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Original Research

Knockdown of *TGF-β* in Pancreatic Cancer Helps Ameliorate Gemcitabine Resistance

Xinxing Wang[1,†], Wei Su[2,†], Chuan Qin[1], Ruxin Gao[1], Shuai Shao[1], Xianwen Xu[1], Zhenhai Zhang[1,*], Junlin Gao[2,*]

[1]Department of Hepatobiliary Surgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, 250021 Jinan, Shandong, China
[2]Liver Gall Bladder and Pancreatic Surgery Ward, Qinghai Red Cross Hospital, 810001 Xining, Qinghai, China
*Correspondence: zhangzhenhai@sdfmu.edu.cn (Zhenhai Zhang); 771410700@qq.com (Junlin Gao)
[†]These authors contributed equally.
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Abstract

Background: The *TGF-β* gene is a gemcitabine (GEM) resistance gene; however, the mechanism by which it regulates GEM resistance in pancreatic cancer remains unclear. **Methods**: The *PANC-1* cell line was treated with GEM and then stimulated with *TGF-β*. Subsequently, we constructed GEM-resistant pancreatic cancer cell lines, knocked down *TGF-β* in these cell lines, and detected changes in the proliferation and apoptosis of drug-resistant cancer cells. In addition, the protein expression levels of *KLF-4*, *GFI-1*, and *ZEB-1* were determined. The xenograft tumor models of nude mice were constructed by subcutaneously injecting GEM-resistant *PANC-1* cells into mouse axilla. The tumors were removed, dissected, and weighed after 6 weeks. The protein levels of *KLF-4*, *GFI-1*, and *ZEB-1* in tumor tissues were quantified. In addition, the percentage of M2 macrophages in tumor tissues was determined using flow cytometry. **Results**: The protein levels of *TGF-β* in pancreatic cancer cells were significantly decreased after GEM treatment. The protein expression of *KLF-4* was downregulated, whereas the expressions of *GFI-1* and *ZEB-1* were upregulated after *TGF-β* stimulation. Apoptosis increased and proliferation decreased after *TGF-β* knockdown in GEM-resistant pancreatic cancer cells, moreover, silencing *TGF-β* promoted the expression of Caspase 3 and Cleaved caspase 3. In addition, the protein expression of *KLF-4* was upregulated, whereas the expressions of *GFI-1* and *ZEB-1* were downregulated. Further, the volume and weight of the transplanted tumor decreased after *TGF-β* knockdown. The protein expression of *KLF-4* was upregulated, whereas the expressions of *GFI-1* and *ZEB-1* were downregulated in tumor tissues. In addition, the percentage of M2 macrophages decreased in tumor tissues after *TGF-β* knockdown. **Conclusions**: The knockdown of *TGF-β* inhibits epithelial-to-mesenchymal transition, suppresses the proliferation and promotes the apoptosis of drug-resistant cancer cells, and decreases the macrophage polarization to the M2 phenotype, consequently ameliorating GEM resistance in pancreatic cancer.

Keywords: *TGF-β*; pancreatic cancer; gemcitabine resistance; EMT; M2-type polarization

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is ranked as the fourth leading cause of cancer-related deaths worldwide [1], and the five-year survival rate is only 9% [2]. Surgical resection combined with postoperative neoadjuvant chemotherapy is the primary treatment option for patients with resectable PDAC. However, several factors, such as genetic instability, metabolic abnormalities, and immunosuppression [3], make chemical therapy less effective, and drug resistance has become a key factor affecting the efficacy of chemotherapeutic drugs.

Gemcitabine (GEM; 2′,2′-difluorodeoxycytidine), a difluoride analog of deoxycytidine, is commonly used for the treatment of PDAC. The drug interferes with DNA synthesis by inhibiting ribonucleotide reductase and DNA polymerase (through diphosphate analogs) or by misincorporation of DNA to prevent chain elongation (through triphosphate analogs) [4]. However, the median progression-free survival for advanced PDAC treated with GEM monotherapy is only 3.7 months [5], and long-term chemotherapy can induce drug resistance in patients, making continued treatment difficult [6].

Chemoresistance induced by GEM is associated with several factors, including bacteria, and metabolic reprogramming [7]. In addition, various transcription factors, cytokines, enzymes, and signaling pathways are involved in the development of GEM resistance [8]. The transforming growth factor-β (*TGF-β*) superfamily comprises various conserved growth factors [9]. *TGF-β* has a key role in tumorigenesis and associated stem cell genesis [10]. It is an important factor involved in GEM resistance of tumors, such as oral squamous cell carcinoma and prostate cancer [11]. In addition, *TGF-β* is associated with GEM resistance in PDAC. The inhibition of *TGF-β* receptor I increases the susceptibility of parental and drug-resistant pancreatic cancer cells to GEM and promotes the apoptosis of GEM-resistant cells [12]. Porcelli *et al*. [11] suggested that crosstalk between mast cells and PDAC cells reduces the survival inhibition of GEM-dependent tumor cells by activating the *TGF-β* signaling pathway.

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TGF-β interacts with specific genes and may indirectly influence tumor therapy. Growth factor independence-1 (*GFI-1*), a cellular proto-oncogene, was originally thought to play a role in T-cell differentiation and lymphoma [13]. Xian *et al.* [14] reported that simvastatin can decrease the resistance of PDAC to GEM by inhibiting the *TGF-β1*/*GFI-1* axis. Kruppel-like factor 4 (*KLF-4*)—a transcription factor containing zinc finger structure—regulates various biological processes including the *TGF-β* signaling pathway [15]. *KLF-4* can be targeted by *TGF-β1* to regulate vascular smooth muscle cells [16]. *TGF-β1* regulates the transcription of zinc finger E-box-binding homologous box (ZEB) family genes involved in the epithelial-to-mesenchymal transition (EMT) of tumor cells. Ursolic acid targets the *TGF-β1*/*ZEB-1* axis and consequently decreases the invasiveness of colorectal cancer cells [17].

Here, we found that *TGF-β* knockdown alleviated the malignant progression of PDAC induced by GEM resistance. Notably, the expressions of *KLF-4* and *ZEB-1* (downstream of *GFI-1*) were altered after *TGF-β* knockdown. Our results suggest a novel target for ameliorating GEM resistance in PDAC to increase the efficacy of treatment. To our knowledge, this is the first report on the role of *TGF-β* in mediating GEM resistance in PDAC.

2. Materials and Methods

2.1 Cell Lines and Animals

Human PDAC cell line *PANC-1* was procured from American Type Culture Collection (ATCC, Manassas, VA, USA), and MIA PaCa-2 was purchased from Wuhan Pricella Biotechnology (No. CL-0627, Wuhan, China). The cell lines used have been tested for mycoplasma and cell STR identification. The cells were cultured in RPMI 1640 medium (HyClone, USA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA), 100 U/mL penicillin, 100 U/mL streptomycin, and 0.03% L-glutamine. The 6-week-old nude SPF Balb/c female mice were purchased from Beijing Weitonglihua Biotechnology Co., Ltd. (Beijing, China) and were adaptively fed for 3 days. Approximately 100 µL of tumor cell suspension (10^6 cells/mL) was subcutaneously injected into the axilla, and tumors were allowed to grow for 6 weeks. The tumor volume was measured every 5 days, and the mice were treated after the experiment.

2.2 Treatment of PANC-1 Cell Line with GEM and TGF-β

The cells were cultured in RPMI 1640 medium containing 50 nmol/L GEM. The state of cells was observed after 2–3 days, and the drug-supplemented medium was replaced with the standard medium until normal cell growth was restored. The same protocol of cell culture was repeated until the cells were stable under GEM exposure. The concentration of GEM was gradually increased to 2 µmol/L. Finally, 10 µg/mL *TGF-β* was added to the medium, and other culture conditions remained same.

2.3 Construction of GEM-Resistant Cell Lines

Actively growing PDAC-1 cells were treated with 100 times IC50 of GEM as the induction dose and subsequently cultured with GEM for 2 h. The drug-containing medium was removed after 2 h, cells were rinsed three times with phosphate-buffered saline, and the standard medium was added. The dead cells were removed by changing the medium every day. The growth of the surviving cells resumed, and the logarithmic growth stage was observed after 15 days. The cells were passaged three times, and the above protocol was repeated four times. Subsequently, the duration of the GEM exposure was extended to 4 h and the above protocol was repeated eight times. The induction lasted 6 months, and the drug-resistant cell line PGAC-1/GEMR was obtained.

2.4 TGF-β Knockdown

TGF-β-silencing lentivirus was purchased from Ghanghai Bolsen Biotechnology Co., Ltd. (BES-20241Ab, Shanghai, China). The adherent cells (1×10^5/well) were plated onto a 24-well plate. The original medium was replaced with 2 mL fresh medium containing 6 µg/mL polybrene, and the viral suspension was added to the medium. The plate was incubated at 37 °C for 24 h, and the virus-containing medium was replaced with fresh medium. The transfection efficiency was measured after 72 h using fluorescence-activated cell sorting.

2.5 Flow Cytometry

Annexin V-PE/7-ADD apoptosis detection kit (MA0429-2; Dalian Boglin Biotechnology Co., Ltd., Dalian, Liaoning, China) was used to detect apoptosis. PDAC cells were treated with GEM for 72 h and suspended in 500 µL of binding buffer. Annexin V-FITC and propidium iodide (5 µL each) were subsequently added. Macrophages were isolated from tumor tissues and the polarization of macrophages was detected by incubating them with F4/80 antibody (1:100; ab6640, Abcam, Waltham, MA, USA). Annexin V-FITC was detected using the PerCP channels. The cells were kept in the dark for 15 min, and the stained cells were analyzed using a flow cytometer (BD Biosciences, San Jose, CA, USA).

2.6 CCK8 Assay

PDAC cells were plated in a 96-well plate and cultured for 12 h. The cells were then treated with a gradient concentration of GEM (0.1, 1,10, 100, 1000, and 10,000 nM) and cultured for 48 h. Finally, the CCK-8 reagent (C0037, Beyotime Biotechnology, Shanghai, China, 10 mL/well) was added to each well, and absorbance was measured at 450 nm using an immunosorbent instrument (BioTek Synergy H1, Agilent, Beijing, China).

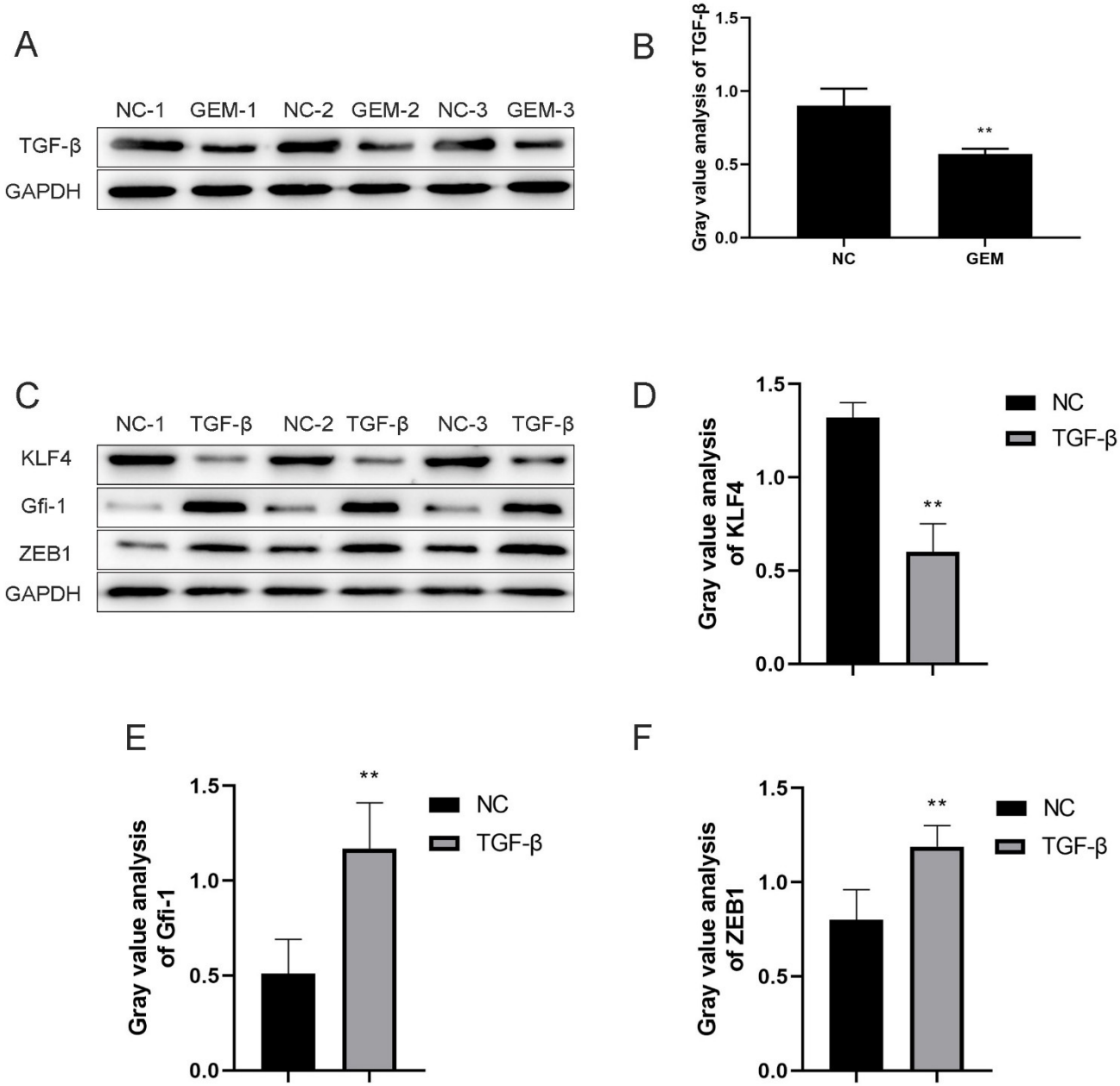

Fig. 1. *TGF-β* is a chemoresistance-associated gene in the GEM-treated *PANC-1* cell line. (A,B) Expression of *TGF-β* was detected using western blotting after *PANC-1* cells were treated with GEM. (C–F) Protein expression levels of *KLF-4*, *GFI-1*, and *ZEB-1* after *TGF-β* induction and their quantitative analysis. $^{**}p < 0.01$, n = 3.

2.7 MTT Assay

MTT cell proliferation assay kit [40206ES76; Yisheng Biotechnology (Shanghai) Co., Ltd., China] was used to detect cell proliferation. The cells were seeded into a 96-well plate and allowed to grow for 24 h. Approximately 20 µL of MTT (5 mg/mL) was added into each well, and the plate was incubated for 4 h. The culture medium was then removed, and 100 µL of dimethyl sulfoxide was added to dissolve Jiazan particles. The plate was oscillated for 2–5 min to ensure proper dissolution of formazan, and the OD value was recorded at 570 nm using an enzyme-label instrument.

2.8 Western Blot Analysis

Cells were lysed in RIPA buffer with a proteinase inhibitor cocktail to extract total cellular protein. The nuclear and cytoplasmic components were separated using nuclear and cytoplasmic extraction reagents, respectively (Thermo Fisher Scientific, Waltham, MA, USA). The protein samples were separated on 10% polyacrylamide gels and transferred to polyvinylidene difluoride membranes. The blotted membranes were then incubated with the primary antibodies against cleaved caspase-3 (1:1000; ab32042, Abcam), caspase-3 (1:1000; ab32351, Abcam), *KLF-4* (1:1000; ab215036, Abcam), *GFI-1* (1:1000; ab21061, Abcam), and

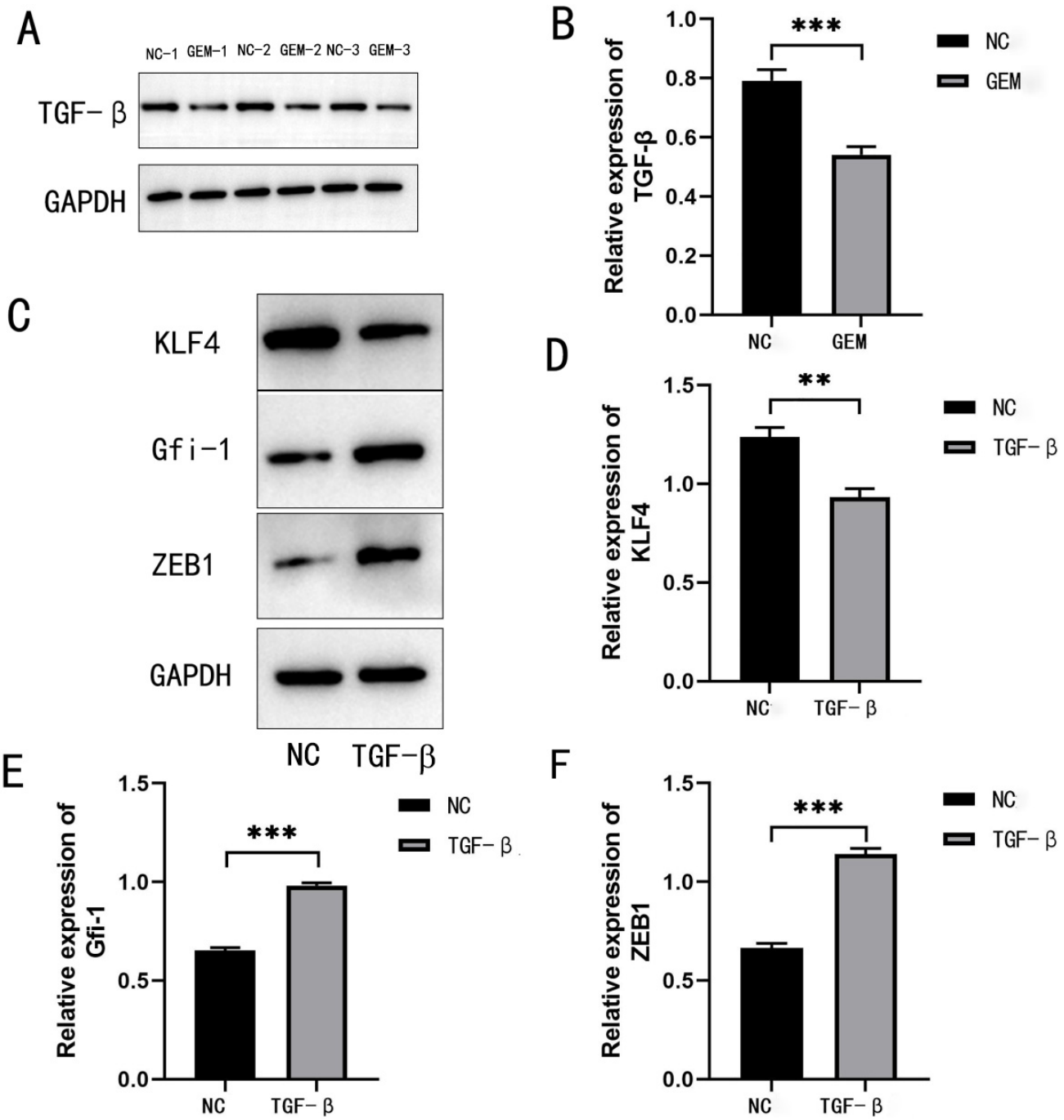

Fig. 2. *TGF-β* is a chemoresistance-associated gene in the GEM-treated MIA PaCa-2 cell line. (A,B) Expression of *TGF-β* was detected using western blotting after MIA PaCa-2 cells were treated with GEM. (C–F) Protein expression levels of *KLF-4*, *GFI-1*, and *ZEB-1* after *TGF-β* induction and their quantitative analysis. $^{**}p < 0.01$, and $^{***}p < 0.001$, n = 3.

ZEB-1 (1:1000; ab203829, Abcam) overnight at 4 °C. Finally, goat anti-rabbit IgG secondary antibodies were added to the membranes for 4 h. The membranes were then developed using an enhanced chemiluminescence kit (20-500-120; Shanghai Xiao Peng Biological Technology Co., Ltd., Shanghai, China).

2.9 Statistical Analysis

Each sample was analyzed in at least 3 independent experiments and at least three technical replicates. Data are reported as the mean ± SEM. One-way ANOVA, two-way ANOVA, or two-tailed Student's *t*-test were performed for pair-wise comparisons. *p*-values of 0.05 or less were considered statistically significant.

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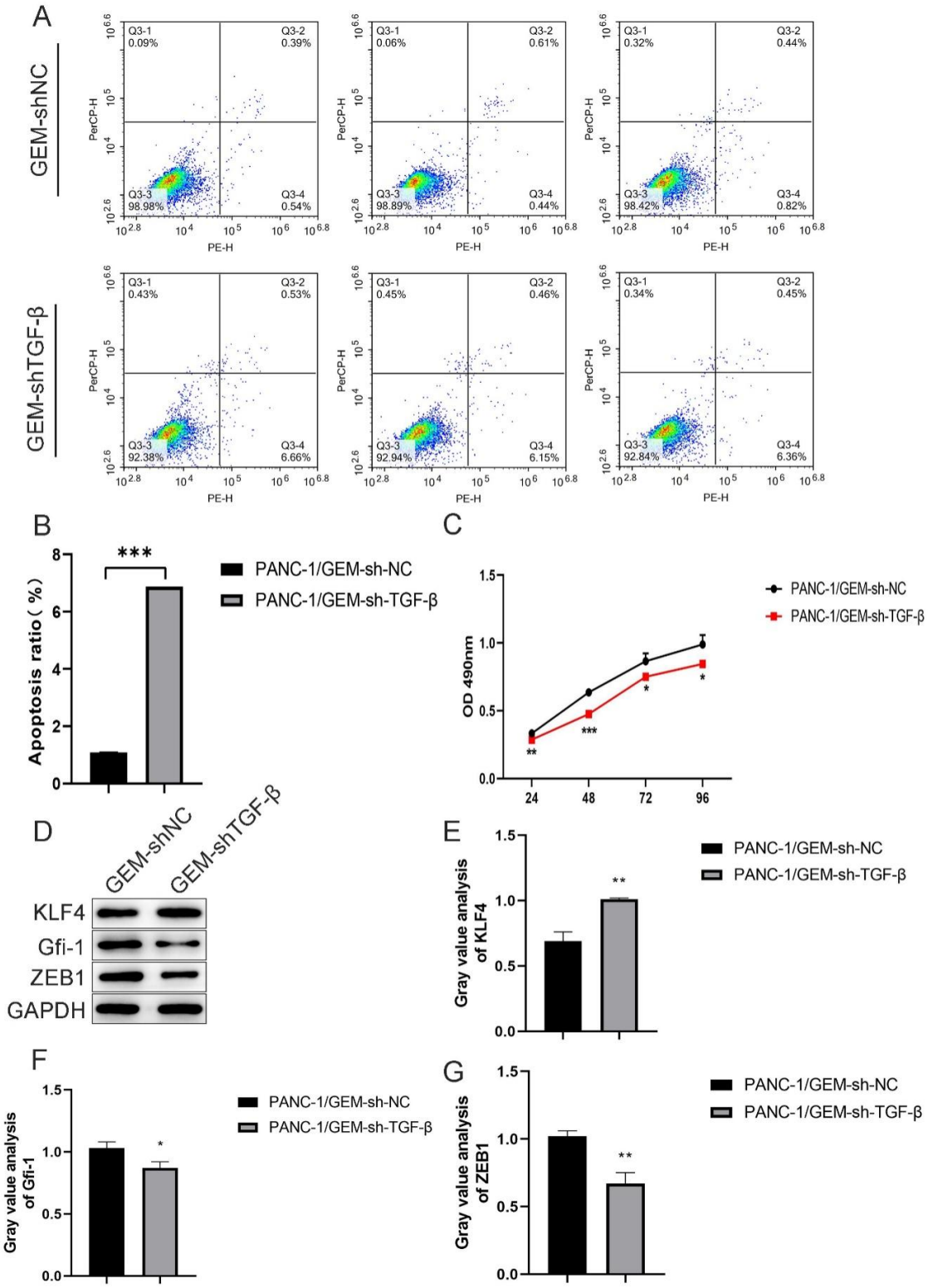

Fig. 3. *TGF-β* knockdown ameliorates GEM resistance in *PANC-1* cells. (A,B) Flow cytometry was performed to detect the percentage of apoptotic drug-resistant *PANC-1* cells after *TGF-β* knockdown. (C) CCK-8 proliferation assay was performed to detect the proliferation of drug-resistant *PANC-1* cells after *TGF-β* knockdown. (D–G) Western blotting was performed to detect the percentage of apoptotic cells after *TGF-β* knockdown. Protein expression and quantification of *KLF-4*, *GFI-1*, and *ZEB-1*. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$; n = 3.

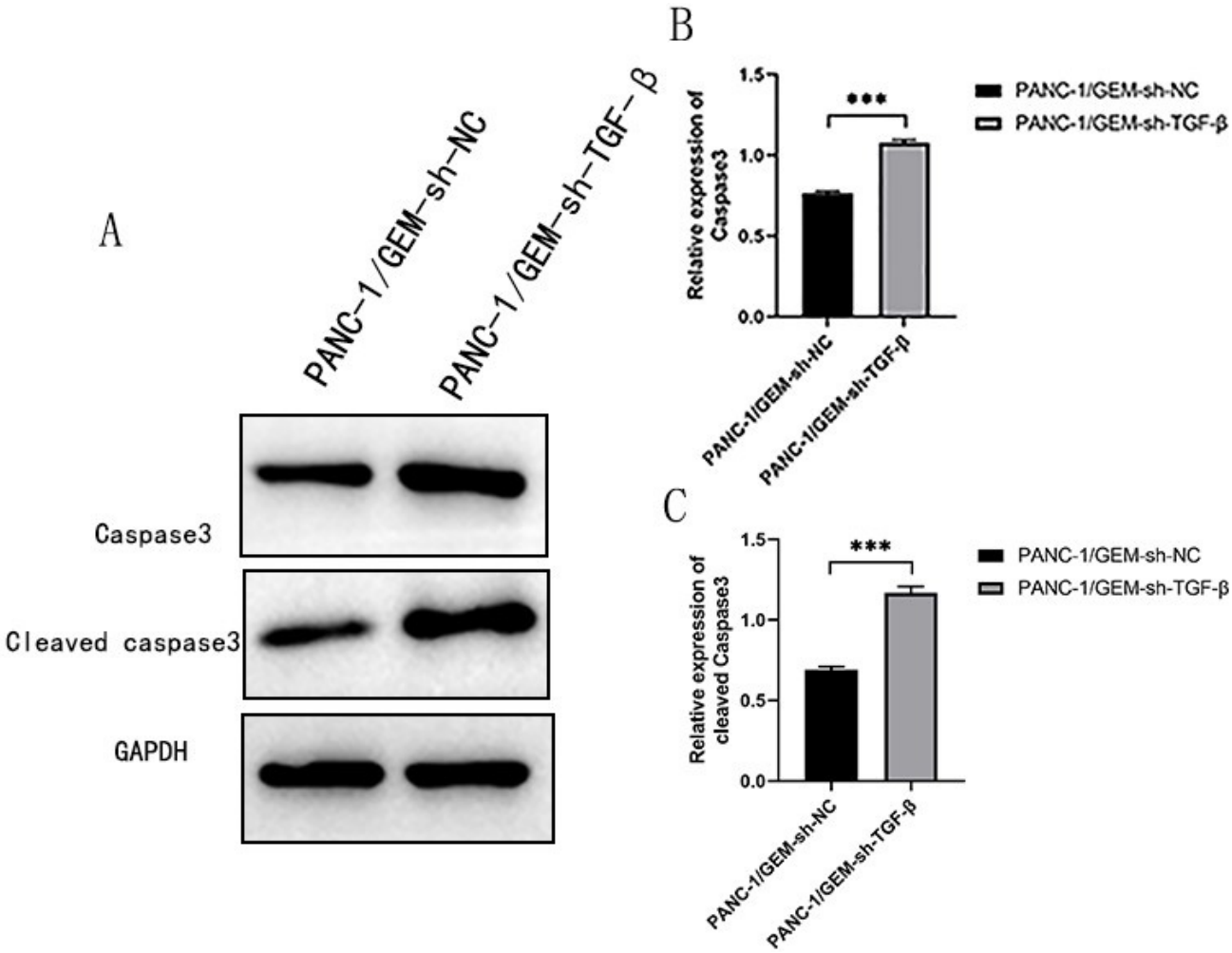

Fig. 4. Downregulating *TGF-β* promotes apoptosis of *PANC-1* cells. (A) Western blotting was performed to detect the expression of caspase-3 and cleaved caspase-3. (B) Caspase-3 and (C) cleaved caspase-3 quantification using Western blotting. ***$p < 0.001$; n = 3.

3. Results

3.1 TGF-β is a Chemoresistance-Associated Gene in the GEM-Treated Pancreatic Cancer Cell Lines

TGF-β has been confirmed as a gene associated with GEM resistance in cancer [16]; however, the mechanism of *TGF-β*-mediated chemoresistance in PDAC remains unclear. The protein expression of *TGF-β* was significantly downregulated in GEM-treated *PANC-1* and MIA PaCa-2 cells, suggesting the critical role of *TGF-β* in this process (Fig. 1A,B and Fig. 2A,B). We then stimulated PNAC-1 cells with *TGF-β* to induce an EMT environment. Western blotting results revealed that the protein expressions of the EMT-promoting transcription factors *ZEB-1* and *GFI-1* (downstream of *TGF-β*) were significantly increased, whereas the expression of EMT-inhibiting *KLF-4* were significantly decreased in pancreatic cancer cells (Fig. 1C–F and Fig. 2C–F). These findings suggest that *TGF-β* may promote EMT through its downstream transcription factors (*ZEB-1*, *GFI-1*, and *KLF-4*) in GEM-treated PDAC cells, thereby playing a role in inducing chemoresistance.

3.2 TGF-β Knockdown Ameliorates GEM Resistance in PDAC Cells

We constructed *PANC-1* and MIA PaCa-2 cell lines with GEM resistance and *TGF-β* knockdown. Compared with the control group (GEM-shNC), the cell proliferation was significantly decreased at 24 ($p < 0.01$), 48 ($p < 0.001$), 72 ($p < 0.05$), and 96 h ($p < 0.05$; Fig. 3C) after *TGF-β* knockdown in the GEM-sh*TGF-β* group. Compared with the control group, the apoptosis of cells was significantly increased after *TGF-β* knockdown in the GEM-sh*TGF-β* group ($p < 0.001$; Fig. 3A,B). In addition, protein expression levels of the *ZEB-1*, *GFI-1*, and *KLF-4* transcription factors were also compared between the two groups. Compared with the control group, the protein expressions of *ZEB-1* ($p < 0.01$) and *GFI-1* ($p < 0.05$) were significantly downregulated, whereas the expression of *KLF-4* was significantly upregulated after *TGF-β* knockdown in the GEM-sh*TGF-β* group ($p < 0.01$; Fig. 3D–G). Western blotting results revealed that *PANC-1*/GEM-sh-*TGF-β* induced the expression of cleaved and total caspase-3 (Fig. 4). Another piece of corroborating evidence found

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the same thing, compared with the control group, the apoptosis of drug-resistant MIA PaCa-2 cells was significantly increased after *TGF-β* knockdown in the GEM-sh*TGF-β* group ($p < 0.001$; Fig. 5A,B). *PANC-1*/GEM-sh-*TGF-β* induced the expression of cleaved and total caspase-3 in *PANC-1*/GEM-sh-*TGF-β* induced the expression of cleaved and total caspase-3 (Fig. 5C–E). Compared with the control group (GEM-shNC), the cell proliferation was significantly decreased at 24 ($p < 0.01$), 48 ($p < 0.001$), 72 ($p < 0.05$), and 96 h ($p < 0.05$; Fig. 6A) after *TGF-β* knockdown in the GEM-sh*TGF-β* group of drug-resistant MIA PaCa-2 cells. Compared with the control group, the protein expressions of *ZEB-1* ($p < 0.01$) and *GFI-1* ($p < 0.05$) were significantly downregulated, whereas the expression of *KLF-4* was significantly upregulated after *TGF-β* knockdown in the GEM-sh*TGF-β* group ($p < 0.01$; Fig. 6B–E). Therefore, *TGF-β* knockdown can inhibit the proliferation, promote apoptosis, and suppress EMT, thereby ameliorating GEM resistance in pancreatic cancer cells.

3.3 TGF-β Knockdown Helps Alleviate GEM Resistance in Pancreatic Cancer Mice

According to Fig. 7D, GEM-resistant PANC-1 cells transfected with the *TGF-β* knockdown plasmid could stably inhibit the expression of *TGF-β* in mice, indicating that we successfully constructed the *TGF-β* knockdown plasmid. We subcutaneously injected sh-*TGF-β*-transfected *PANC-1* cells in nude mice to examine the effect of silencing *TGF-β* expression on tumor formation, the tumor formation in nude mice is shown in Fig. 7A. The tumor volume and mass were the highest in the GEM-resistant group (GEM-shNC). However, the tumor volume ($p < 0.05$; Fig. 7B) and mass ($p < 0.01$; Fig. 7C) were decreased in the *TGF-β*-knockdown group (GEM-sh*TGF-β* group). In addition, protein expression levels of *ZEB-1*, *GFI-1*, and *KLF-4* were compared among the three groups (Fig. 7D–H). Compared with the GEM-resistant group, the protein expressions of *ZEB-1* ($p < 0.01$) and *GFI-1* ($p < 0.05$) were significantly downregulated, whereas the expression of *KLF-4* was significantly upregulated ($p < 0.01$) after *TGF-β* knockdown in the GEM-sh*TGF-β* group. These findings were similar to those of cell line experiments. Therefore, *TGF-β* knockdown ameliorates GEM resistance in a pancreatic cancer mouse model by inhibiting EMT in cancer cells.

3.4 TGF-β Knockdown Regulates the Polarization of Macrophages

M2-type macrophages (tumor-associated macrophages) produce several cytokines that promote the survival, angiogenesis, and metastasis of malignant tumor cells to maintain tumor growth [18]. Qiaofei Liu reported that *TGF-β* regulates the M0/M2 polarization of macrophages in pancreatic cancer [19]. Therefore, we detected the percentage of M2 macrophages in tumor tissues obtained from the three groups of nude mice. The percentage of M2 macrophages was the highest in the GEM-resistant group. However, the percentage of M2 macrophages was significantly decreased after the knockdown of *TGF-β* in the GEM-resistant group ($p < 0.001$; Fig. 8A,B). These findings suggested that *TGF-β* knockdown ameliorates GEM resistance in pancreatic cancer mice by decreasing the polarization of macrophages to the M2 phenotype.

4. Discussion

The activation of the *TGF-β* signaling pathway enhances the progression of pancreatic cancer and promotes GEM resistance; however, the specific mechanism has not been clarified [13,20]. *TGF-β* can regulate tumor invasion and metastasis by regulating the EMT signaling pathway. In addition, it can directly promote the proliferation and inhibit the apoptosis of tumor cells, thereby regulating the malignant progression of cancer. Lou *et al.* [21] reported that naringin downregulates the mRNA and protein levels of EMT markers by inhibiting the *TGF-β*1/Smad3 signaling pathway in pancreatic cancer cells. This downregulation inhibits the activity of cancer cells and reverses their resistance to GEM. *TGF-β*1 secreted by tumor-associated fibroblasts upregulates ATF4 expression in PDAC cells and induces pancreatic cancer progression (proliferation, colony formation, and migration) and GEM resistance [10]. BRAP inhibits the proliferation, migration, and self-renewal of glioma stem cells [22]. Reserpine has potential therapeutic value in inhibiting DNA repair, cell proliferation, and invasion while inducing cell apoptosis by regulating the *TGF-β* signaling [23]. LINC00665 is overexpressed in gastric cancer cells, and the activation of gastric cancer cell lines was inhibited by the *TGF-β* signal after knocking down the LINC00665 gene. Moreover, apoptosis was promoted in cancer cells [24]. In addition, the authors indicated that downregulating *TGF-β* can inhibit cell proliferation and promote cell apoptosis in GEM-resistant pancreatic cancer cell lines. The volume and weight of transplanted tumors were markedly decreased after *TGF-β* downregulation, suggesting that the downregulation of *TGF-β* can ameliorate GEM resistance in mice.

Components of the *TGF-β* signaling pathway are expressed in most liver cancer cells, and the activation of this pathway promotes cell migration and invasion. Regulating the expression of *KLF-4* can block *TGF-β* signal transduction [25]. *KLF-4* depletion inhibits the mesenchymal characteristics of stem cells and *TGF-β*1 pathway activation, whereas the overexpression of *KLF-4* can activate the phosphorylation of *TGF-β*1, expression of Smad 2/3 and Snail, and restore the stem cell and mesenchymal phenotype [26]. The *TGF-β* signaling pathway promotes the expression of IL-7Rα and the differentiation of CD8+ T cells through downstream *GFI-1* and plays a regulatory role in the immune microenvironment of tumors [27]. *ZEB-1* is

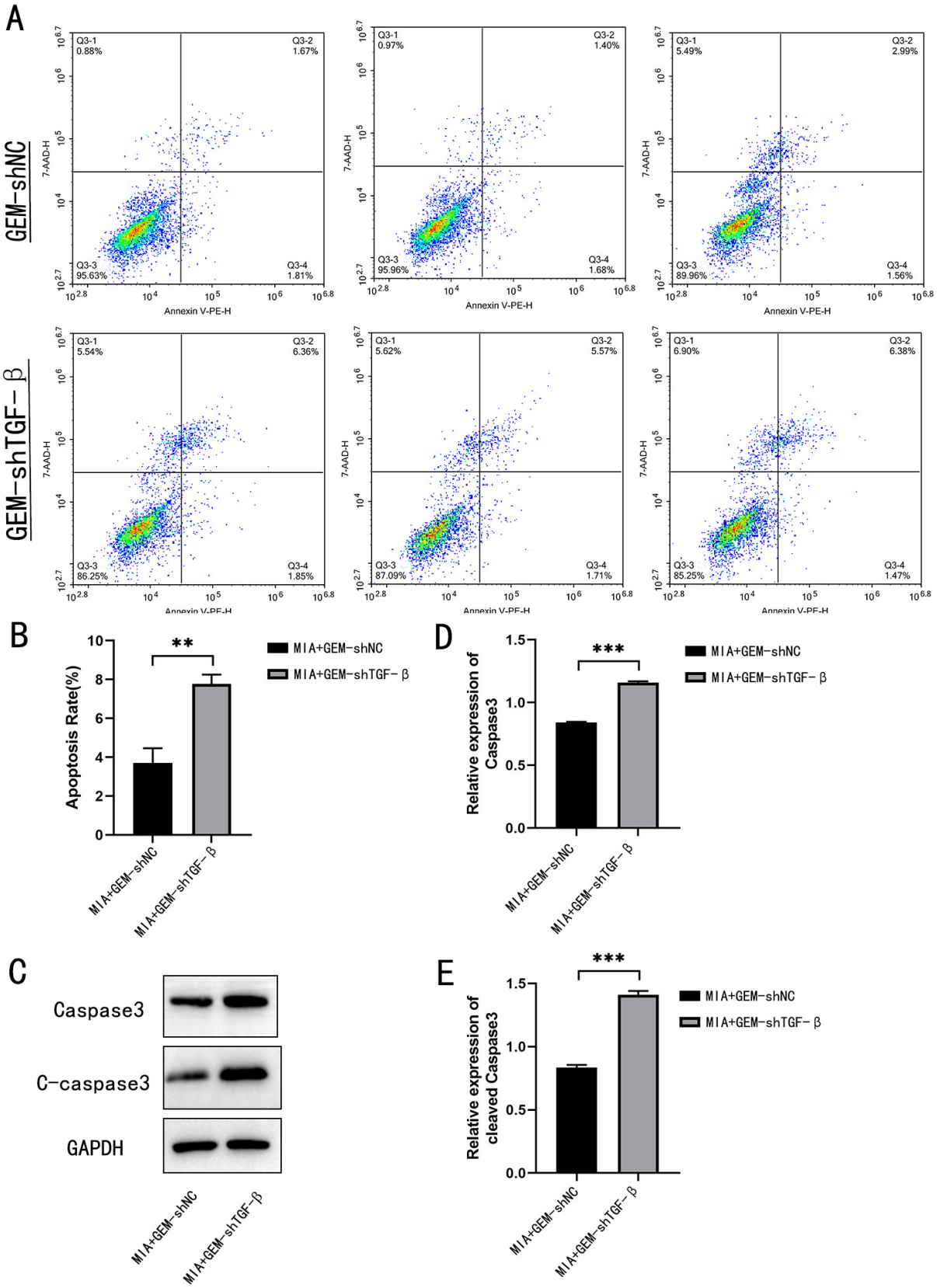

Fig. 5. *TGF-β* knockdown promotes apoptosis of MIA PaCa-2 cells. (A,B) Flow cytometry was performed to detect the percentage of apoptotic drug-resistant MIA PaCa-2 cells after *TGF-β* knockdown. (C) Western blotting was performed to detect the expression of caspase-3 and cleaved caspase-3. (D) Caspase-3 and (E) cleaved caspase-3 quantification using western blotting. $^{**}p < 0.01$, $^{***}p < 0.001$; n = 3.

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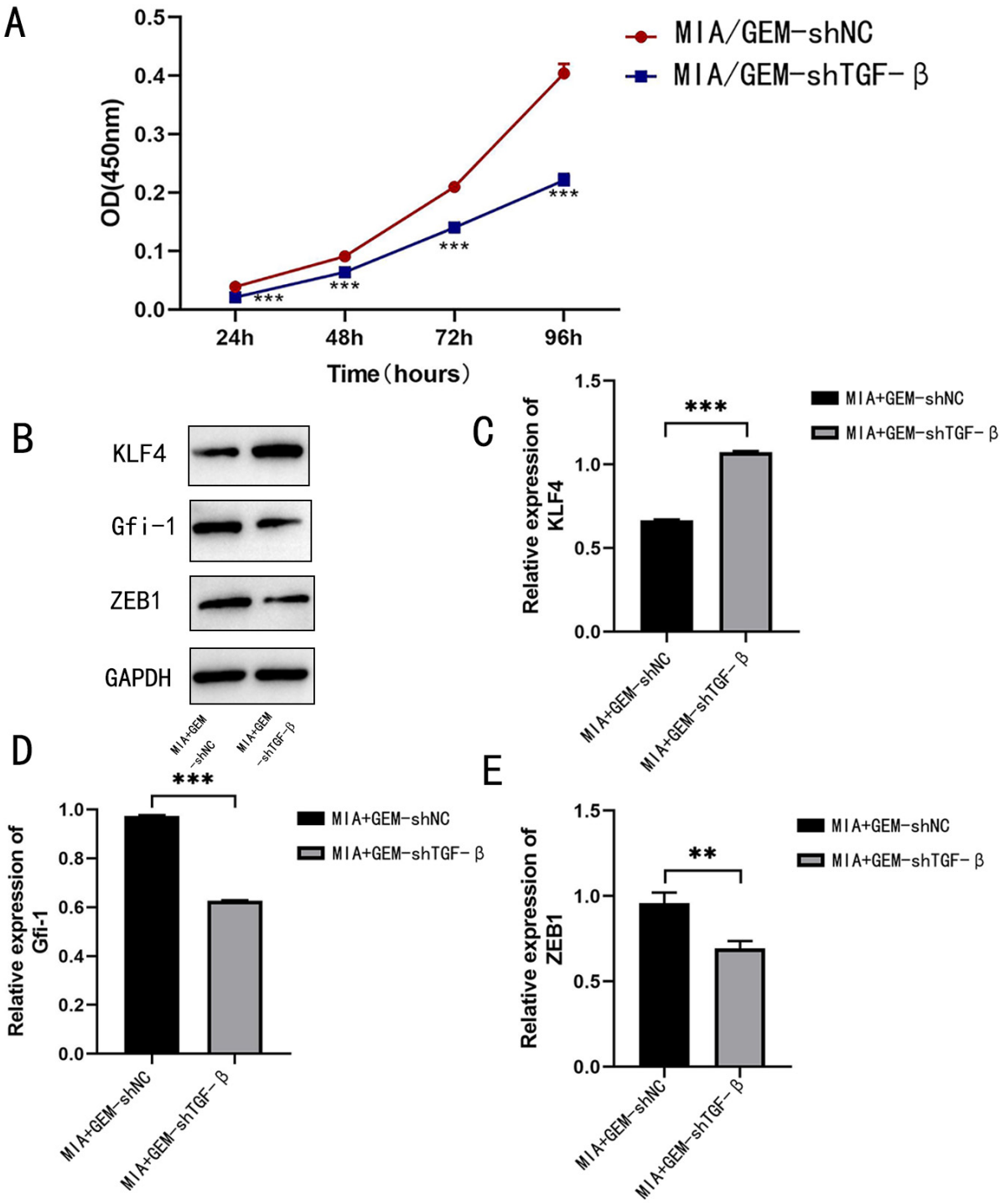

Fig. 6. *TGF-β* knockdown ameliorates GEM resistance in MIA PaCa-2 cells. (A) CCK-8 proliferation assay was performed to detect the proliferation of drug-resistant MIA PaCa-2 cells after *TGF-β* knockdown (B–E) Western blotting was performed to detect the percentage of apoptotic cells after *TGF-β* knockdown. Protein expression and quantification of *KLF-4*, *GFI-1*, and *ZEB-1*. $^{**}p < 0.01$, and $^{***}p < 0.001$; n = 3.

an EMT marker gene, which can significantly promote the metastasis and progression of pancreatic cancer [28]. In addition, it acts as an oncogene to promote the activation of pancreatic cancer [29].

Several studies on the three downstream transcription factors of *TGF-β*, namely *KLF-4*, *GFI-1*, and *ZEB-1* have been reported in recent years. The clinical manifestations of head and neck squamous cell carcinoma are closely related to EMT, and *TGF-β*1 promotes tumor progression through the EMT pathway by downregulating the expression of anti-EMT factor *KLF-4* [30]. Downregulation of the *GFI-1* transcription factor driven by *TGF-β* promotes

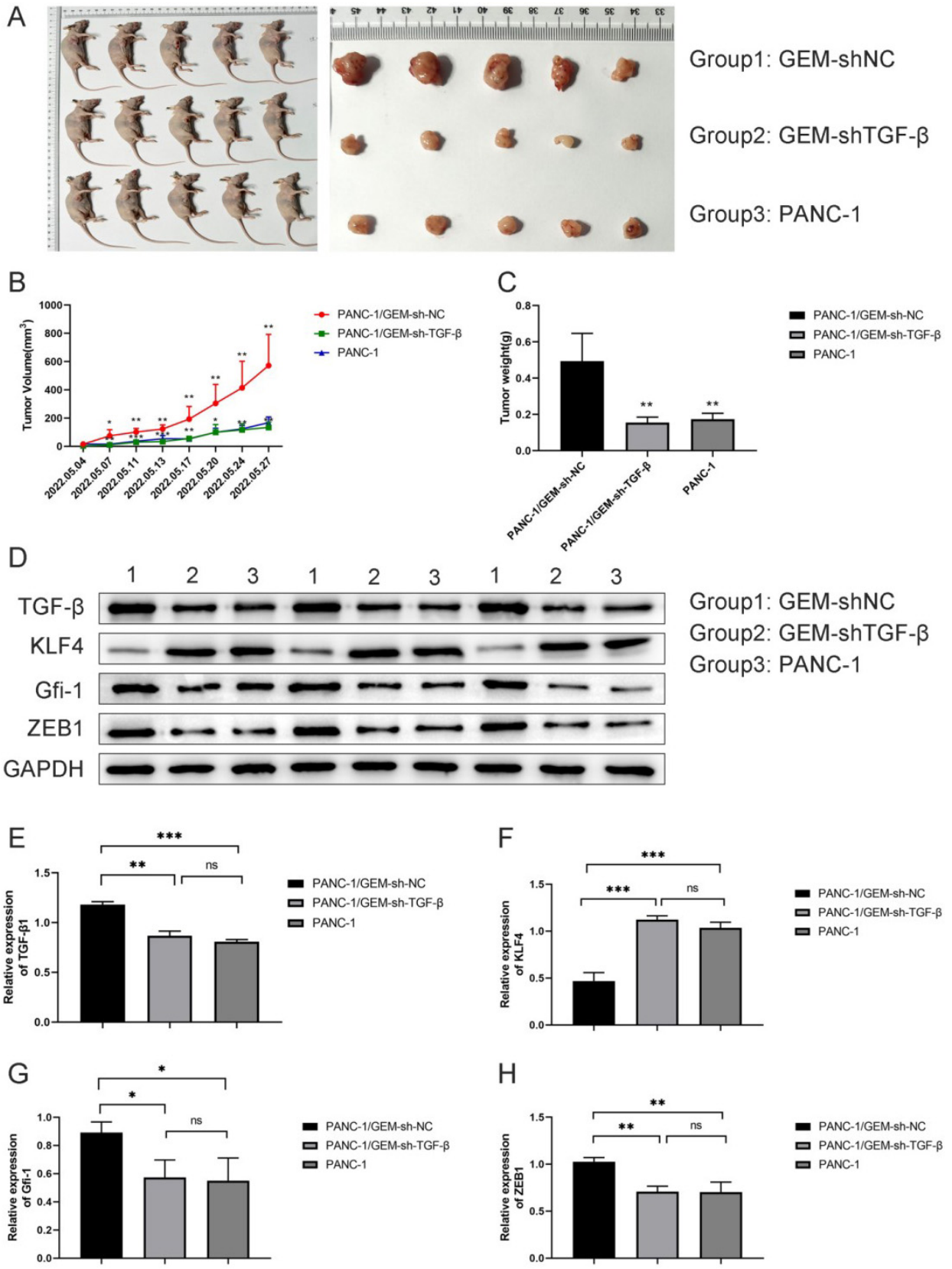

Fig. 7. *TGF-β* knockdown helps alleviate GEM resistance in pancreatic cancer mice. (A) Pancreatic cancer mouse model was constructed by subcutaneously injecting GEM-resistant *PANC-1* cells in mouse axilla. (B) Tumor volume and mass (C) measurements in each group. (D–H) Expression and quantification of the *KLF-4*, *GFI-1*, and *ZEB-1* proteins in tumor tissues using western blot analysis. ns means no difference significance, $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$; n = 3.

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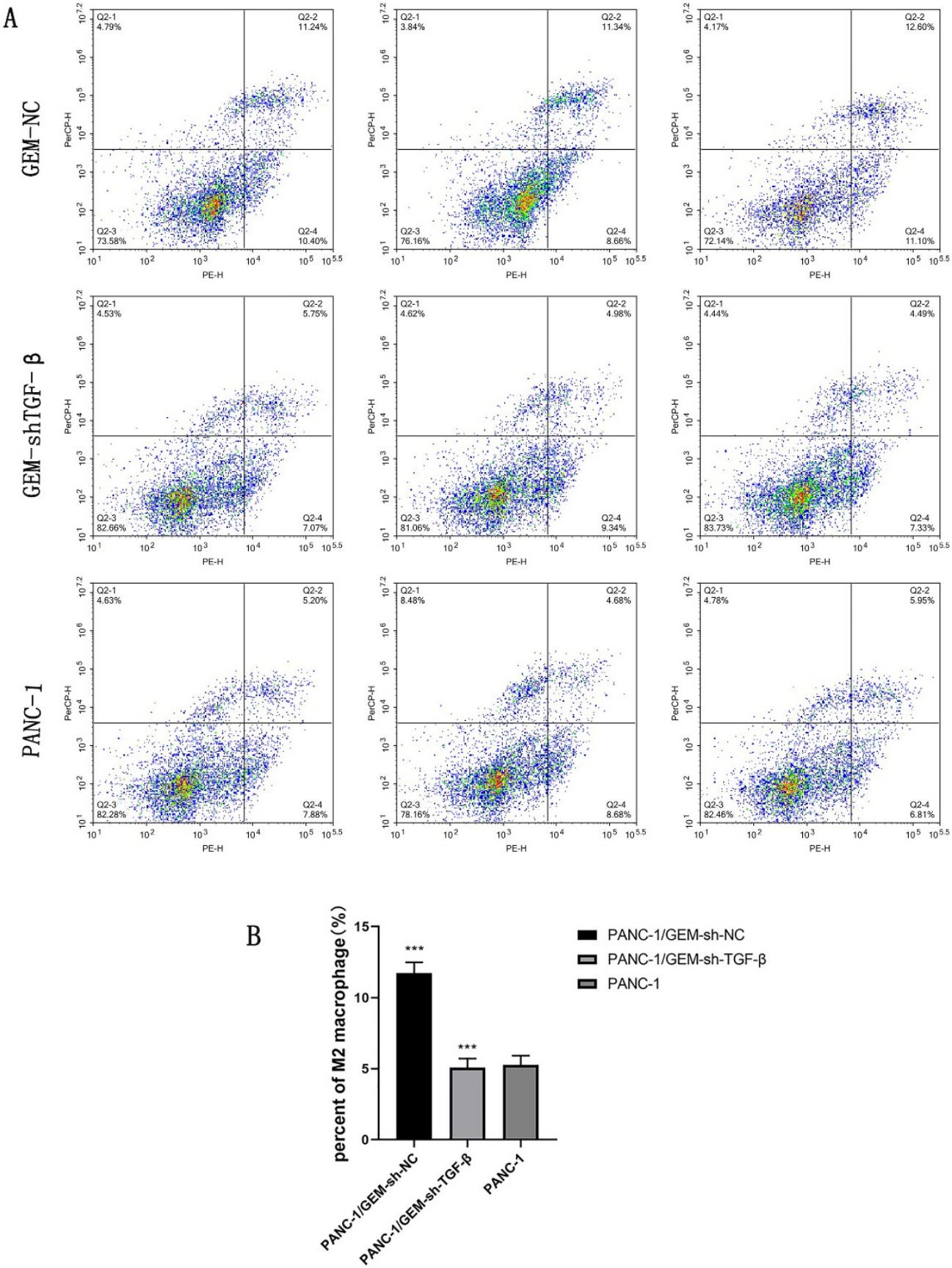

Fig. 8. ***TGF-β* knockdown regulates the polarization of macrophages to the M2 phenotype.** (A,B) Percentage of M2-type macrophages after *TGF-β* knockdown detected using flow cytometry; ***$p < 0.001$; n = 3.

Th17-cell differentiation and subsequently promotes tumor growth [31]. *ZEB-1*, a downstream transcription factor of *TGF-β*, plays a key role in EMT and tumor metastasis. Consistent with these findings, our results also revealed that the protein expressions of *ZEB-1* and *GFI-1* were significantly increased, whereas the expression of *KLF-4* was significantly decreased in a *TGF-β*-induced environment. Moreover, the knockdown of *TGF-β* upregulated *KLF-4* expression and downregulated *ZEB-1* and *GFI-1* expression in GEM-resistant pancreatic cancer cell lines or mouse models with transplanted tumors. Therefore, *TGF-β* may be involved in GEM resistance in pancreatic cancer through the EMT pathway by regulating the three downstream transcription factors (*ZEB-1*, *GFI-1*, and *KLF-4*).

Conditioned medium treated with gemcitabine can promote the infiltration, growth, and M0/M2 polarization of macrophages in pancreatic tumors, thus forming an immunosuppressive microenvironment. Simultaneous blocking of *TGF-β*1 and GM-CSF improves the efficacy of chemotherapy by decreasing the concentration of the M2-polarized tumor-associated macrophages and inducing CD8+ T cells in mice with normal immunity [13]. Notably, our study also indicated that the knockdown of *TGF-β* ameliorated GEM resistance in pancreatic cancer mice by inhibitng the M0/M2 polarization of macrophages. Furthermore, the stability of HIF-1α regulated by mucin 1 mediates metabolic reprogramming of PDAC, and targeting HIF-1α or neopyrimidine biosynthesis and combining this approach with GEM therapy can significantly reduce the tumor burden. PDAC tumors with high mucin-1 levels responded to *TGF-β*-neutralizing antibodies, leading to a substantial decrease in tumor growth. However, tumors with low mucin-1 levels did not respond to *TGF-β*-neutralizing antibodies. However, we did not explore the mechanism of mucin-1-mediated resistance to gemcitabine, and our future studies will focus on this aspect.

5. Conclusions

This study revealed that the knockdown of *TGF-β* inhibits EMT, suppresses the proliferation and promotes the apoptosis of drug-resistant cancer cells, and decreases the polarization of macrophages to the M2 phenotype, thereby ameliorating the GEM resistance in pancreatic cancer cells.

Abbreviations

PDAC, pancreatic ductal adenocarcinoma; *TGF-β*, transforming growth factor-*β*; *GFI-1*, growth factor independence-1; *KLF-4*, Kruppel-like factor 4; *ZEB-1*, zinc finger E-box-binding homologous box-1.

Availability of Data and Materials

All data analysed during this study are included in this published article. Analysed data of flow cytometry could be found in **Supplementary Material**. Further enquiries can be directed to the corresponding author.

Author Contributions

XW contributed to the conception of the study. XW, ZZ and WS designed the study. CQ, RG and SS participated in data collection. XX and JG performed data analysis, prepared the figures and tables. XW and WS wrote the manuscript. ZZ and JG do the writing – review and supervised the project. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Experiments of 4–5 weeks SPF Balb/c female nude mice were been reviewed and approved by the Animal Protection and Use Committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University. Approval number: NO.2019039.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2907269.

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