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Research article

Tumor-derived exosome PPP3CB induce gemcitabine resistance by regulating miR-298/STAT3 in pancreatic cancer

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ABSTRACT

Purpose: Due to resistance to gemcitabine (GEM), patients with pancreatic cancer (PC) usually have poor prognosis and low survival rate. The purpose of our research was to explore the impact of exosome PPP3CB on GEM resistance in PC, and concurrently analyze the regulatory role of the miR-298/STAT3 signaling pathway.

Methods: Exosomes isolated from PC cells were verified by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA) and western blotting (WB). The interaction between PPP3CB and miR-298 was verified using dual-luciferase reporter gene assay, followed by evaluation of cell growth and death using CCK8 assay, EdU staining, and flow cytometry.

Results: Increased PPP3CB expression was observed in GEM-resistant PC cells. Exosomes from PC cells and GEM-resistant PC cells were successfully extracted by ultra-high speed centrifugation. Confocal microscopy showed internalization of fluorescein amide (FAM)-labeled GEM-resistant exosomes by PC cells. PPP3CB enhanced the proliferation of GEM-resistant PC cells and inhibited their apoptosis, whereas down-regulation of PPP3CB promoted the death of PC cells and inhibited the proliferation of GEM-resistant PC cells, and enhance the susceptibility of PC cells to GEM. Additionally, PPP3CB positively regulated STAT3 expression in PC cells by down-regulating miR-298, thus promoting the growth and inhibiting the death of PC cells.

Conclusion: PC cell-derived exosome PPP3CB enhances STAT3 expression by downregulating miR-298, stimulating cell growth, and suppressing cell death, thereby increasing the resistance of PC cells to GEM.

1. Introduction

Pancreatic cancer (PC) is fourth common causes of cancer death worldwide. Despite ongoing advancements in its treatment modalities including chemotherapy, radiotherapy and molecular biology technology, the 5-year survival rate is only 12 %. Moreover, there has been a global annual rise in the number of both confirmed cases and deaths of PC [1,2]. Its persistently high death rates can be imputed to factors such as high incidence, delayed diagnosis, early metastasis, and chemotherapy resistance during treatment, etc. Encouragingly, the combination of surgical resection and chemotherapy has shown promise in enhancing the 5-year survival rate of PC patients [3,4]. Although gemcitabine (GEM) serves as the primary chemotherapy drug for PC, and has shown significant efficacy in

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improving the prognosis of advanced PC, the occurrence of chemotherapy resistance still leads to a poor prognosis for PC patients [5,6]. This resistance is believed to arise from a complex interplay of multiple factors involving the tumor microenvironment, autophagy, cancer cells, and RNA [7,8].

Recent researches have emphasized the role of intercellular communication in the tumor microenvironment in cancer chemotherapy resistance [9,10]. Tumor-associated M2 macrophages promote chemotherapy resistance in PC by inducing epithelial-mesenchymal transition (EMT) [11]. High expression of IL1β in tumor-associated stroma is associated with poor survival of PC, with IL1β activating IRAK4 promotes sensitivity of PC to GEM [12]. Tumor-derived exosomes contain DNA, mRNA and proteins, etc., all of which play important regulatory roles in cell communication related to tumor development and chemotherapy resistance, and can be used as the grading basis and prognostic index of tumor patients [13–15]. Exosomes from chemoresistant PC cells express high levels of EphA2, increasing PC cell susceptibility to GEM [16]. Exosomal AFAP1-AS1 enhances trastuzumab susceptibility in breast cancer by inducing ERBB2 translation [17]. Furthermore, the stimulation of the β-catenin pathway by exosome H19 through sponge miR-141 aids chemotherapy susceptibility in colorectal cancer cells [18]. These findings collectively demonstrate exosomes' significant role in chemotherapy resistance in cancer.

Although the important role of exosomes in cancer chemotherapy resistance has been confirmed, their mechanism of action remain very complex and has not been fully elucidated. Therefore, by constructing GEM-resistant PC cell lines, this research aimed to probe the impact of exosomes on GEM resistance in PC and analyze the specific regulatory mechanism involved, providing experimental support for improving the therapeutic outcomes of GEM-resistant patients with PC.

2. Materials and methodology

2.1. Experimental materials

GEM (#1288463), RNase A (#10109142001), and Triton™ X-100 (#9036-19-5) were from Sigma; Antibodies CD63 (ab68418), CD81 (ab79559), Bcl-2 (ab32124), Caspase-3 (ab32351), Bax (ab32503), Cleared Caspase-3 (ab2302), and GAPDH (ab8245) all originate from Abcam; Cell Counting Kit-8 (#C0037), BeyoClick™ EdU-488 (C0071S), and Annexin V-FITC (#C1062S) were from Beyotime Biotechnology; Trizol (15596026CN) was from Thermo Fisher Scientific; Evo M-MLV RT Premix for qPCR (AG11706) was from Shandong Ke Ai Rui Biotechnology Co., Ltd; miRNA First-strand cDNA synthesis kit (B532453-0020) was from Sangon Biotech (Shanghai) Co., Ltd; miR-298 inhibitor (UGGGAGAACCUCCCUGCUUCUGCU)/NC (CAGUACUUUUGUGUAGUACAA) was synthesized by Gemma gene.

2.2. Cell culture

Human PC cell lines PANC-1 and SW1990 are from the Cell Bank of the Chinese Academy of Sciences. GEM-resistant PC cells, PANC-1/GEM and SW1990/GEM, were established by exposing PANC-1 and SW1990 cells to gradient concentrations of GEM [19]. PANC-1 or SW1990 cells were initially exposed to 5 μM GEM, with concentrations little by little increased (5, 10, 20, 40, 80, 160, and 320 μM) every fortnight until the cells became tolerant to 320 μM GEM. PANC-1, SW1990, PANC-1/GEM and SW1990/GEM cells were cultured using DMEM medium and placed in a thermostatic incubator at 37 °C with 5 % CO_2.

2.3. Cell transfection

For PPP3CB silencing, cells were inoculated in 6-well plates. The serum-free medium containing si-PPP3CB/si-NC was replaced when the cells are not fully covered, and the complete medium was displaced 6 h after transfection. For PPP3CB overexpression, when the cells are not fully covered, plasmid containing PPP3CB/Vector was added and replaced with complete medium after transfection for 12 h. For miR-298 silencing, cells were inoculated in 6-well plates. The serum-free medium containing si-PPP3CB/si-NC was replaced once the cells are not fully covered. After transfection for 6 h, the serum-free medium containing miR-298 inhibitor/miR-NC was replaced, and after continuous culture for 6 h, the complete medium was displaced.

2.4. CCK-8 assay

Inoculate the grouped cells onto a 96 well plate, cell viability was detected by the CCK-8 method as per the kit instructions. Cells were cultivated in DMEM containing 10 % CCK-8 solution for 2 h, followed by measurement of the intensity of absorption at 450 nm using a Microplate spectrophotometer. The IC50 value was calculated using the SPSS statistical program.

2.5. EdU staining

PC cells were inoculated in 6-well culture plates at a concentration of 5×10^4 cells/well, followed by incubation with EdU for 3 h. Following fixation with 4 % paraformaldehyde for 15 min, cells were stained with Hoechst for 10 min. The well plates were scanned and pictures taken with a microscope.

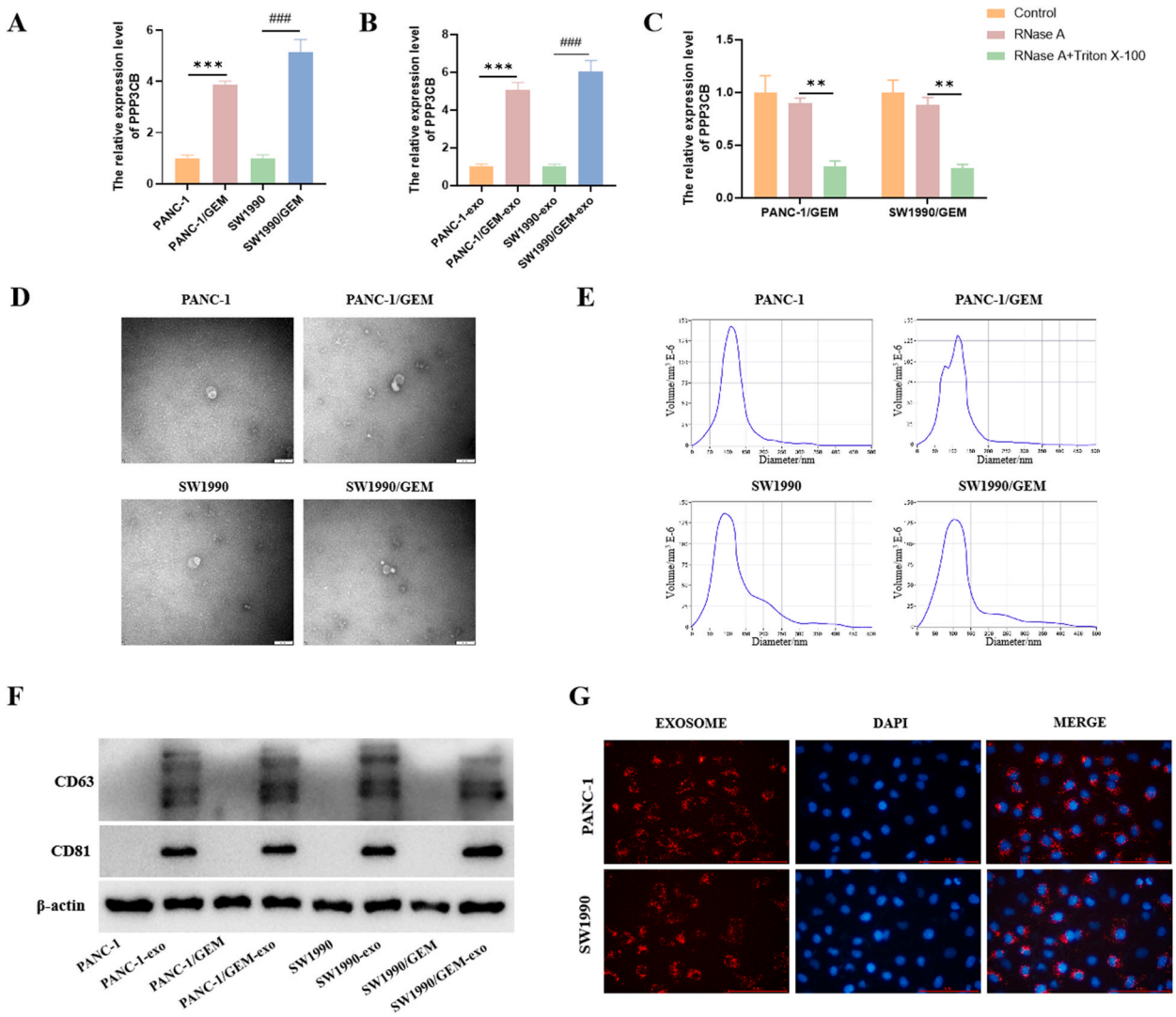

Fig. 1. PPP3CB level is elevated in GEM-treated PC cells. A: The expression levels of PPP3CB in GEM-sensitive cell lines (PANC-1 and SW1990) and GEM-resistant cell lines (PANC-1/GEM and SW1990/GEM) detected by qRT-PCR; B: The expression levels of PPP3CB in exosomes derived from GEM-sensitive cell lines and GEM-resistant cell lines determined by qRT-PCR; C: qRT-PCR detection of PPP3CB levels in GEM-resistant cell lines treated with the conjunction of RNase and Triton X-100; D: The morphology of exosomes derived from GEM-sensitive cell linesand GEM-resistant cell lines under a transmission electron microscope; E: NTA of exosome particle size in GEM-sensitive cell lines and GEM-resistant cell lines; F: The protein expression levels of exosome markers in GEM-sensitive cell lines and GEM-resistant cell lines detected by Western blot (The original image is provided in the Supplementary file); G: Exosome internalization detected by confocal microscopy.

2.6. Detection of apoptosis by flow cytometry

Cells (1×10^6 cells/well) were cultured in culture dishes with a diameter of 6 cm, incubated with Annexin V-FITC, and thereafter dyed with propidium iodide (PI). Finally, flow cytometry was used to analyze cell apoptosis.

2.7. Isolation and identification of PC cell-derived exosomes

The culture medium containing PC cells was gathered and exosomes were isolated by ultrahigh-speed centrifugation. Exosomes were identified using western blotting to measure surface markers (CD63 and CD81). Subsequently, the exosomes were observed using Transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA).

2.8. Western blot analysis

Total protein was collected from PC cells using RIPA lysis solution, followed by quantification using the BCA protein quantification Kit. 60 μg of protein were segregated by SDS-PAGE and then shifted to a PVDF membrane. 5 % skimmed milk was used for blocking. Then incubated with 1000 fold diluted primary antibodies CD63, CD81, Caspase3, Bax, Cleaved Caspase-3, Bcl2, and GAPDH at 4 °C for 15 h, then incubate the secondary antibody at room temperature for 2 h. The immunoblots were ultimately viewed using an enhanced chemiluminescence kit.

2.9. Starbase analysis

In the Starbase database, mRNA-miRNA interactions involving PPP3CB are selected. Sieve severity and tumor type were specified, and results were displayed by selecting "Targetsite". Finally, miRNA interacting with PPP3CB were shown by selecting "Cancer Num".

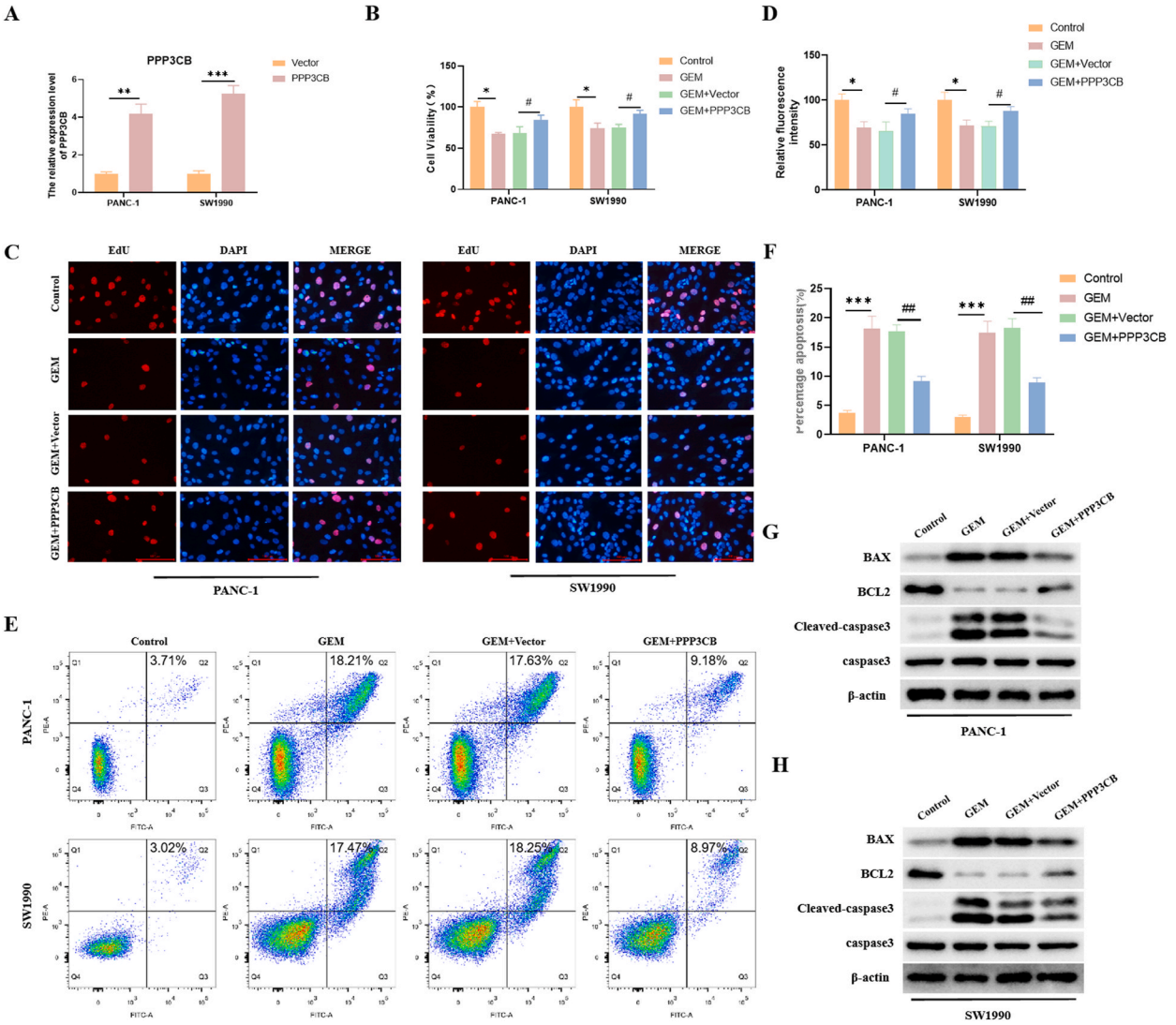

Fig. 2. PPP3CBenhances the resistance of PC cells to GEM. A: qRT-PCR assessment of PPP3CB level in ANC-1/SW1990 cells treated with PPP3CB overexpression plasmid or empty vector; B: Cell viability gauged by CCK8 assay; C–D: Cell proliferation gauged by EDU staining after PPP3B overexpression; E–F: Cell apoptosis gauged by flow cytometry after PPP3B overexpression; G: The expression of apoptosis proteins in GEM-treated PANC-1 cells detected by Western blot after PPP3B overexpression (The original image is provided in the Supplementary file); H: The expression of apoptosis proteins in GEM-treated SW1990 cells detected by Western blot after PPP3B overexpression (The original image is provided in the Supplementary file).

2.10. qRT-PCR analysis

Cell RNA was collected using Trizol. Evo M-MLV RT Premix for qPCR kit was used for reverse transcription into cDNA, and miRNA reverse transcription using the first-strand cDNA synthesis kit (stem ring method). The ExiLENT SYBR Green Master Mix Kit was used for detecting miR-298 expression, whereas Roche Master Mix containing SYBR Green was employed for detecting mRNA expression. U6 and GAPDH serve as internal references for miR-298 and mRNA, separately. The primer sequences of PPP3CB, STAT3, miR-298, U6 and GAPDH are as follows: miR-298 forward primer: TCAGGTCTTCAGCAGAAGC, miR-298 reverse primer: TAGTTCCTCA-CAGTCAAGG; U6 forward primer: ATACAGAGAAAGTTAGCACGG, U6 reverse primer: GGAATGCTTCAAAGAGTTGTG; PPP3CB forward primer: GAGGCTGAGGCAGGAGAATGG, PPP3CB reverse primer: CGGAGTCTCGCTCTGTCACC; STAT3 forward primer: CGCACTTTAGATTCATTGATGC, STAT3 reverse primer: AGGTGAGGGACTCAAACTG; GAPDH forward primer: TCAA-GATCATCAGCAATGCC, GAPDH reverse primer: CGATACCAAAGTTGTCATGGA. Relative expression levels of miRNA and mRNA were determined using the $2^{-\Delta\Delta Ct}$ technique.

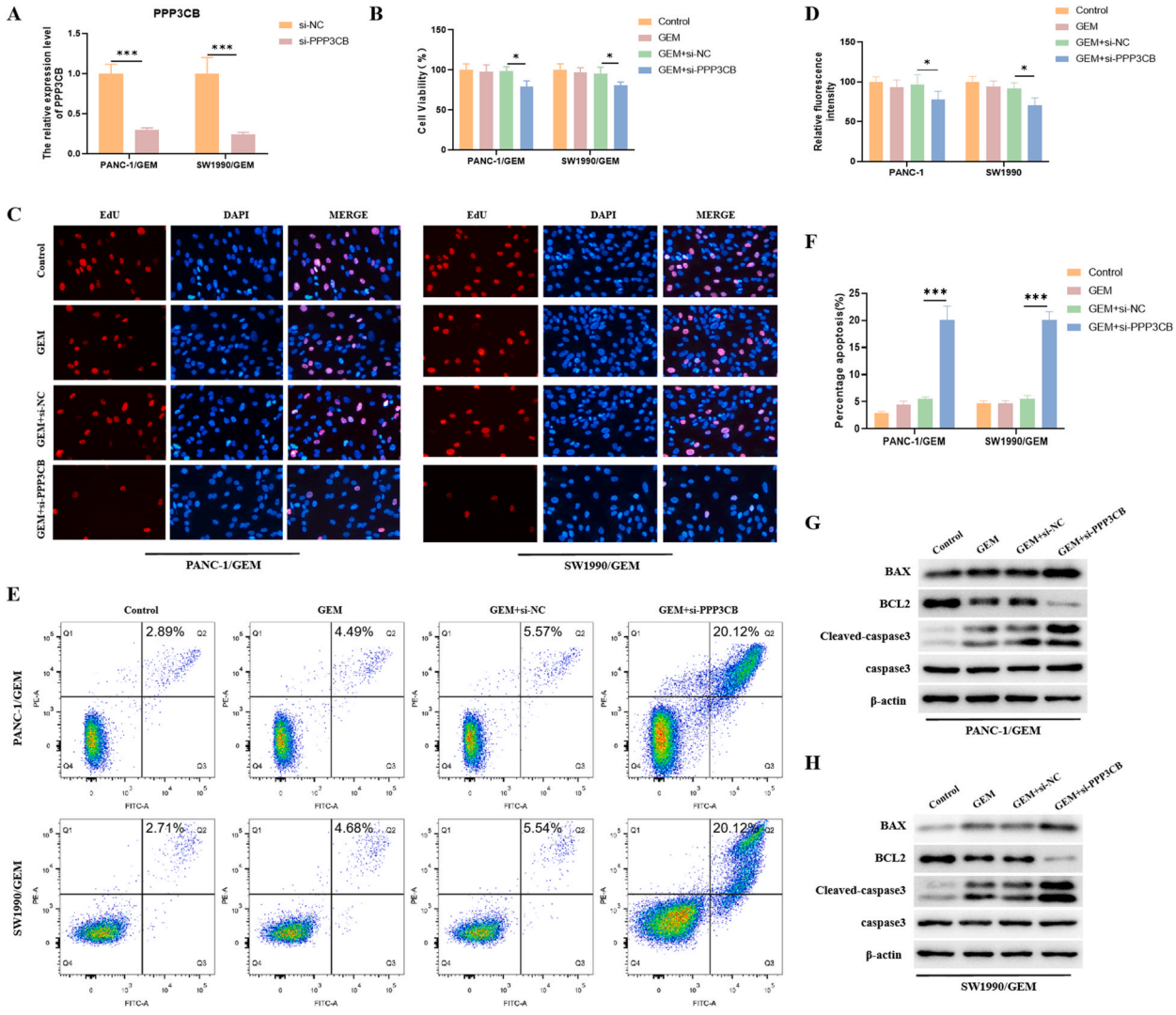

Fig. 3. Down-regulation of PPP3CB strengthens the susceptibility of GEM-resistant PC cells to GEM. A: Detection of PPP3CB level in PANC-1/GEM or SW1990/GEM cells transfected with siPPP3CB or control via qRT-PCR; B: Assessment of cell viability via CCK8 assay; C–D: Evaluation of cell proliferation via EdU staining after PPP3CB knockdown; E–F: Detection of apoptosis via flow cytometry after PPP3CB knockdown; G: Detection of the expression level of apoptosis proteins in PANC-1/GEM cells via Western blot after PPP3CB knockdown (The original image is provided in the Supplementary file); H: Detection of the expression level of apoptosis proteins in SW1990/GEM cells via Western blot after PPP3CB knockdown (The original image is provided in the Supplementary file).

2.11. Dual-luciferase reporter gene detection

PC cells were co-transfected with 100 nmol/L miR-298 mimic or NC-miRNA, 100 ng luciferase reporter plasmid (wild-type PPP3CB (WT-PPP3CB: GCTGACTGTAAATTTCACAGTCTCTCTGAAGAAACCATTGTGCTTCTGAGACCCTAGCCCCCTTCCTGGATGGAG) or mutant PPP3CB (MT-PPP3CB:GCTGACTGTAAATTTCACAGTCTCTCTCATCATTGGAAAGACGAAGACAGACCCTAGCCCCCTTCCTGGATGGAG), 10 ng pRLCMV Renilla fluorescence Primer enzyme reporter plasmid using Lip3000 reagent for 48 h. Luciferase activity was detected by the Lucifer Reporter Analysis System.

2.12. Statistical evaluation

The data are expressed as mean ± standard deviation (M ± SD) of three replicates. Statistical analysis was conducted using t-tests (between two groups) and one-way ANOVA (between three or more groups). SPSS 18.0 software was used for analysis, with a conspicuousness of $P < 0.05$ applied.

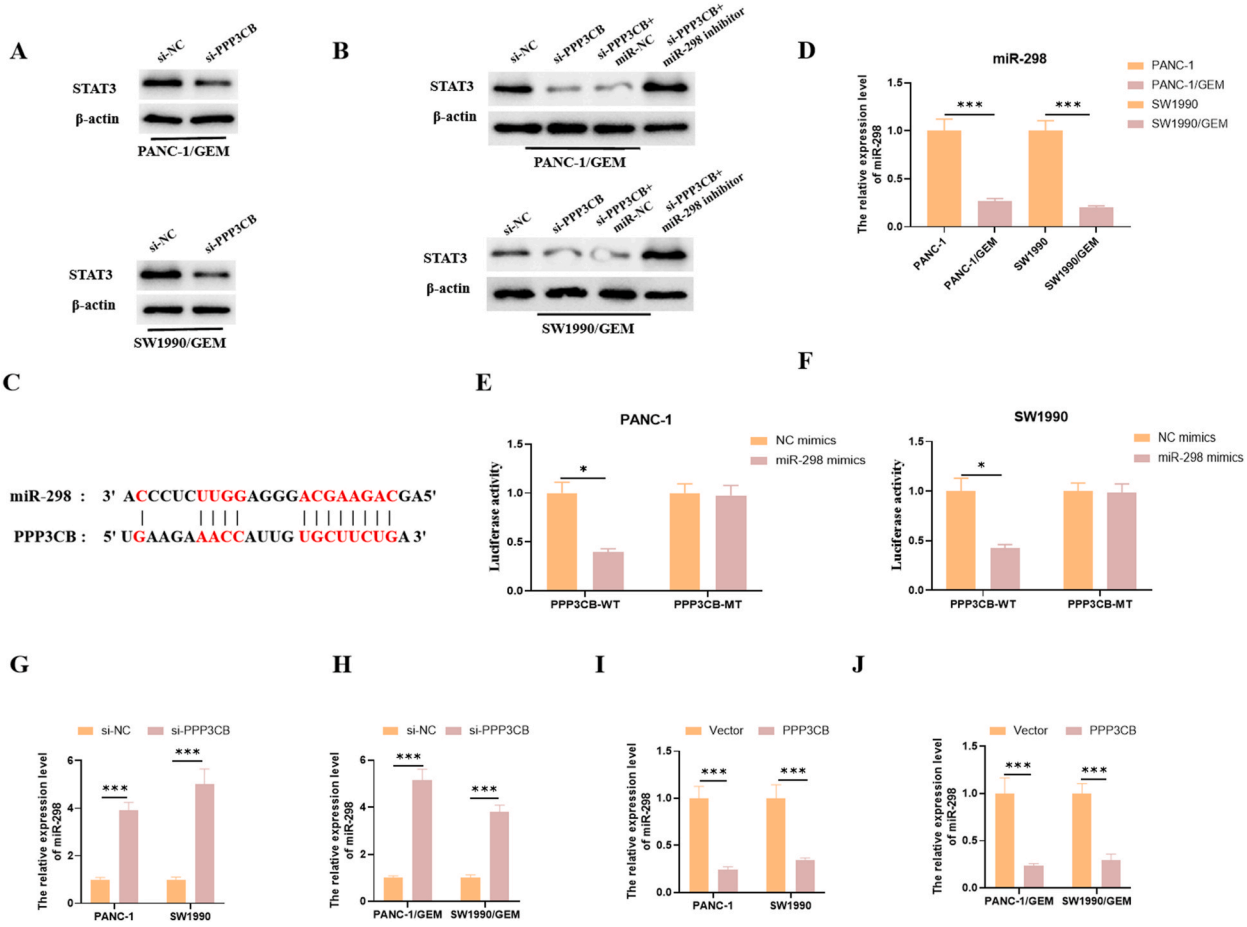

Fig. 4. PPP3CB regulates STAT3 expression by sponge adsorption of miR-298. A: Detection of the expression of STAT3 protein by Western blot after transfection of si-PPP3CB/si-NC into PANC-1/GEM or SW1990/GEM cells for 48h (The original image is provided in the Supplementary file); B: Detection of the expression of STAT3 protein by Western blot after PANC-1/GEM or SW1990/GEM cells were co-transfected with miR-298 inhibitor and si-PPP3CB (The original image is provided in the Supplementary file); C: Binding sequence of miR-298 and PPP3CB; D: qRT-PCR detection of the level of miR-298 in GEM-resistant PC cells; E: Determination of the enzyme activity by luciferase reporter gene assay after co-transfecting PANC-1 cells with WT-PPP3CB or MT-PPP3CB reporter plasmids and NC mimics or miR-298 mimics respectively for 48 h; F: Assessment of enzyme activity by luciferase reporter gene assay after co-transfecting SW1990 cells with WT-PPP3CB or MT-PPP3CB reporter plasmids and NC mimics or miR-298 mimics respectively for 48 h; G: Detection of the level of miR-298 by qRT-PCR after co-transfecting PANC-1/SW1990 cells with siPPP3CB for 48 h; H: Detection of the level of miR-298 by qRT-PCR after co-transfecting PANC-1/GEMH and SW1990/GEM cells with siPPP3CB for 48 h; I: Detection of the level of miR-298 by qRT-PCR after PPP3CB overexpression in PANC-1/SW1990 cells for 48 h; J: Detection of the level of miR-298 by qRT-PCR after PPP3CB overexpression in PANC-1/GEM and SW1990/GEM cells for 48 h.

3. Results

3.1. PPP3CB level is elevated in GEM-resistant PC cells

Through qRT-PCR assay for the detection of PPP3CB level in GEM-sensitive cell lines, GEM-resistant cell lines, exosomes derived from GEM-sensitive cell lines, and exosomed derived from GEM-resistant cell lines, it was revealed that GEM-resistant cells had higher levels of PPP3CB (Fig. 1A), and exosomes derived from GEM-resistant cell lines had higher levels of relative PPP3CB expression (Fig. 1B). To eliminate interference from non-specific sediments, GEM-resistant cells were deal with RNase A along or in conjunction with Triton X-100. qRT-PCR assay demonstrated decreased PPP3CB levels in GEM-resistant cells treated with RNase A and Triton X-100 compared to those treated with RNase alone (Fig. 1C), confirming that PPP3CB was encapsulated by exosomes. PC cells and GEM-resistant PC cell-derived exosomes were successfully extracted via ultrahigh-speed centrifugation, with an obvious lipid bilayer membrane observed under a transmission electron microscope (Fig. 1D), whereas NTA showed a particle size of approximately 100 nm

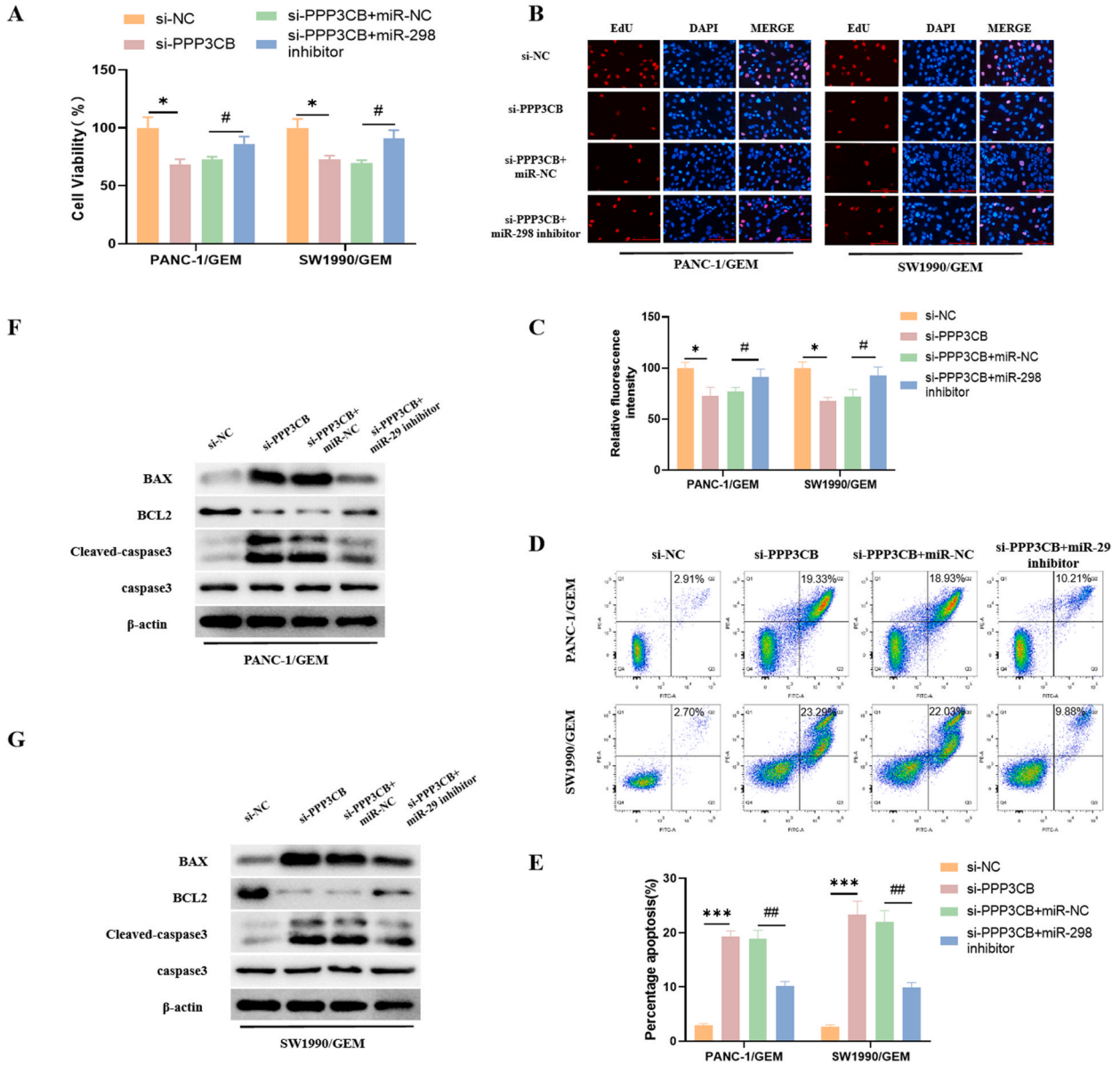

Fig. 5. PPP3CB regulates GEM resistance in PC through miR-298/STAT3. A: Cell viability gauged by CCK8 assay; B–C: Cell proliferation evaluated by EdU dyeing; D–E: Cell apoptosis detected by flow cytometry; F: The expression levels of apoptosis proteins in PANC-1/GEM cells detected by Western blot (The original image is provided in the Supplementary file); G: The expression levels of apoptosis proteins in SW1990/GEM cells detected by Western blot (The original image is provided in the Supplementary file).

in PC cells and GEM-resistant PC cell-derived exosomes (Fig. 1E). Furthermore, Western blot analysis showed expression of exosome-labeled proteins CD63 and CD81 exclusively in exosomes derived from PC cells and GEM-resistant PC cells (Fig. 1F). Co-transfection of PC cells with fluorescein amide (FAM)-labeled PPP3CB and exosome marker CD63 revealed exosome internalization by PC cells under confocal microscopy (Fig. 1G).

3.2. PPP3CB enhances the resistance of PC cells to GEM

To investigate PPP3CB's role in GEM resistance in PC, we successfully constructed an overexpressed PPP3CB plasmid and its corresponding empty vector. Upon transfection of GEM-treated PANC-1/SW1990 cells with the constructed PPP3CB overexpression plasmid, PPP3CB levels were significantly increased (Fig. 2A). CCK-8 assay revealed increased viability of GEM-treated PANC-1/SW1990 cells following PPP3CB overexpression (Fig. 2B). EdU staining further demonstrated enhanced proliferation of GEM-treated PANC-1/SW1990 cells after PPP3CB overexpression (Fig. 2C–D). Moreover, flow cytometry analysis indicated a prominent reduce in the apoptosis rate of GEM-treated PANC-1/SW1990 cells after PPP3CB overexpression (Fig. 2E–F). Western blot analysis revealed raised expression of BCL2, and reduced expression of BAX and cleaved caspase3 following PPP3CB overexpression (Fig. 2G–H). These results indicated that PPP3CB enhances the resistance of PC cells to GEM.

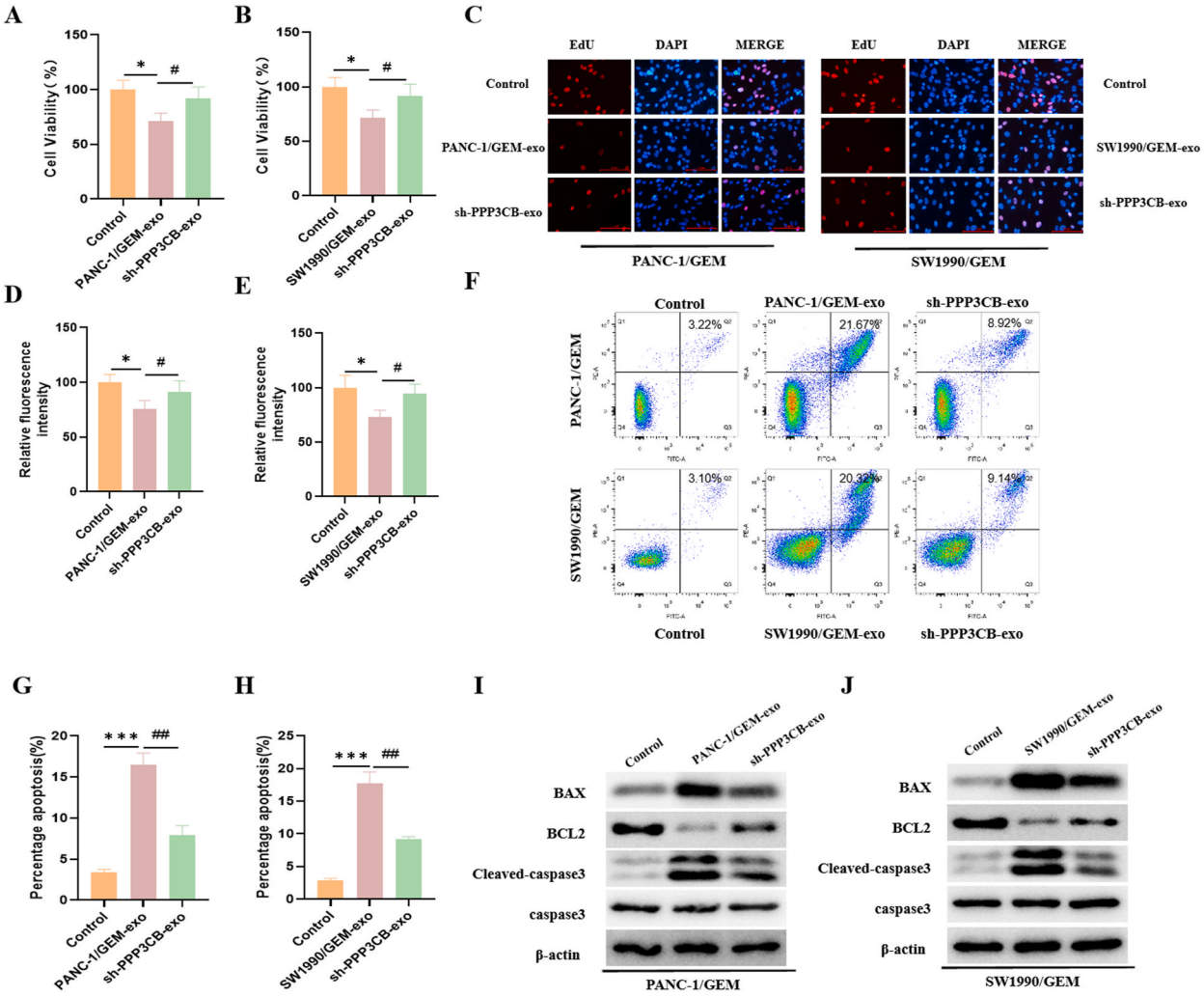

Fig. 6. Knockdown of exosome PPP3CB strengthens the susceptibility of GEM-resistant PC cells to GEM. A: Cell viability (PANC-1/GEM) gauged by CCK8 assay; B: Cell viability (SW1990/GEM) gauged by CCK8 assay; C–E: Cell proliferation evaluated by EdU dyeing; F–H: Cell apoptosis gauged by flow cytometry; I: The expression levels of apoptosis proteins in PANC-1/GEM cells detected by Western blot (The original image is provided in the Supplementary file); J: The expression levels of apoptosis proteins in SW1990/GEM cells detected by Western blot (The original image is provided in the Supplementary file).

3.3. Knockdown of PPP3CB strengthens the susceptibility of GEM-resistant cells to GEM

In this study, siPPP3CB and its corresponding control were successfully constructed, and a reduction was observed in the level of PPP3CB after transfection of siPPP3CB into PANC-1/GEM or SW1990/GEM cells (Fig. 3A). Interestingly, compared with the Control group, no remarkable changes were observed in cell viability, proliferation and apoptosis in the GEM group (Fig. 3B–F), indicating that PANC-1/GEM and SW1990/GEM resistant cell lines exhibited enhanced resistance to GEM. However, knocking down PPP3CB in PANC-1/GEM or SW1990/GEM cells resulted in a decrease in cell viability(Fig. 3B). EdU staining results showed inhibited cell proliferation after siPPP3CB was transfected into PANC-1/GEM or SW1990/GEM cells (Fig. 3C–D). Flow cytometry results revealed a remarkable raise in cell apoptosis after siPPP3CB was transfected into PANC-1/GEM or SW1990/GEM cells(Fig. 3E–F), while Western blot analyses suggested a decrease in BCL2 expression and a steady raise in the expression of BAX and Cleaved-caspase3 (Fig. 3G–H). The aforementioned findings suggest that knocking down PPP3CB strengthens the susceptibility of GEM-resistant PC cells to GEM.

3.4. PPP3CB regulates STAT3 expression by sponge adsorption of miR-298

STAT3 has once been reported as the target gene of miR-298 [20]. To determine whether PPP3CB regulates STAT3 expression through sponge adsorption of miR-298, PPP3CB was knocked down in PC cells, resulting in reduced STAT3 expression (Fig. 4A). To investigate whether miR-298 plays an significant role in the relationship between PPP3CB and STAT3, PANC-1/GEM or SW1990/GEM cells were co-transfected with miR-298 inhibitor and si-PPP3CB. It was revealed that miR-298 inhibitor remarkably returned the inhibitory action of siPPP3CB on STAT3 protein (Fig. 4B), indicating that PPP3CB's sponge adsorption of miR-298 regulates STAT3 expression. The bioinformatics database, Starbase, was used to predict miRNAs bound by PPP3CB, revealing a binding sequence between miR-298 and PPP3CB (Fig. 4C). In GEM-resistant PC cells, miR-298 level was significantly reduced (Fig. 4D). However, after co-transfection of miR-298 mimics with WT-PPP3CB or MT-PPP3CB into PC cells, the luciferase activity of WT-PPP3CB was considerably reduced by the miR-298 mimics, while the miR-298 binding site mutation plasmid MT-PPP3CB showed no effect on luciferase enzyme activity (Fig. 4E–F). Furthermore, knockdown of PPP3CB significantly increased the level of miR-298 (Fig. 4G–H), while overexpression of PPP3CB visibly reduced the level of miR-298 (Fig. 4I–J). These results prompt that PPP3CB down-regulates the level of miR-298 through sponge adsorption, thereby promoting STAT3 expression.

3.5. PPP3CB regulates GEM resistance in PC through miR-298/STAT3

The miR-298 inhibitor returned the inhibitory action of si-PPP3CB on the viability and growth of PANC-1/GEM and SW1990/GEM cells, as shown by CCK-8 and EdU staining (Fig. 5A–C). In addition, flow cytometry data illustrated that the miR-298 inhibitor returned the auxo-action of si-PPP3CB on the death of these cells (Fig. 5D–E), whereas Western blot displayed that miR-298 reversed the inhibitory effect of siPPP3CB on BCL2 protein expression while enhancing the effects on BAX and cleaved-caspase3 proteins in PANC-1/GEM and SW1990/GEM cells (Fig. 5F–G).

3.6. Knockdown of exosome PPP3CB enhances the susceptibility of GEM-resistant PC cells to GEM

GEM-resistant PC cell-derived exosomes increased the viability and proliferation of GEM-treated GEM-resistant PC cells, while knocking down exosome PPP3CB inhibited their viability (Fig. 6A–B) and proliferation (Fig. 6C–E). Furthermore, GEM-resistant PC cell-derived exosomes inhibited the death of GEM-treated GEM-resistant PC cells, while downregulation of exosome PPP3CB promoted apoptosis of these cells (Fig. 6F–H). Concurrently, downregulation of exosome PPP3CB reduced the expression of BCL2 protein and increased the expression of BAX and cleaved-Caspase-3 proteins (Fig. 6I–J). The aforementioned findings indicate that knocking down exosome PPP3CB may enhance the susceptibility of GEM-resistant PC cells to GEM.

4. Discussion

GEM is a deoxycytidine nucleoside analogue that primarily functions by inhibiting several types of DNA synthesis. This inhibition leads to the suppression of cell proliferation and the blockade of cell cycle progression at the G1/S phase boundary [21]. GEM has been considered the standard treatment for PC for nearly two decades, and is widely used as a first-line therapeutic drug. Compared to 5-fluorouracil, GEM significantly improves patient survival rate. The clinical efficacy response of GEM is notably satisfactory, approximately 5 times higher than that of 5-fluorouracil [4]. However, despite its effectiveness in patients with advanced and metastatic PC, GEM resistance has seriously limited the effectiveness of chemotherapy. Multiple enzymes are involved in the activation, metabolism, and transport of GEM. Additionally, the development of drug resistance is adjusted by multiple factors, including epithelial mesenchymal transition, microRNA and tumor microenvironment, etc. [22]. Accordingly, it is necessary to explore the regulatory mechanism of GEM resistance and screen out new targets for the regulation of GEM resistance. This study highlights a novel finding that a novel exosome inclusion PPP3CB may act as an oncogenic gene in PC by increasing cell resistance to GEM, and inhibition of PPP3CB may be considered as a promising clinical approach for overcoming GEM resistance.

Phosphoprotein phosphatases (PPPs) are part of the protein phosphatase family and participate in various biological processes including gene expression, mitosis and cancer development [23]. The PPP family is composed of catalytic subunits, among which PPP3CB has been rarely reported and was originally described as abnormally expressed in both primary and metastatic PC [24,25] Studies have demonstrated higher transcription level of PPP3CB in PC compared to normal pancreas [26]. Here, we report a novel

function of PPP3CB in GEM resistance, where it enhances the resistance of PC cells to GEM. In addition, knockdown of exosome PPP3CB strengthens the sensitivity of PC cells to GEM. Therefore, our data prompt that PPP3CB may regard as a latent prognosis and predictive marker for GEM-resistant PC.

MicroRNAs (miRNAs) are small non-coding RNAs that adjust the expression of downstream targets post-transcriptionally [27]. They play regulatory roles in multiple biological processes, comprising cell death, infiltration, and cancer cell growth [28]. Among these, miR-298 is a tumor-associated miRNA known to regulate tumor cells by targeting CDK6, CDK9 and MYB18-20 [29]. Previous studies have shown that miR-298 binds to the 3′UTR of MDR1, down-regulates the expression of MDR1, and enhances the cytotoxicity of doxorubicin (DOX) in DOX-resistant cancer cells [30,31]. Additionally, miR-298 has been found to inhibit thyroid cells proliferation and promote apoptosis by regulating the expression of CDK6 [32]. Despite these findings, the effect of miR-298 on PC remains uninvestigated. Therefore, this research aims to explore the effects of miR-298 on PC and analyze its mechanism of action. In this study, PPP3CB-binding miRNAs were predicted using StarBase, revealing binding sequences between miR-298 and PPP3CB. Therefore, we hypothesized that miR-298 could act as a competing endogenouse RNA of PPP3CB sponge, facilitating proliferation and decreasing apoptosis of GEM-resistant PC cells, thereby enhancing the resistance of these cells to GEM.

As reported, miR-298 targets the STAT3 gene, and the miR-298/STAT3 feedback pathway regulates epithelial-to-mesenchymal transition and the growth of liver malignant cells [20]. Emerging findings demonstrates the significance of STAT3 in cancer progression, infiltration, and metastasis [33]. Blocking miR-660-3p enhances GEM resistance in PC by activating the STAT3 signaling pathway in PC cells, whereas blocking the STAT3/NF-κB signaling pathway improves PC cells' sensitivity to GEM [34,35]. STAT3 inhibitors significantly restrain the growth and movement of PC both in vivo and in vitro. In the preclinical PC model, these inhibitors completely regress human tissue xenograft tumors and remarkably extend the survival of mice [36]. In GEM-treated PC cells, STAT3 inhibition enhances mitochondria-dependent apoptosis [37]. Based on these findings, STAT3 was identified as the downstream target of miR-298 in this research. Nevertheless, the dual-luciferase reporter gene test corroborated the hypothesis that miR-298 serves as a direct target of PPP3CB and is responsible for PPP3CB-mediated GEM resistance. Additionally, our data demonstrated that knockdown of PPP3CB reduced STAT3 expression. Interestingly, when miR-298 was restrained, the influence of PPP3CB downregulation on STAT3 expression was eliminated. These data prompt that PPP3CB may function through the miR-298/STAT3 axis. Moreover, knockdown of exosome PPP3CB strengthened the sensitivity of PC cells to GEM. Collectively, our data provide evidence supporting the notion that the PPP3CB/miR-298/STAT3 axis plays a role in regulating PC cell resistance to GEM. As a result, therapeutic interventions targeting PPP3CB have the potential to enhance the efficacy of PC therapy.

5. Conclusion

In summary, this study concludes that exosome PPP3CB derived from GEM-resistant PC cells enhances STAT3 expression by down-regulating miR-298, which promotes cell proliferation and inhibit cell apoptosis, thereby enhancing resistance of PC cells to GEM. The results of this research support that the level of exosome PPP3CB may serve as a latent biomarker for PC, which is of great meaning for optimizing the clinical efficacy of PC and also valuable for developing novel treatment strategies for PC patients resistant to GEM.

Funding

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Ethics approval

Review and/or approval by an ethics committee was not needed for this study, because this study only involved cell experiments.

Data availability statement

All data accessed and analyzed in this study are available in the article and its supplementary materials.

CRediT authorship contribution statement

Chaojun Wang: Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Shengqian Xu:** Writing – review & editing, Validation, Software, Investigation, Data curation. **Yong Qin:** Writing – review & editing, Supervision, Resources, Project administration, Methodology.

Declaration of competing interest

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

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36434.

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