potential relationship between *SLFN11* expression and survival outcomes.

Additionally, among the *SLFN* family members, *SLFN5*, *SLFN12*, and *SLFN13* exhibited elevated expression in PC and were linked to poorer overall survival. In contrast, *SLFN14* showed no significant variation in expression levels or survival outcomes when comparing normal and PC tissues (Supplementary Fig. S1). Although *SLFN11* mRNA expression exhibited a tendency to increase with tumor stage, this trend failed to achieve statistical significance (Fig. 1E). Based on these findings, *SLFN11* protein expression levels were examined afterward in non-cancerous immortalized HPDE cells and eight PC cell lines (AsPC-1, BxPC-3, MIA PaCa-2, PANC-1, Panc10.05, HPAF-II, Capan-1, and Capan-2) using western blotting. Compared to HPDE cells, *SLFN11* protein levels were lower in AsPC-1, BxPC-3, MIA PaCa-2, HPAF-II, and Capan-2 cells but higher in PANC-1, Panc10.05, and Capan-1 cells (Fig. 1F). According to these results, we examined the effect of *SLFN11* expression in PC cells and hypothesized that it is related to cell death.

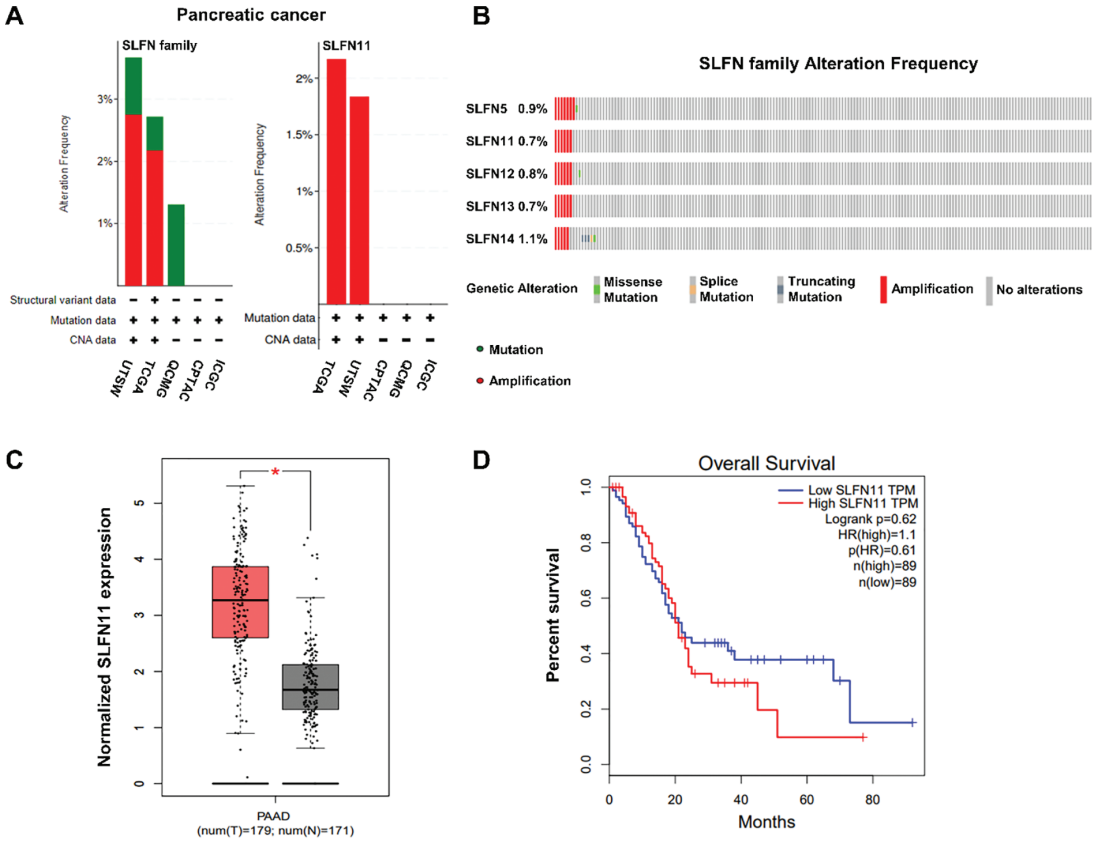

Figure 1: (Continued)

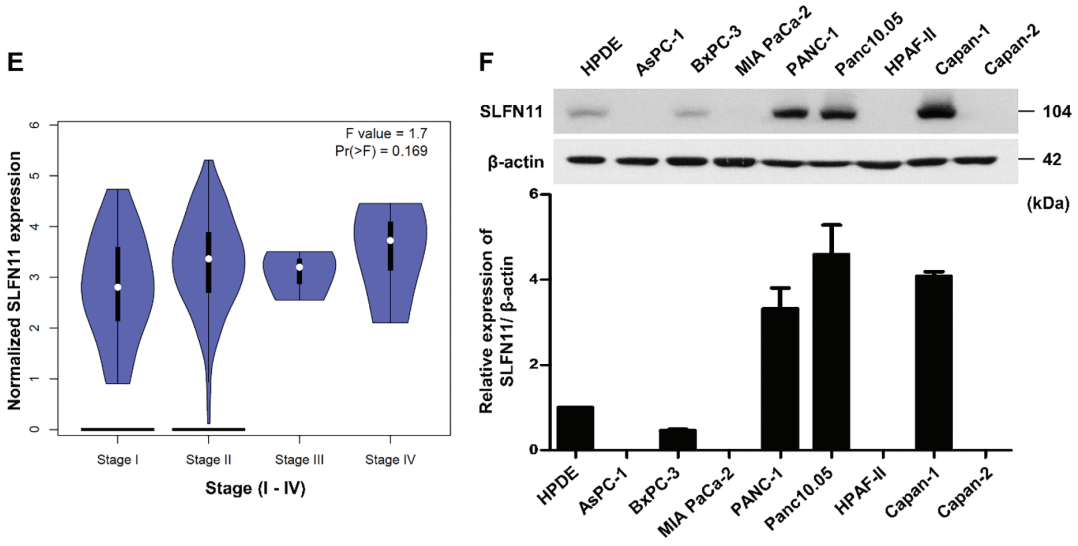

Figure 1: Analysis of genetic variation, gene expression pattern, and prognostic outcome of *SLFN11* in PC patients. (A) Distribution of amplifications and mutations in the *SLFN* family in human PC, analyzed in the ICGC (n = 99), QCMG (n = 456), CPTAC (n = 140), UTSW (n = 109), and TCGA (n = 186) datasets from cBioPortal. Green bars indicate mutational events, while red bars represent gene amplification. (B) Oncoprint analysis from the cBioPortal database shows the proportion and distribution of *SLFN* family samples with genetic alterations. The numbers represent the overall frequency of all changes. (green: Missense mutation, orange: Splicing mutation, blue: Loss-of-function mutation, red: Amplification, gray: No detectable alterations). (C) *SLFN11* mRNA expression levels in tumor (n = 179, red box) and normal tissues (n = 171, gray box) from the TCGA and GTEx databases, assessed through GEPIA. Dots represent each sample. $^*p < 0.05$. (D) Overall survival analysis of PC patients from the TCGA database. Each dot denotes the *SLFN11* expression level in an individual sample. Kaplan-Meier survival analysis along with log-rank tests were performed to assess the link between *SLFN11* expression and patient outcome in PC patients. (E) Comparative analysis of *SLFN11* mRNA expression across different tumor stages in PC, utilizing pathological stage plots derived from GEPIA dataset. (F) Western blot analysis of *SLFN11* protein levels in HPDE and various PC cell lines, including AsPC-1, BxPC-3, MIA PaCa-2, PANC-1, Panc10.05, HPAF-II, Capan-1, and Capan-2. Densitometric quantification of *SLFN11* relative to β-actin was measured via ImageJ software (version 1.50i, National Institutes of Health, Bethesda, MD, USA), with error indicators representing the variation measure from two independent biological replicates

3.2 SLFN11 Deficiency Induces G1 Cell Cycle Dysregulation and Apoptotic Sub-G1 Phase Arrest

To investigate how *SLFN11* influences cell cycle progression and apoptosis in PC cells, we used Panc10.05 cells with high *SLFN11* expression and evaluated them using western blotting, CCK-8 assay, and FACS. We used three separate siRNAs to silence *SLFN11* expression, all of which inhibited SLFN11 protein expression (Supplementary Fig. S2A). Cell growth was monitored for 72 h following siRNA transfection, revealing showing no significant impact on proliferation (Supplementary Fig. S2B). Next, we measured the cell cycle and the effect of siRNA treatment on apoptosis by assessing the hypodiploid (sub-G1) peak using PI staining in *SLFN11*-knockdown Panc10.05 cells. Apoptotic sub-G1 phase cells comprised 2.5% of the control and 4.8% of the *SLFN11*-knockdown PC cells, while polyploidy cells were found in 11.4% of the control and 17.3% of the *SLFN11*-knockdown PC cells. In addition, the percentage of cells arrested at the G0/G1 phase was 44.7% in the control and 34.4% in *SLFN11*-knockdown PC cells, while the percentage in the S phase was 11.4% in the control and 12.7% in *SLFN11*-knockdown PC cells (Supplementary Fig. S2C,D).

To investigate whether *SLFN11* affects cell cycle progression in PC cells, we conducted a double thymidine block (DTB) assay. Pyrimidine deoxynucleoside was used as thymidine to synchronize cells arrested at the G1/S transition phase. In the DTB assay, cells were first synchronized at the G1/S phase

boundary, and upon release from the second thymidine block, they progressed into the S phase (0–4 h) and subsequently entered the G2/M phase by 8 h. In Panc10.05 cells, *SLFN11* expression was knocked down using siRNA, followed by DTB synchronization. Cells were then harvested at 0, 2, 4, 6, 8, and 10 h following the removal of the second thymidine block (Fig. 2A). Western blot analysis was conducted to examine the expression of key cell cycle regulators, including CDC6 and cyclin A, which are critical for DNA replication and checkpoint maintenance. In *SLFN11*-knockdown PC cells, CDC6 accumulation was observed at 0–4 h but rapidly decreased at 6–8 h compared to the control group. Similarly, cyclin A expression increased at 0–2 h and declined at 8 h in *SLFN11*-knockdown PC cells relative to the control group (Fig. 2B, Supplementary Fig. S3).

Next, after evaluating the cell cycle before release after DTB, most of the cells detected in the control group showed accumulation in the G0/G1 phase (54.6%), then in the S (18%), G2/M (20.9%), and sub-G1 phases (6.2%). However, in *SLFN11*-knockdown PC cells, the percentage of cells arrested at the G0/G1 was significantly reduced (37%), while those in the S (21.4%) and G2/M (24.4%) phases showed a partial increase. Additionally, the sub-G1 population (17.1%) exhibited an important rise relative to the control group at 0 h (Fig. 2C,D). Four hours after the release of the second thymidine block, the number of *SLFN11*-knockdown PC cells accumulated in the G0/G1 phase reduced (control siRNA: 45.8%, *SLFN11* siRNA: 31.7%) while those in the sub-G1 phase (control siRNA: 4.4%, *SLFN11* siRNA: 16%) increased, compared with control cells (Fig. 2C,D, 4 h). These data demonstrate that *SLFN11* contributes to the regulation of PC cell transition to the G1 phase and apoptosis.

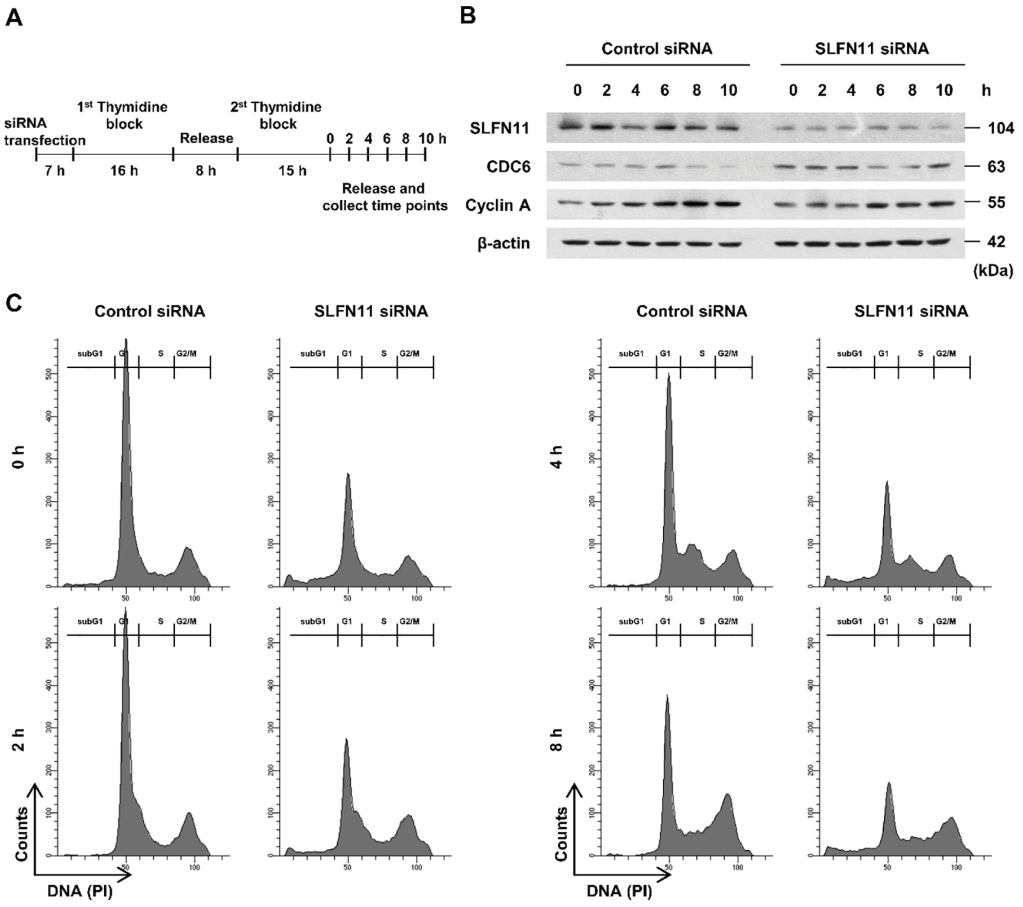

Figure 2: (Continued)

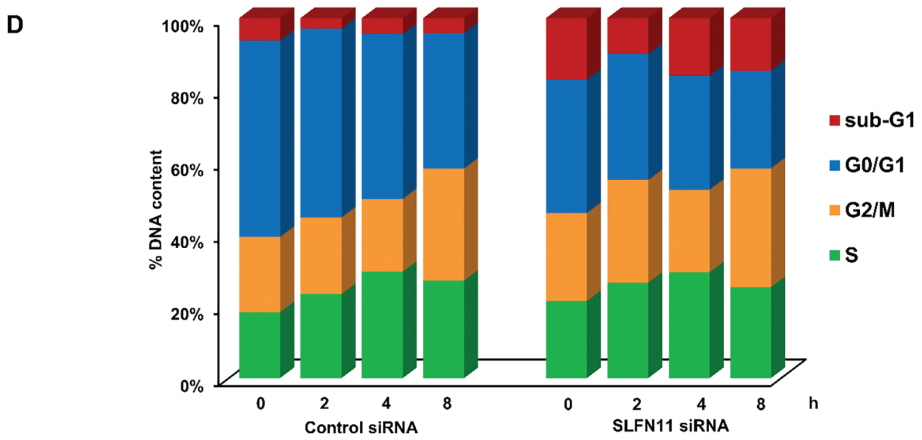

Figure 2: Influence of *SLFN11* knockdown in cell cycle regulation in PC cells. (A) Cell synchronization at the G1/S transition was performed using the DTB method, and a brief overview of the procedure. (B) Panc10.05 cells were transfected with either control or *SLFN11* siRNA, subsequently synchronized through the DTB method. Cells were collected at designated intervals (0, 2, 4, 6, 8, and 10 h) after the second thymidine treatment. Western blot assessment was performed to assess the expression of SLFN11, CDC6, cyclin A, and β-actin. (C) Panc10.05 cells underwent FACS analysis at the designated time points (0, 2, 4, and 8 h) after the second thymidine treatment. (D) Quantification of cell cycle phase distribution based on FACS analysis. The percentage of the cell population in sub-G1 (apoptosis, red), G0/G1 (blue), G2/M (orange), and S (green) phases was determined

3.3 SLFN11 Deficiency Contributes to Gemcitabine Resistance in PC Cells

Gemcitabine, a DDA, is a standard chemotherapeutic agent for PC [40,41]. To investigate the influence of *SLFN11* on gemcitabine sensitivity, we analyzed the association between *SLFN11* mRNA expression and gemcitabine response across various cancer types using the CellMiner Cross-Database (https://discover.nci.nih.gov/cellminercdb/ (accessed on 1 January 2025)). A statistically significant association was identified between *SLFN11* mRNA levels and gemcitabine sensitivity using the CTRP and CCLE datasets (r = 0.43, $p = 3.1 \times 10^{-34}$, Fig. 3A). This correlation was further supported by analyses using the GDSC and CCLE datasets (r = 0.29, $p = 1.3 \times 10^{-13}$, Supplementary Fig. S4A) and the NCI-60 dataset (r = 0.69, $p = 7.8 \times 10^{-10}$, Supplementary Fig. S4B). These findings indicate a possible function of *SLFN11* in modulating drug response across multiple cancer types.

Next, we analyzed gemcitabine sensitivity in PC cell lines according to *SLFN11* mRNA expression levels using the GDSC and CCLE datasets. PC cell lines with high *SLFN11* expression, such as Panc10.05 and Capan-1, exhibited greater gemcitabine sensitivity, whereas AsPC-1 and Capan-2 cells, which have low *SLFN11* expression, were less sensitive (Fig. 3B). Furthermore, a strong statistical association was identified between *SLFN11* expression and gemcitabine sensitivity in PC cell lines (r = 0.54, p = 0.013, Fig. 3B), further supporting the role of *SLFN11* in gemcitabine response.

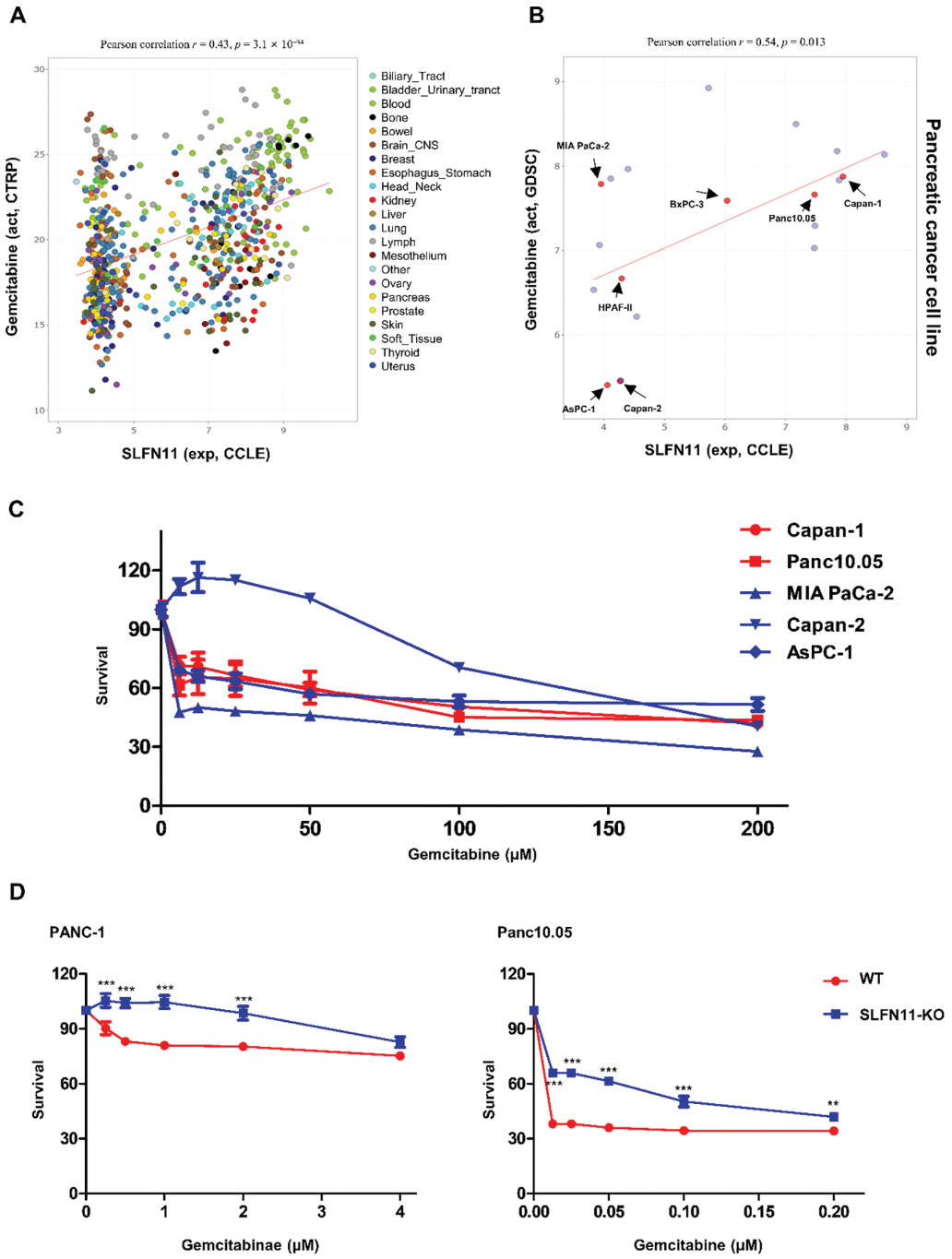

Figure 3: Correlation between *SLFN11* expression and gemcitabine sensitivity, and development of gemcitabine resistance in *SLFN11*-KO PC cells. (A) Correlation between gemcitabine sensitivity (CTRP database) and *SLFN11* mRNA expression (CCLE database) across various tumor types. Pearson correlation coefficient: $r = 0.43$, $p = 3.1 \times 10^{-34}$. (B) Correlation between gemcitabine sensitivity (GDSC database) and *SLFN11* mRNA expression (CCLE database) in PC cell lines. Pearson correlation coefficient: $r = 0.54$, $p = 0.013$. Red dots indicate PC cell lines analyzed in this study, while lilac dots represent additional PC cell lines from public datasets. (C) Gemcitabine sensitivity in PC cell lines (Capan-1, Panc10.05, MIA PaCa-2, Capan-2, and AsPC-1) was evaluated using the CCK-8 assay at 48 h. Curves were generated from biological triplicates, with values represented as means ± SEMs. (D) Gemcitabine sensitivity in PANC-1 and Panc10.05 PC cell lines was assessed using the CCK-8 assay at 48 h. Curves were generated from triplicate biological experiments, and the values are displayed as means ± SEMs. $^{**}p < 0.01$, $^{***}p < 0.001$

To further validate these findings, we assessed gemcitabine sensitivity in PC cell lines using the CCK-8 assay. Consistent with the results from Fig. 3B, Panc10.05 and Capan-1 cells exhibited high gemcitabine sensitivity, whereas Capan-2 cells displayed lower sensitivity (Fig. 3C). Interestingly, MIA PaCa-2 and AsPC-1 cells, despite low *SLFN11* expression, showed relatively high gemcitabine sensitivity (Fig. 3B,C), suggesting that additional factors may contribute to drug response. Additionally, although PANC-1 cells exhibit high *SLFN11* expression, their gemcitabine sensitivity was lower than that of Panc10.05 and Capan-1 cells (Fig. 3B,C). Conversely, Capan-2, another low-*SLFN11*-expressing cell line, exhibited the lowest gemcitabine sensitivity, further suggesting that *SLFN11* expression alone does not fully predict drug response and that additional regulatory mechanisms are involved. This suggests that *SLFN11* expression alone may not fully determine gemcitabine response, with other factors, such as DNA repair pathways or drug efflux mechanisms, may influence drug sensitivity in PC. To further assess the direct link between *SLFN11* levels and gemcitabine response, we established *SLFN11*-KO PC cells through the CRISPR/Cas9 system in two PC cell lines, PANC-1 and Panc10.05. Western blot and immunofluorescence assays confirmed the successful knockout of *SLFN11* in Panc10.05 cells (Supplementary Fig. S4C,D). According to the CCK-8 assay results, *SLFN11*-KO cells exhibited increased resistance to gemcitabine compared to WT Panc10.05 cells. However, in PANC-1 cells, the difference in survival between WT and KO was less pronounced, with WT survival leveling off at approximately 80% (Fig. 3D). This suggests that *SLFN11* knockout alone may not be sufficient to significantly alter gemcitabine sensitivity in certain PC cell lines and that additional resistance mechanisms may be involved. These findings indicate that *SLFN11* plays a significant role in modulating gemcitabine sensitivity in PC cells, although additional factors may also contribute to drug response.

3.4 SLFN11 Expression Correlates with Cisplatin and ATR Inhibitor Sensitivity

To further explore the relationship between *SLFN11* levels and drug sensitivity, we examined data from the NCI-60, GDSC, and CCLE datasets in the CellMiner Cross-Database for cisplatin (DDA), olaparib (PARP inhibitor), ceralasertib (ATR inhibitor), and adavosertib (Wee1 inhibitor). *SLFN11* expression was strongly correlated with cisplatin sensitivity (Pearson correlation $r = 0.64$, $p = 4.3 \times 10^{-8}$) and a moderately correlated with ceralasertib sensitivity ($r = 0.29$, $p = 0.027$). Conversely, *SLFN11* expression exhibited a weak and non-statistically meaningful relationship with olaparib ($r = 0.11$, $p = 0.4$) and adavosertib ($r = -0.03$, $p = 0.85$) sensitivity (Fig. 4A, Supplementary Fig. S4E,F). Next, the effects of cisplatin, olaparib, ceralasertib, and adavosertib on the growth of PC cell lines (AsPC-1, Capan-1, MIA PaCa-2, Capan-2, and Panc10.05) were confirmed using the CCK-8 assay. The treatment concentration ranges of cisplatin, olaparib, ceralasertib, and adavosertib were determined using *IC50* values from the CellMiner Cross-Database (Supplementary Fig. S5), which served as a reference for experimental conditions. As a result, Panc10.05 and Capan-1 cells (high *SLFN11* expression) showed high cisplatin and ceralasertib sensitivity, while Capan-2 cells (low *SLFN11* expression) showed low cisplatin and ceralasertib sensitivity. MIA PaCa-2 and AsPC-1 cells had low *SLFN11* expression, but showed high sensitivity to cisplatin and ceralasertib. On the other hand, *SLFN11* expression was moderately associated with adavosertib sensitivity but not with olaparib sensitivity (Fig. 4B). Thus, *SLFN11* expression appears to be partially associated with how PC cells respond to cisplatin and DDR inhibitors.

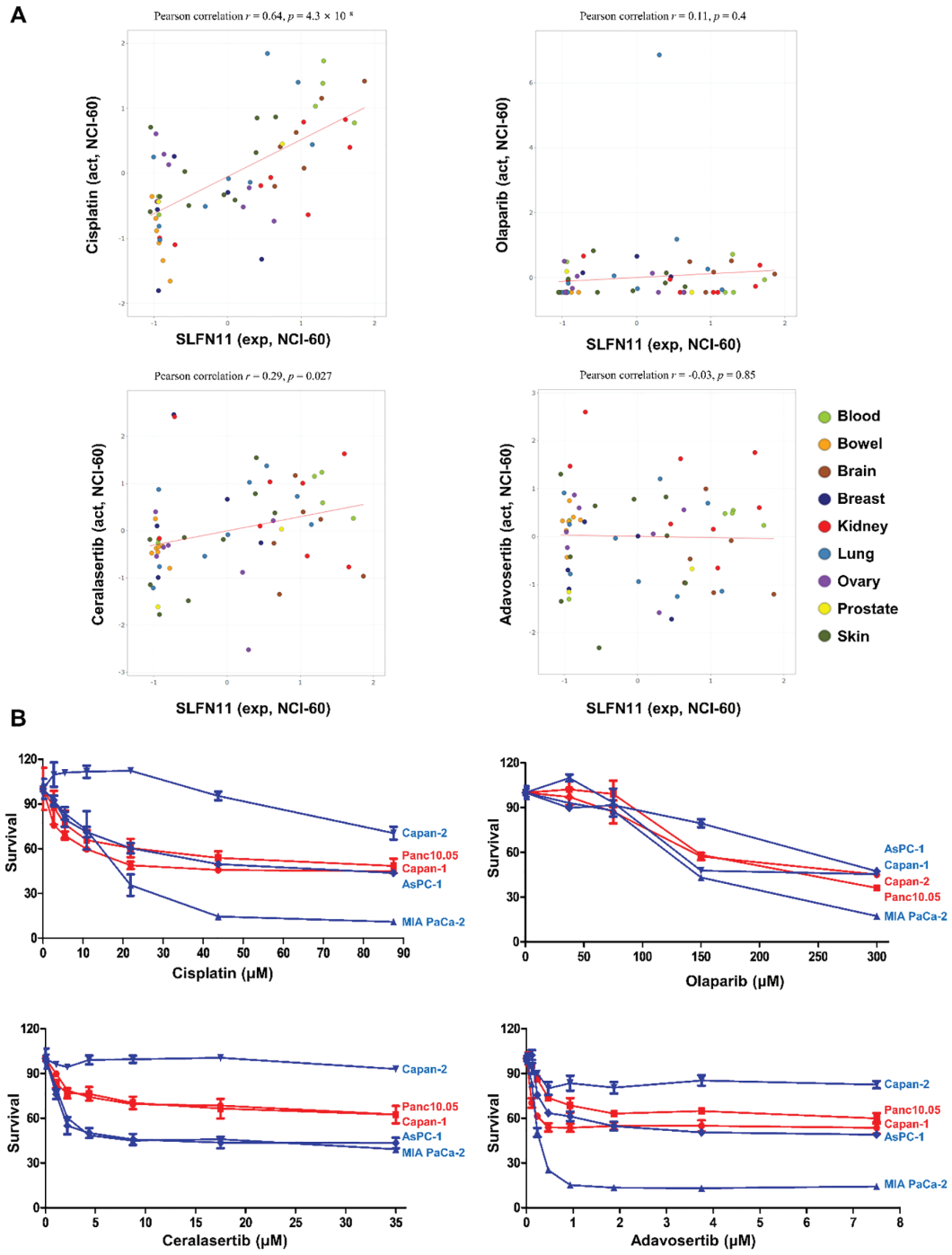

Figure 4: *SLFN11* expression correlates with drug sensitivity to cisplatin and DDR inhibitors. (A) Correlation analysis between drug sensitivity (NCI-60 database) and *SLFN11* mRNA expression (NCI-60 database) for cisplatin, olaparib, ceralasertib, and adavosertib across various tumor types. *SLFN11*-cisplatin: Pearson correlation coefficient $r = 0.64$, $p = 4.3 \times 10^{-8}$; *SLFN11*-olaparib: $r = 0.11$, $p = 0.4$; *SLFN11*-ceralasertib: $r = 0.29$, $p = 0.027$; *SLFN11*-adavosertib: $r = -0.03$, $p = 0.85$. (B) Drug sensitivity to cisplatin, olaparib, ceralasertib, and adavosertib was assessed using the CCK-8 assay at 48 h in AsPC-1, MIA PaCa-2, Panc10.05, Capan-1, and Capan-2 cells. Curves were generated from biological triplicates, with values represented as means ± SEMs

3.5 The Increased Resistance to Gemcitabine in SLFN11-KO Cells Is Reversed by Cisplatin and DDR Inhibitors

To investigate how *SLFN11* expression influences the responsiveness of PC cells to DDR-targeted agents, we treated WT and *SLFN11*-KO Panc10.05 and PANC-1 cells with cisplatin, olaparib, ceralasertib, and adavosertib, either as monotherapies or in combination with gemcitabine, and assessed their impact on cell proliferation using the CCK-8 assay. When WT and *SLFN11*-KO cells were treated with cisplatin, olaparib, ceralasertib, and adavosertib alone, *SLFN11*-KO cells showed decreased responsiveness to cisplatin and adavosertib relative to WT cells, whereas no significant difference was observed in olaparib or ceralasertib treatment (Fig. 5A,B). In PANC-1 cells, *SLFN11*-KO cells inhibited cell proliferation only after high-concentration ceralasertib treatment (Fig. 5B). When cisplatin, olaparib, ceralasertib, and adavosertib were co-administered with gemcitabine (+G), *SLFN11*-KO cells exhibited greater sensitivity compared to WT cells across all tested drugs (Fig. 5C,D). The obtained results suggest that *SLFN11* deficiency enhances the cytotoxic effects of these agents in the presence of gemcitabine. The obtained results indicate that targeting *SLFN11* may serve as a potential strategy for improving the efficacy of various anticancer drugs in the treatment of gemcitabine-resistant PC.

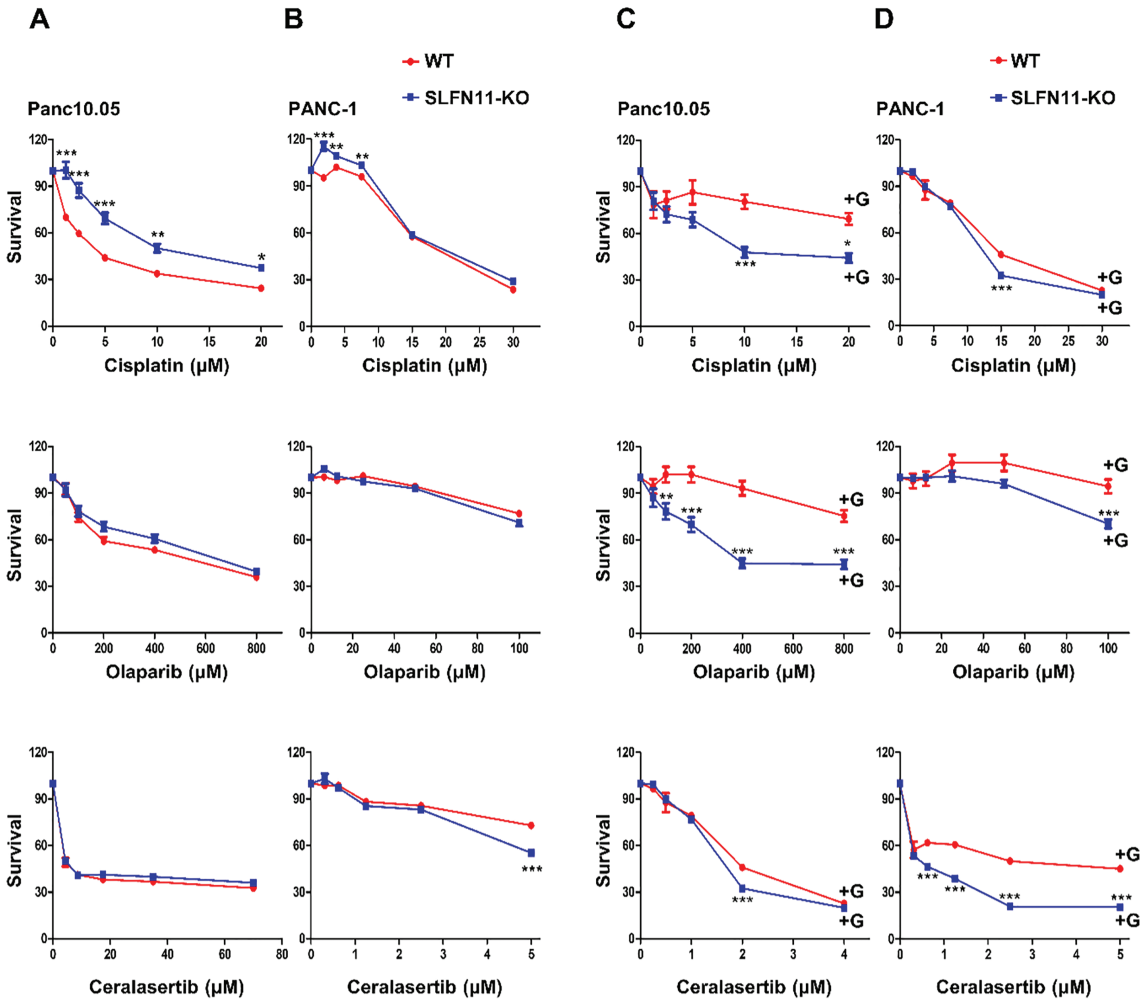

Figure 5: (Continued)

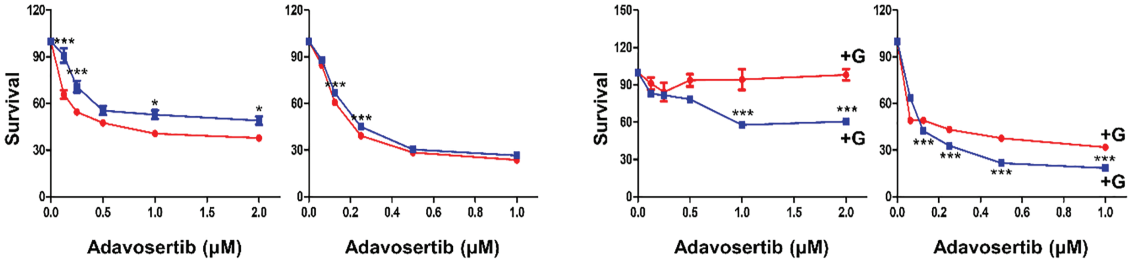

Figure 5: Resistance to gemcitabine obtained in *SLFN11*-knockdown PC cells is reversed when combined with cisplatin and DDR inhibitors. (A, B) The effects of cisplatin, olaparib, ceralasertib, and adavosertib treatment in Panc10.05 and PANC-1 cells were measured using the CCK-8 assay at 48 h. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (C, D) The effects of combination treatment with gemcitabine (+G) and cisplatin, olaparib, ceralasertib, or adavosertib in Panc10.05 (gemcitabine concentration: 0.025 μM) and PANC-1 (gemcitabine concentration: 1 μM) were analyzed using the CCK-8 assay at 48 h. Curves were generated from biological triplicates, with values represented as means ± SEMs. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

4 Discussion

The present study investigated the role of *SLFN11* in PC and its relationship with anticancer drug response. Our database analyses indicate that elevated levels of SLFN11 expression are significantly associated with poor survival outcomes, suggesting its potential as a prognostic marker in PC. In contrast, our functional data reveal that *SLFN11*-proficient cells display increased sensitivity to gemcitabine, supporting a predictive role in chemotherapy response. We observed that *SLFN11* expression varied among PC tissues and cell lines, suggesting potential heterogeneity in its biological function. While *SLFN11* is recognized for its role in cell cycle regulation and apoptosis, its prognostic significance in PC remains unclear. Notably, the discrepancy between the poor survival associated with elevated levels of SLFN11 expression and the enhanced gemcitabine sensitivity observed in functional assays implies that *SLFN11* may have distinct roles in tumor progression vs. therapy response. Furthermore, gemcitabine resistance was observed in *SLFN11*-KO PC cells, while combination treatments with cisplatin and DDR agents improved drug sensitivity. This observation suggests that high *SLFN11* expression, despite being linked to an aggressive tumor phenotype, may predict a favorable chemotherapy response. Additionally, the induction of apoptosis following *SLFN11* knockdown indicates a disruption in normal cell cycle progression; this paradoxical finding might reflect compensatory mechanisms that contribute to the overall poor prognosis observed in high *SLFN11*-expressing tumors. While these results suggest a possible function for *SLFN11* in modulating both tumor aggressiveness and drug response, further studies are needed to delineate these distinct aspects and validate their clinical relevance in PC.

According to previous studies in humans, a high expression of *SLFN* family members (*SLFN5*, *SLFN11*, *SLFN12*, *SLFN13*, and *SLFN14*) has been observed in renal cell carcinoma, gastric cancer, and PC [14,42]. In gastric cancer, elevated *SLFN* family expression has been linked to disease progression, such as stage of the tumor, histological categorization, and lymphatic metastasis. Notably, the upregulation of *SLFN5* and *SLFN13* correlates with poor prognosis [42]. According to a representative study of *SLFN* family members, the knockout of *SLFN5* in PC cells significantly reduced cell viability. *SLFN5* was also shown to participate in cell cycle progression by binding to E2F7 [29]. *SLFN11* shows elevated expression in certain cancers, where it has been linked to tumor stage, histological grade, and metastasis. Additionally, it has been shown to inhibit the proliferation of cancer cells [27,42]. Although numerous studies have explored the effects and mechanisms of specific genes with elevated expression in PC [38,43–46] the function of *SLFN11* in PC remains largely uncharacterized. In our research, we analyzed the function of *SLFN11* in PC and found that its elevated expression levels were associated with higher disease stages and poor patient prognosis in patients.

In addition, when the DTB experiment was performed on *SLFN11*-knockdown PC cells, the G1 phase did not proceed normally. Moreover, the number of cells within the cell population in the apoptotic sub-G1 phase increased relative to the control group. Our findings indicate that elevated *SLFN11* expression correlates with unfavorable patient prognosis and suggests a crucial involvement of *SLFN11* in regulating PC cell cycle progression and programmed cell death.

The influence of *SLFN11* on various cancer types has been studied, including gastric cancer [21,27,42], colorectal cancer [19,47] bladder cancer [48], lung cancer [23,49–51], liver cancer [52,53], prostate cancer [20], breast cancer [17,54], and ovarian cancer [55]. It has been described that the *SLFN11* expression in colorectal and gastric cancer is controlled by methylation and suppression of cancer cell growth and is related to cisplatin sensitivity [27,56]. Resistance of *SLFN11* knockout cells to platinum-based anticancer drugs including cisplatin has been described in bladder, prostate, and ovarian cancers [20,48,55]. Despite investigations into the role of *SLFN11* in various cancer types, studies on its relationship with drug sensitivity in PC are limited.

A recent analysis of drug sensitivity data from GDSC and AstraZeneca revealed that *SLFN11* mRNA expression across 738 tumor-derived cell lines was associated with responses to anticancer monotherapies targeting approximately 589 compounds, including DDAs and DDR inhibitors [57]. Similar to previous studies, our result analyzed four publicly available datasets: NCI60, CTRP, GDSC, and CCLE. *SLFN11* expression in various cancer types was closely correlated with sensitivity to drugs such as gemcitabine, cisplatin, and ATR inhibitors. Furthermore, in this study, variations in *SLFN11* expression were observed in PC cell lines, and their responses to gemcitabine, cisplatin, and DDR inhibitors differed. This suggests that factors beyond *SLFN11* expression may influence drug response. While our results demonstrate a statistically significant association between *SLFN11* levels and gemcitabine responsiveness, additional research is required to clarify the interplay between *SLFN11* and these potential regulatory mechanisms. On the other hand, contrary to our findings, in head and neck squamous cell carcinoma (HNSCC), the *SLFN11*-positive group revealed better overall survival than the *SLFN11*-negative group; however, cisplatin sensitivity was reduced in *SLFN11* knockout HNSCC cells [58]. Our findings contrast with those of Fischietti et al. [29], who found no meaningful association between *SLFN11* levels and patient overall survival in PC. This discrepancy may be owing to differences in datasets, patient cohorts, or analytical approaches [59,60]. Further validation using independent cohorts is necessary to clarify this relationship. Additionally, the association between high *SLFN11* expression and poor survival, despite increased chemosensitivity, may result from factors such as tumor aggressiveness or interactions with the tumor microenvironment [23,61]. Additional research is required to clarify these mechanisms. Our results imply that SLFN11 may serve as a predictive biomarker for drug sensitivity, despite its variable expression across different cancer types.

SLFN11 was strongly connected with the response to DDAs in upper gastrointestinal and genitourinary malignancies, whereas the correlation was significantly weaker with Wee1 inhibitors or DDR inhibitors such as olaparib [57]. Similar to our results, *SLFN11*-KO PC cells developed drug resistance after treatment with gemcitabine and cisplatin alone. However, only marginal effects were observed for olaparib, ceralasertib, and adavosertib. The increased sensitivity of *SLFN11*-proficient cells to cisplatin and ceralasertib suggests that *SLFN11* plays a pivotal role in modulating DNA damage responses. While the precise mechanisms remain under investigation, previous studies have proposed two primary pathways through which *SLFN11* influences drug sensitivity: (1) translation inhibition via tRNA cleavage and (2) replication stalling. *SLFN11* has been shown to cleave specific tRNAs, leading to global translational suppression and increased susceptibility to DNA-damaging agents [62]. Additionally, *SLFN11* promotes replication fork stalling and collapse, further enhancing cytotoxicity in response to chemotherapeutic agents that induce replication stress [61]. These mechanisms may underlie the differential drug responses observed in this study. In addition, sensitivity to

PARP inhibitors and expression of *SLFN11* are highly connected; specifically, *SLFN11* inactivation confers resistance to PARP inhibitors, which is overcome by ATR inhibition [25]. In addition, the loss of *SLFN11* leads to resistance to DDAs, which can be counteracted by inhibitors targeting ATR, Wee1, and Chk pathways; these effect was validated in PC cells [57]. Importantly, gemcitabine-induced drug resistance in *SLFN11*-KO PC cells was enhanced by cisplatin and DDR inhibitors. Our results indicate that *SLFN11* has potential as a predictive marker for assessing response to DDR-targeted therapies in PC; however, further validation is required for clinical application. The variability observed in *SLFN11* expression and drug response underscores the need for multi-omic approaches and patient-derived models to accurately assess its role in chemotherapy sensitivity. Future studies integrating *SLFN11* expression analysis with functional assays and clinical outcomes will be critical to determining its utility in guiding personalized treatment strategies for PC patients. Furthermore, the relationship between *SLFN11* expression and cancer responsiveness to DDAs should be thoroughly examined through clinical trials, and the underlying mechanisms by which *SLFN11* influences DDA sensitivity require further investigation in additional studies.

Our study elucidates the relationship between *SLFN11* expression and PC, highlighting its potential role in modulating sensitivity to DDAs and DDR inhibitors. These findings provide evidence that may help in the establishment of strategies to overcome chemoresistance in PC. Furthermore, this study demonstrates the role of *SLFN11* in PC cells and its possible contribution to drug sensitivity. Ongoing experiments aim to further investigate the function of *SLFN11* in PC, while its role in animal models and clinical settings remains to be fully established.

5 Conclusion

To conclude, our research elucidates the function of *SLFN11* in PC and its potential impact on drug sensitivity. We demonstrated that SLFN11 expression is associated with PC advancement and adverse overall survival, indicating its value as a predictive biomarker. Moreover, its loss contributes to gemcitabine resistance, supporting its role as a marker for predicting chemotherapy response, particularly for DDAs and DDR inhibitors. These findings, although seemingly conflicting, highlight the dual nature of *SLFN11* in modulating both tumor aggressiveness and treatment sensitivity. However, further investigations are required to establish its clinical utility and to explore the underlying mechanisms through which SLFN11 regulates drug sensitivity. Subsequent research should concentrate on validating these findings in independent cohorts, employing patient-derived models, and conducting clinical trials to resolve this ambiguity and firmly establish *SLFN11* as a clinical biomarker for both prognosis and drug response in PC.

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Availability of Data and Materials: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

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