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Original Article

Downregulation of MicroRNA-455-3p Links to Proliferation and Drug Resistance of Pancreatic Cancer Cells via Targeting TAZ

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Drug resistance is a major cause of treatment failure in pancreatic cancer. The limited evidence indicates the involvement of miR-455-3p in chemotherapy resistance of cancer. Here we observed by qPCR that miR-455-3p was significantly decreased in pancreatic cancer tissues and cell lines. We then confirmed that the inhibition of miR-455-3p increased cell proliferation and gemcitabine resistance of pancreatic cancer, whereas forced overexpression of miR-455-3p had the opposite effect. Furthermore, we demonstrated that TAZ, which is associated with drug resistance of pancreatic cancer, is a new direct downstream target of miR-455-3p. Our present study suggests that miR-455-3p contributes to cell proliferation and drug resistance in pancreatic cancer cells via targeting TAZ.

INTRODUCTION

Pancreatic cancer is one of the most aggressive tumors with a high mortality rate and short survival as a result of the early metastasis and reduced chemotherapeutic efficacy.[1] Drug resistance is one of the main causes of failure in the chemotherapeutic treatment of different types of cancer, including pancreatic cancer. The molecular mechanism underlying the drug resistance includes drug inactivation, drug target alteration, drug efflux, DNA damage repair, cell death inhibition, epithelial-mesenchymal transition, inherent cell heterogeneity, and epigenetic effects.[2] Elucidation of the mechanism of drug resistance in pancreatic cancer is important for improving chemotherapeutic efficacy against pancreatic cancer.

MicroRNAs act as post-transcriptional regulators of gene expression, and they are involved in the regulation of genes related to various kinds of biological processes, such as development, differentiation, proliferation, and apoptosis of cells. Based on the different targeted genes by different microRNAs in different types of cells, microRNAs exhibit either a positive or negative effect on drug resistance. Accumulating data have demonstrated that microRNAs contribute to the development progression, metastasis, and drug resistance of pancreatic cancer.[3–8] It has been reported that miR-455-3p is implicated in acquired temozolomide resistance in glioblastoma multiforme cells.[9] Our recent microRNA microarray study showed that miR-455-3p was downregulated in human ovarian cancer cells (data not shown). However, it is not clear how miR-455-3p plays its role in drug resistance associated with pancreatic cancer.

Transcriptional co-activator with PDZ-binding motif (TAZ), also known as WW domain-containing transcriptional regulator 1 (WWTR1), is a key downstream component of the Hippo pathway.[10–12] The expression of TAZ is upregulated in a variety of tumors, such as lung cancer,[13] breast cancer,[14] colorectal cancer,[15] oral cancer,[16] and pancreatic cancer.[17,18] Growing evidence suggests that TAZ promotes resistance to various anti-cancer therapies, including cytotoxic chemotherapy.[19] It has been known that many microRNAs, including miR-9-3p, miRNA-125a-5p, miRNA-141, and miRNA-338-3p, directly regulate the expression of TAZ.[20–23] It is unclear whether miR-455-3p also exerts the post-transcriptional regulation of the TAZ gene.

In this study, we show that miR-455-3p is downregulated whereas TAZ is upregulated during the development of drug resistance in pancreatic cancer cells. Furthermore, we reveal that miR-455-3p acts as a direct post-transcriptional regulator of TAZ to contribute to the proliferation and drug resistance of pancreatic cancer cells. The data presented here provide new insights into the role of miR-455-3p and its downstream target TAZ in drug resistance of pancreatic cancer.

RESULTS

miR-455-3p Is Downregulated in Pancreatic Cancer and Contributes to Cell Proliferation of Pancreatic Cancer

miR-455-3p has recently been implicated in acquired temozolomide resistance of glioblastoma multiforme cells and chondrogenesis of mesenchymal stem cells.[9,24] Our recent microRNA (miRNA) microarray study showed that miR-455-3p was downregulated in human

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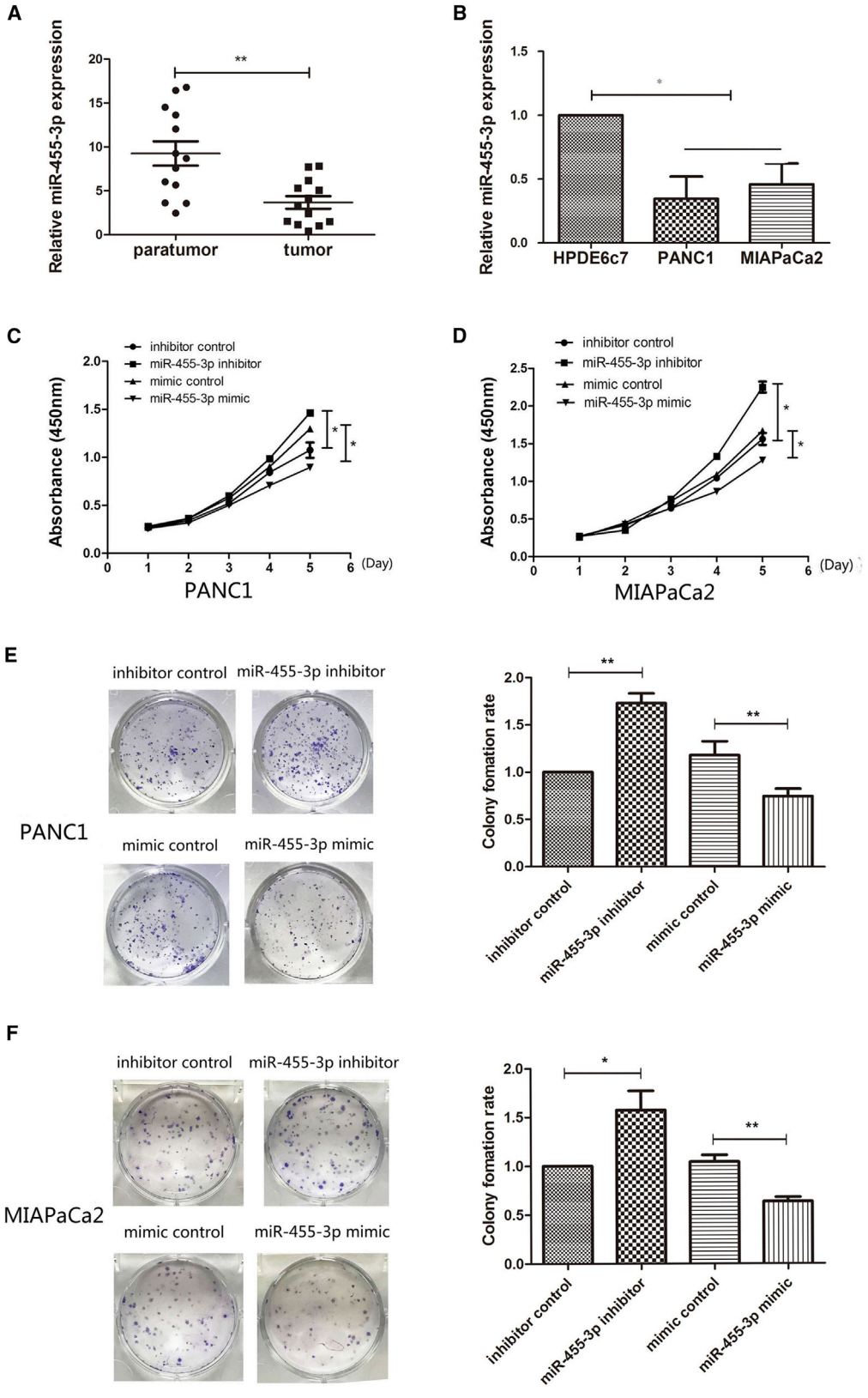

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ovarian cancer cells (data not shown). To test whether miR-455-3p is involved in human pancreatic cancer, we used qPCR to analyze the expression of miR-455-3p in human pancreatic cancer tissues. We found that miR-455-3p levels in 13 human pancreatic tissues were markedly lower than those in 13 counterparts ($p < 0.01$) (Figure 1A). We then used qPCR to analyze the expression of miR-455-3p in two human pancreatic cancer cell lines, PANC-1 and MIAPaCa-2. As expected, miR-455-3p was downregulated in both PANC1 and MIAPaCa2 compared with the normal pancreatic ductal epithelial cell line, HPDE6c7 (Figure 1B). To evaluate the effect of miR-455-3p on cell proliferation in pancreatic cancer, we transfected both PANC1 and MIAPaCa2 cells with miR-455-3p mimics, miR-455-3p inhibitors, or their negative control (NC), and then we measured the cell proliferation by CCK-8 assays and colony formation assay. We observed that the miR-455-3p overexpression decreased the proliferation of both PANC1 and MIAPaCa2, whereas the miR-455-3p inhibition increased the proliferation of both PANC1 and MIAPaCa2 (Figures 1C–1F). These data suggest that miR-455-3p is downregulated in pancreatic cancer and contributes to cell proliferation in pancreatic cancer.

miR-455-3p Decreases Gemcitabine Resistance in Pancreatic Cancer Cell Lines

Since our previous study showed that miR-455-3p was upregulated in drug-resistant human ovarian cancer cells (data not shown), we hypothesized that miR-455-3p might be involved in the drug resistance of pancreatic cancer. Gemcitabine has been widely used in the treatment of pancreatic cancer, and it is an antimetabolite that inhibits processes required for DNA synthesis phase.[25] We conducted qPCR to detect the expression of miR-455-3p in pancreatic cancer cells, and we found that miR-455-3p was downregulated in PANC1 and MIAPaCa2 treated with gemcitabine (20, 40, and 80 μM) for 6 hr (Figure 2A). We then used CCK-8 assay to measure the IC_{50} values of gemcitabine in PANC1- and MIAPaCa2-transfected miR-455-3p mimics, and we observed that miR-455-3p significantly decreased the IC_{50} values of gemcitabine in pancreatic cancer cells (Figures 2B and 2C). Furthermore, we analyzed the cell apoptosis by flow cytometry, and we demonstrated that overexpression of miR-455-3p increased the number of apoptotic cells in both PANC1 and MIAPaCa2 and inhibition of miR-455-3p reduced the number of apoptotic cells when these cells were treated with 20 μM gemcitabine for 48 hr (Figures 2D and 2E). These results indicate that miR-455-3p contributes to the development of gemcitabine resistance in pancreatic cancer cells.

TAZ Is Associated with the Drug Resistance of Pancreatic Cancer Cells

To investigate how miR-455-3p regulates proliferation and drug resistance in pancreatic cancer cells, we predicted TAZ as one potential target of miR-455-3p using TargetScan (http://www.targetscan.org). TAZ is a key downstream component of the Hippo pathway, and it is associated with cancer cell proliferation, invasion, metastasis, epithelial-mesenchymal transition (EMT), and cancer stem cell maintenance.[13,14,26] It has been reported that TAZ is markedly upregulated in pancreatic cancer cell and promotes the growth of pancreatic cancer cells.[17] In this study, we examined the expression of TAZ in pancreatic cancer cell lines via qPCR and western blot, and we found significantly higher expression levels of TAZ mRNA and protein in both PANC1 and MIAPaCa2 compared to HPDE6c7 (Figures 3A and 3B). We also observed that the expression of TAZ mRNA and protein increased in both PANC1 and MIAPaCa2 treated with gemcitabine (Figures 3C–3E).

To determine whether TAZ is involved in the drug resistance of pancreatic cancer cells, we transfected both PANC1 and MIAPaCa2 with TAZ expression vector or TAZ small interfering RNA (siRNA) lentiviral vector, and then we treated the cells with gemcitabine. We showed that inhibition of TAZ decreased the IC_{50} values of gemcitabine and increased the number of apoptotic cells in pancreatic cancer cells (Figures 3F and 3H), whereas overexpression of TAZ increased the IC_{50} values of gemcitabine and decreased the number of apoptotic cells in pancreatic cancer cells (Figures 3G and 3I). These data indicate that TAZ is involved in the drug resistance of pancreatic cancer cells.

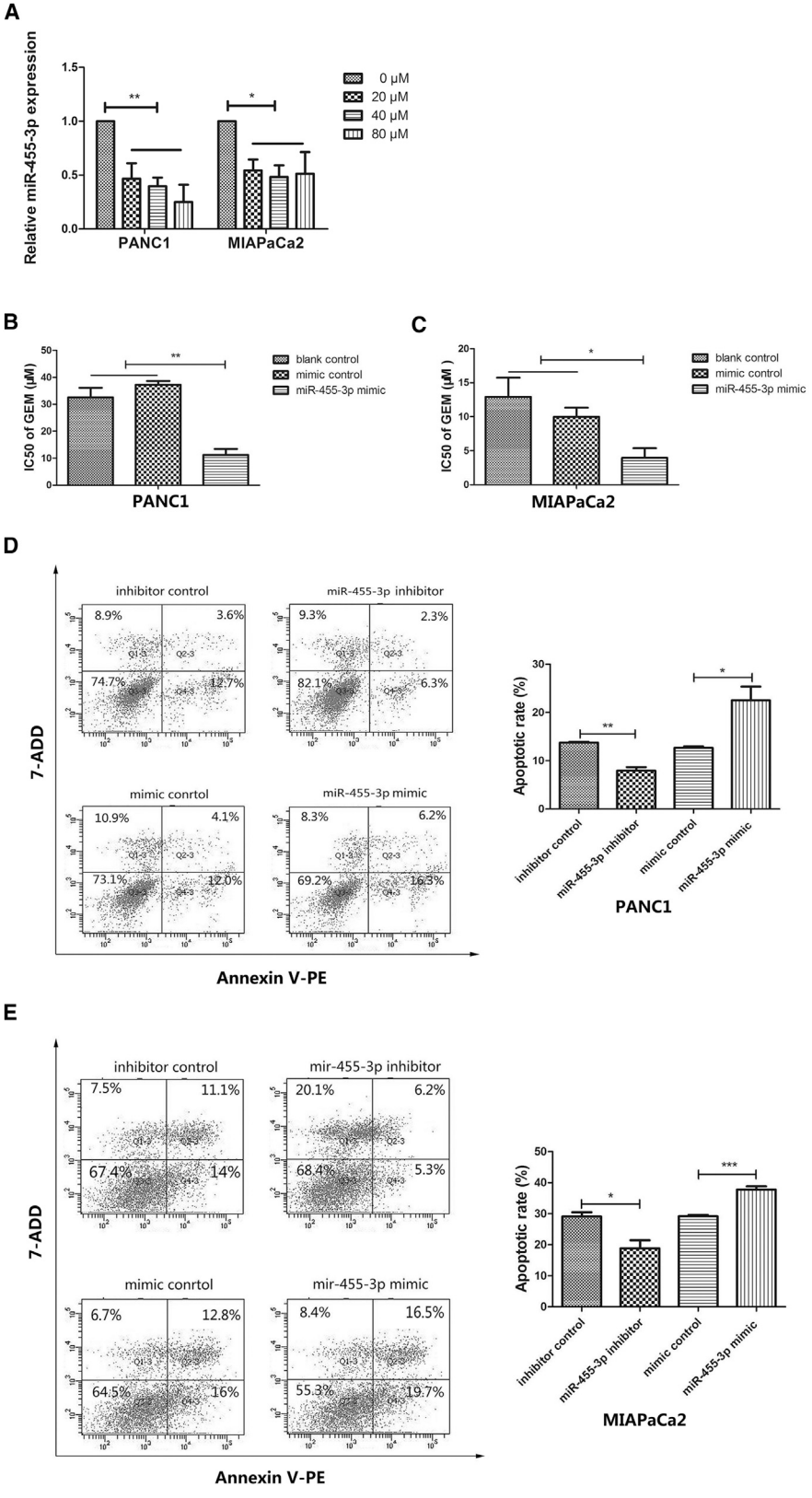
miR-455-3p Participates in Cell Proliferation and Drug Resistance of Pancreatic Cancer Cells via Targeting TAZ

To investigate whether miR-455-3p regulates the expression of TAZ, we examined the effect of miR-455-3p on the TAZ expression level in both PANC-1 and MIAPaCa2 cells. The qPCR and western blot analysis demonstrated that forced overexpression of miR-455-3p suppressed the mRNA and protein levels of TAZ (Figures 4A–4C). Luciferase activity assays revealed that miR-455-3p significantly inhibited luciferase activity of the wild-type (WT), but not mutant (mt), 3′ UTR of TAZ (Figures 4D and 4E). To further determine whether miR-455-3p regulates the TAZ expression, we transfected both PANC1 and MIAPaCa2 cells with miR-455-3p mimics or miR-455-3p mimics plus TAZ expression vector without 3′ UTR of TAZ. We found that miR-455-3p suppressed the endogenous expression of TAZ mRNA and protein, but not the forced overexpression of TAZ expression vector without 3′ UTR of TAZ (Figures 5A–5C). These data indicate that TAZ is a new direct downstream target of miR-455-3p.

To verify whether miR-455-3p participates in cell proliferation and drug resistance of pancreatic cancer cells via targeting TAZ, we tested the cell proliferation and IC_{50} of gemcitabine in both PANC-1 and MIAPaCa2 cells, which were transfected with miR-455-3p mimics

Figure 1. miR-455-3p Is Downregulated in Pancreatic Cancer

(A) Expression of miR-455-3p in pancreatic cancer tissue and the adjacent non-neoplastic tissues. (B) Expression of miR-455-3p in HPDE6c7, PANC-1, and MIAPaCa-2 cells via qPCR. (C and D) The effect of miR-455-3p on proliferation in PANC1 cells (C) and MIAPaCa2 cells (D) transfected with miR-455-3p mimics or inhibitors via CCK-8 assay. (E and F) The effect of miR-455-3p on proliferation in PANC1 cells (E) and MIAPaCa2 cells (F) transfected with miR-455-3p mimics or inhibitors via colony formation assay. The statistical significance was evaluated using Student's t test or one-way ANOVA. All data are presented as the mean ± SD. *$p < 0.05$, **$p < 0.01$.



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or miR-455-3p mimics plus TAZ expression vector. We confirmed that overexpression of miR-455-3 produced the cell proliferation and IC_{50} of gemcitabine in pancreatic cancer cells, whereas the overexpression of TAZ inversed the inhibition of miR-455-3p to endogenous TAZ (Figures 5D–5H). Taken together, these data clearly suggest that miR-455-3p participates in cell proliferation and drug resistance of pancreatic cancer cells via targeting TAZ.

miR-455-3p Functions *In Vivo*

To examine the regulatory effects of miR-455-3p on pancreatic cancer *in vivo*, miR-455-3p was delivered intratumorally in xenografts formed by the relatively more invasive pancreatic cancer cell line PANC-1. We found that the growth of xenografts formed by PANC-1 was significantly inhibited when treated with miR-455-3 agomir compared to agomir NC (Figures 6A–6D). qPCR showed that expression of miR-455-3p in the miR-455-3p agomir treatment was upgraded compared to the agomir NC treatment (Figure 6E). Moreover, miR-455-3p agomir treatment decreased expression levels of Ki67, which serves as a proliferation index, and expression levels of TAZ mRNA and protein in xenografts when compared to those treated with agomir NC (Figures 6F and 6G). These findings indicate that miR-455-3p might inhibit the growth of pancreatic cancer *in vivo* via TAZ.

DISCUSSION

In this report, we provide evidence that miR-455-3p is downregulated in pancreatic cancer and silencing of miR-455-3p increases cell proliferation and reduces cell apoptosis, whereas the forced expression of miR-455-3p reduces cell proliferation and increases cell apoptosis. We have for the first time demonstrated that miR-455-3p participates in cell proliferation and drug resistance of pancreatic cancer cells via directly targeting TAZ.

MicroRNAs are small non-coding RNAs of approximately 22 nt that act as post-transcriptional regulators of gene expression. It has been estimated that microRNAs regulate 30%–60% of protein-coding genes. MicroRNAs are involved in the regulation of genes related to various kinds of biological processes, such as cell proliferation, apoptosis, and drug resistance. Drug resistance is the main cause of failure in the chemotherapeutic treatment of cancer, including pancreatic cancer. Several earlier reports suggested that a few microRNAs, such as miR-21, miR-141, miR-184, miR-199a, miR-205, miR-214, and miR-421, contribute to drug resistance in cancer.[27–33] Based on the different targeted genes by different microRNAs in different types of cells, microRNAs exhibit either a positive or negative effect on drug resistance. The limited data show that miR-455-3p has been implicated in cell proliferation, apoptosis, migration, and invasion of cancer cells.[34–37] An earlier report demonstrated that miR-455-3p was upregulated in glioblastoma multiforme cells with temozolomide resistance and suppression of miR-455-3p led to a cell-killing effect of temozolomide.[9] In this study, we reveal that downregulated miR-455-3p in pancreatic cancer is associated with the proliferation and drug resistance of pancreatic cancer cells. The paradoxical regulation of miR-455-3p on chemotherapy resistance may be due to different types of cancer cells and different drugs.

It has been reported that the direct downstream targets of the miR-455 family include RAB18,[34] Runx2,[35] UBE2B,[36] and ZEB1.[37] miR-455 plays a role in many biological processes via suppressing its downstream targets. Here we identified TAZ as a new direct downstream target of miR-455-3p. TAZ as well as Yes-associated protein (YAP) is the major downstream effector of the Hippo pathway, which regulates tissue homeostasis, organ size, regeneration, and tumorigenesis.[38] Transcriptional upregulation of TAZ appears to be a critical mechanism in the development of drug resistance in cancer cells.[18] Several microRNAs, including miR-9-3p, miRNA-125a-5p, miRNA-141, and miRNA-338-3p, directly regulate the expression of TAZ.[20–23] The data presented in this study suggest miR-455-3p as a new regulator that participates in the growth and drug resistance of pancreatic cancer via directly suppressing TAZ.

Since many potential targets of miR-455-3p and other drug resistance-related genes exist, miR-455-3p may contribute to drug resistance of cancer through targeting other downstream genes. It is well known that YAP, which is similar to its homolog TAZ, functions as a transcriptional cofactor and the core of the Hippo-YAP-signaling pathway, which is implicated in the regulation of organ size, tissue growth, cell proliferation, migration, stem cell self-renewal, tissue regeneration, as well as drug resistance.[39] Because no potential binding sites of miR-455-3p in the 3′ UTR of YAP were predictable, we did not examine the role of YAP in gemcitabine resistance of pancreatic cancer cells. However, we hypothesized that other potential microRNAs may lead to drug resistance via targeting YAP.

In conclusion, our present study provides novel insights into the role of miR-455-3p and its post-transcriptional regulation to TAZ in the drug resistance of pancreatic cancer. Biological or pharmacological intervention based on miR-455-3p may be a promising new strategy to inverse the drug resistance and improve the chemotherapeutic efficacy in human pancreatic cancer cells.

MATERIALS AND METHODS

Cell Lines and Cell Culture

The human pancreatic cancer cell lines PANC-1 and MIAPaCa-2, human normal pancreatic ductal epithelial cell line HPDE6c7, and

Figure 2. miR-455-3p Decreases GEM Resistance in Pancreatic Cancer Cell Lines

(A) qPCR analysis of relative expression levels of mir455-3p in PANC1 and MIAPaCa2 cells treated with GEM (0, 20, 40, and 80 μM) for 6 hr. (B and C) The IC_{50} values of GEM in PANC1 (B) and MIAPaCa2 cells (C) transfected with miR-455-3p mimics, mimic control, or blank control for 72 hr using CCK-8 assay. (D and E) Flow cytometry assessment of apoptosis in PANC1 (D) and MIAPaCa2 cells (E) transfected with miR-455-3p mimics or inhibitors and treated with 20 μM GEM for 48 hr. The total events shown in the lower right-hand and upper right-hand quadrants are apoptotic cells. The statistical significance was evaluated using Student's t test or one-way ANOVA. All data are presented as the mean ± SD. *$p < 0.05$, **$p < 0.01$. GEM, gemcitabine.

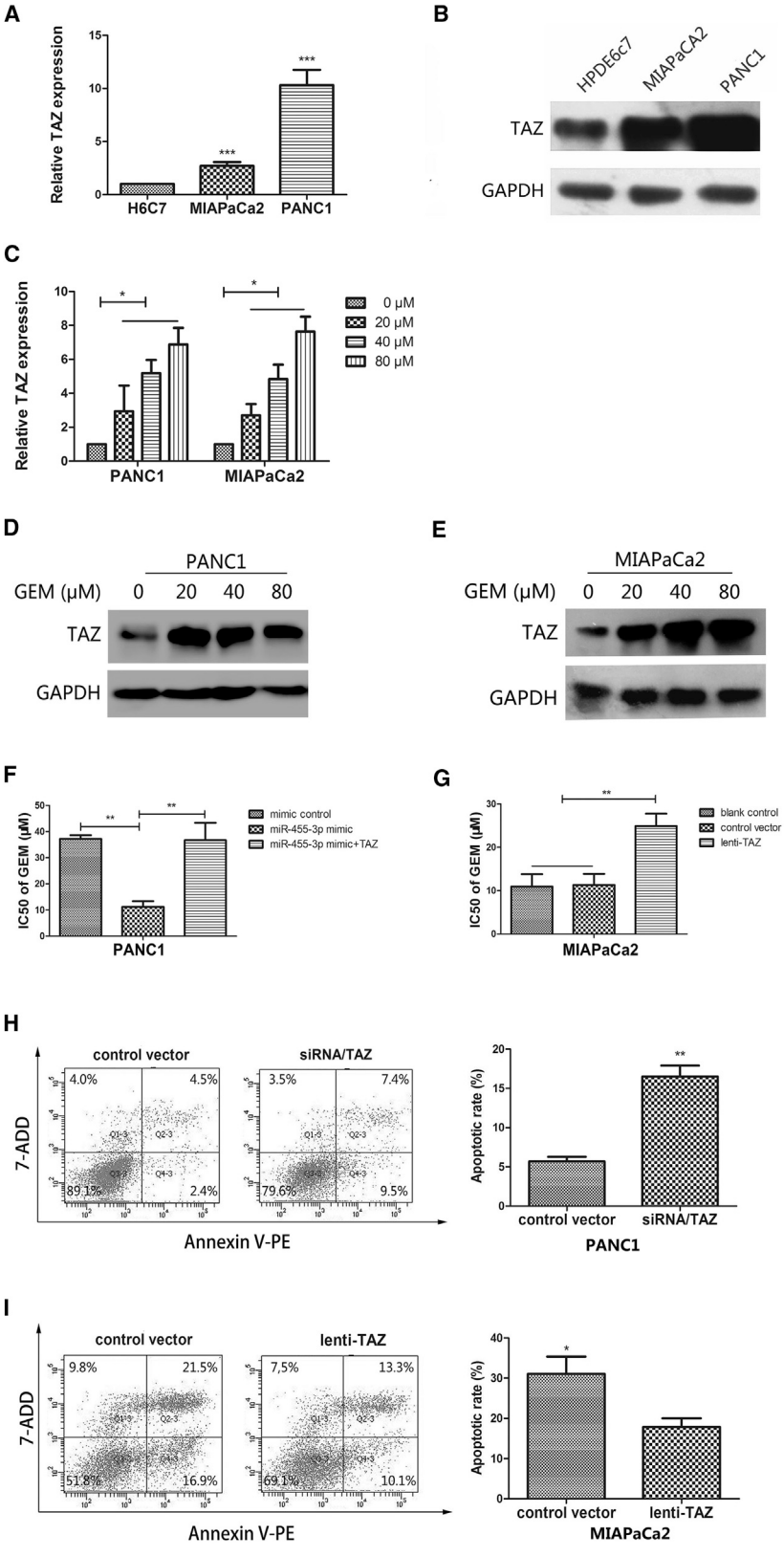


Figure 3. TAZ Is Associated with the Drug Resistance of Pancreatic Cancer Cells

(A and B) Expression of TAZ mRNA and protein in PANC-1 and MIAPaCa-2 cells was detected by qPCR (A) and western blot (B). (C–E) Expression of TAZ mRNA and protein in PANC-1 and MIAPaCa-2 cells treated with GEM (0, 20, 40, and 80 μM) for 6 hr was detected by qPCR (C) and western blot (D and E). (F and G) The IC_{50} values of GEM in PANC1 (F) and MIAPaCa2 cells (G) transfected with miR-455-3p mimics or lenti-TAZ for 72 hr using CCK-8 assay. (H and I) Flow cytometry assessment of apoptosis in PANC1 cells (H) transfected with lenti-siRNA/TAZ and MIAPaCa2 cells (I) transfected with lenti-TAZ, and both of them treated with 20 μM GEM for 48 hr. The total events shown in the lower right-hand and upper right-hand quadrants are apoptotic cells. The statistical significance was evaluated using Student's t test or one-way ANOVA. All data are presented as the mean ± SD. *$p < 0.05$, **$p < 0.01$.GEM, gemcitabine.

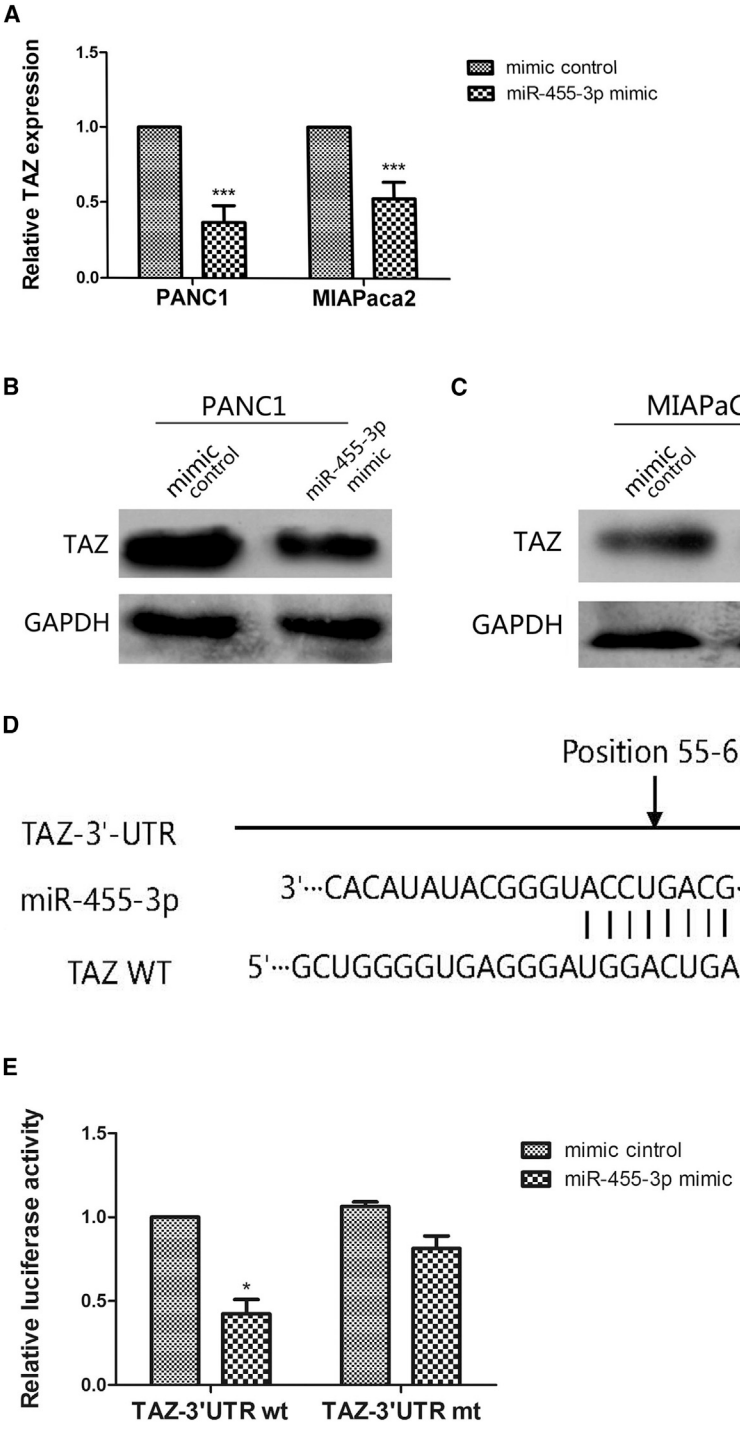


Figure 4. TAZ Is a New Direct Downstream Target of miR-455-3p

(A–C) qPCR (A) and western blot (B and C) analyses of TAZ expression in PANC1 and MIAPaCa2 cells transfected with miR-455-3p mimics. (D) TAZ 3′ UTR contained potential binding sites of miR-455-3p. (E) HEK293T cells were co-transfected with miR-455-3p and wild-type (WT) or mutant (mt) TAZ 3′ UTR. The dual luciferase activity was determined after 48 hr. The statistical significance was evaluated using Student's t test or one-way ANOVA. All data are presented as the mean ± SD. *$p < 0.05$, **$p < 0.01$.

HEK293T cells were provided by the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). All of the cells were cultured in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (NQBB, USA), 100 μg/mL streptomycin, and 100 IU/mL penicillin (Thermo Fisher Scientific, USA). Cells were incubated in a humidified incubator at 37°C with 5% CO_2.

Patients and Tissue Samples

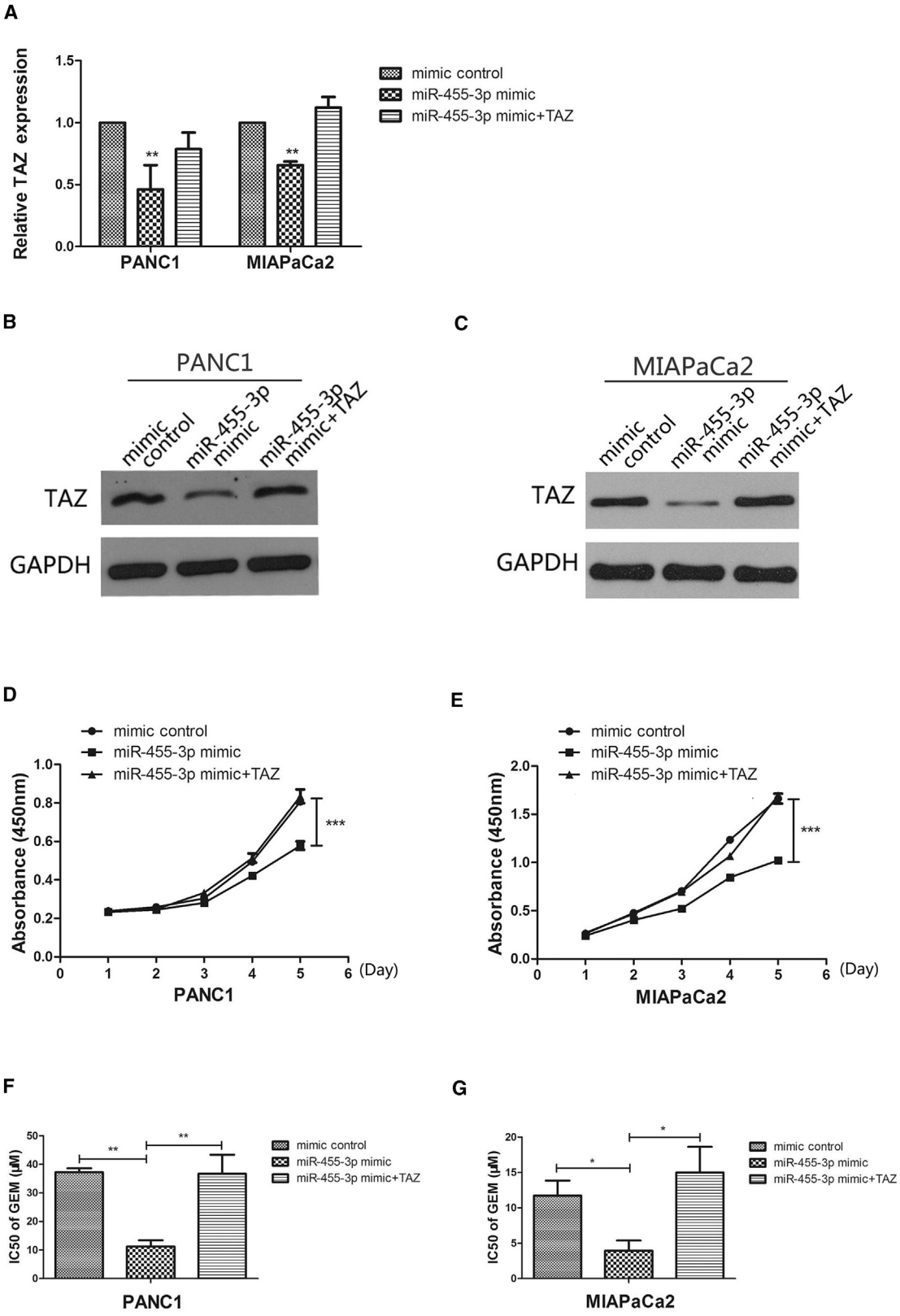
Human pancreatic cancer and corresponding noncancerous tissue used in this study were obtained from patients who underwent surgical resection. All patients whose tumor tissue was examined had not had any neoadjuvant chemotherapy prior to surgical resection. A total of 13 paired tissues involved in qPCR assay were from the Renmin Hospital of Wuhan University (n = 8) and the Central Hospital of Wuhan (n = 5). These samples were snap-frozen in liquid nitrogen immediately and stored at −80°C until RNA extraction. The use of tissues for this study was approved by the Ethics Committee of Renmin Hospital of Wuhan University and the Central Hospital of Wuhan.

Cell Transfection

Hsa-miR-455-3p mimics (sense: 5′-GCAGUCCAUGGGCAUAUACAC-3′; antisense: 5′-GUAUAUGCCCAUGGACUGCUU-3′), hsa-miR-455-3p inhibitors (sense: 5′-GUGUAUAUGCCCAUGGACUGC-3′), hsa-miR mimics NC (sense: 5′-UUCUCCGAACGUGUCACGUTT-3′; antisense: 5′-ACGUGACACGUUCGGAGAATT-3′), and hsa-miR inhibitors NC (5′-CAGUACUUUUGUGUAGUACAA-3′) were synthesized by Genepharma (Shanghai, China). PANC-1 and MIA paCa2 cells were transfected with miR-455-3p mimics (40 nM), miR-455-3p inhibitors (80 nM), or their NC using Lipofectamine 2000 (Invitrogen, USA), according to the manufacturer's instructions. The TAZ lentiviral expression vector (lenti-TAZ) and TAZ siRNA lentiviral expression vector (lenti-siRNA/TAZ) were constructed by Shanghai GeneChem (Shanghai, China). The lentiviruses were transfected according to the manufacturer's instructions.

Total RNA Extraction and qPCR Analysis

Total RNA from tissues and cell lines was extracted using TRIzol Reagent (Invitrogen, USA), and subsequent synthesis of cDNA was



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carried out according to the manufacturer's protocols (TOYOBO, Japan). To evaluate TAZ mRNA levels, the reverse transcription products were analyzed using UltraSYBR Mixture (ComWin Biotech, China) on an ABI StepOne Plus qPCR System (Applied Biosystems, USA). GAPDH mRNA was used as an endogenous control. The expression level of miR-455-3p was evaluated using the same qPCR system. Endogenous U6 small nuclear RNA (snRNA) was used for normalization. The primers for amplification were as follows: GAPDH (sense: 5′-TCTGACTTCAACAGCGACAC-3′; antisense: 5′-CAAATTCGTTGTCATACCAG-3′); and TAZ (sense: 5′-GTCA CCAACAGTAGCTCAGATC-3′; antisense: 5′-AGTGATTACAGC CAGGTTAGAAAG-3′). Primers for miR-455-3p and U6 were obtained from Ribobio (Guangzhou, China). The $2^{-\Delta\Delta}$CT method was used to calculate changes in mRNA expression levels.

Western Blot Analysis

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitors (Beyotime, Shanghai, China), and the protein concentration was measured using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, USA). Soluble lysate was mixed with loading buffer and boiled for 10 min. Proteins were separated by 10% SDS-PAGE, transferred to NC membranes, and then incubated with primary antibodies against TAZ (1:1,000, CST, USA) or GAPDH (1:1,000, Santa Cruz Biotechnology, USA) overnight at 4°C. The membranes were further incubated with secondary antibodies (LI-COR Biosciences, USA) for 1 hr at 37°C, and the protein levels were detected by the enhanced chemiluminescence (ECL) Western Blot Analysis Detection System (Amersham, USA).

Analysis of Cell Proliferation and IC_{50}

The Cell Counting Kit-8 Assay Kit (CCK-8, Dojindo, Japan) was used to determine the cell proliferation and drug resistance. Cells were seeded in 96-well plates at a density of 2×10^3 cells per well, and 10 μL CCK-8 solution was added to each well the next day. The cells were incubated for 2 hr at 37°C, and the absorbance at 450 nm was determined using EnSpire Multimode Plate Reader (PerkinElmer, USA).

Colony Formation Assay

Cells were seeded in 6-well plates at a density of 500 cells per well; visible cell colonies appeared after 2 weeks in complete medium at 37°C. The cell colonies were then fixed with methanol for 15 min and stained with 0.1% crystal violet for 30 min.

Analysis of Cell Apoptosis

Pancreatic cancer cells were transfected in 6-well plates and 20 mg gemcitabine (Sigma, USA) was added to each well the next day. After incubating for 48 hr, cells were stained using the Annexin V-PE Detection Kit (BD Biosciences, USA), according to the manufacturer's protocols. All the samples were analyzed using the FACS Caliber II Sorter and Cell Quest FACS System (BD Biosciences, USA).

Luciferase Activity Assay

Cells were seeded in 24-well plates for 24 hr. The miR-455-3p mimics or mimics NC and the psiCHECK2-UTR vector (Promega, USA) were co-transfected into HEK293T cells using Lipofectamine 2000 (Invitrogen, USA) and cultured for 48 hr. Then the luciferase activities were measured using Dual-Luciferase Reporter Assay System (Promega, USA), according to the manufacturer's protocols.

Tumor Formation in Xenograft Model

The 6-week-old male BALB/c nude mice were purchased from Beijing Vital River Laboratory Animal Technology (Beijing, China) and housed under specific pathogen-free conditions. 5×10^6 PANC1 cells suspended in 100 μL PBS-containing 25% Matrigel were subcutaneously injected into the flank of the nude mice. When the tumor volume reached approximately 100 mm^3, miR-455-3 agomir and agomir NC (GenePharma, Shanghai, China) were respectively injected into the tumor at multiple sites three times every 5 days. Each treatment group had five animals. Tumor volume was measured every 5 days. All mice were sacrificed after 30 days. Tumor grafts from nude mice were dissected for immunohistochemistry (IHC) and qPCR analysis. Tumor volume was monitored by measuring the length (L) and width (W) with calipers and calculated using the following formula: tumor volume = $L \times W^2/2$.

Histological Analysis

IHC analysis was performed per standard protocols. The primary antibodies used for IHC analysis were a TAZ antibody (1:100, CST, USA) and Ki67 antibody (1:100, CST, USA).

Statistical Analysis

All statistical analyses were conducted using SPSS software and illustration data were performed by GraphPad Prism. The statistical significance was evaluated using Student's t test or one-way ANOVA, and it is presented as the mean ± SD; $p < 0.05$ was considered statistically significant.

AUTHOR CONTRIBUTIONS

Y.Z. and T.Z. conceived and designed the experiments. T.Z., X.T., X.C., and Y.D. performed the experiments. Y.Z., T.Z., X.H., and H.L. analyzed data. Y.Z. and T.Z. wrote the manuscript.

Figure 5. miR-455-3p Participates in Cell Proliferation and Drug Resistance of Pancreatic Cancer Cells via Targeting TAZ

(A) qPCR analysis of TAZ in PANC1 and MIAPaCa2 cells transfected with miR-455-3p mimic or miR-455-3p mimic plus TAZ expression vector. (B and C) Western blot analysis of TAZ in PANC1 (B) and MIAPaCa2 cells (C) transfected with miR-455-3p mimic or miR-455-3p mimic plus TAZ expression vector. (D and E) The cell proliferation of PANC1 (D) and MIAPaCa2 cells (E) transfected with miR-455-3p mimic or miR-455-3p mimic plus TAZ expression vector. (F and G) The IC_{50} values of GEM in PANC1 (F) and MIAPaCa2 cells (G) transfected with miR-455-3p mimic or miR-455-3p mimic plus TAZ expression vector. The statistical significance was evaluated using one-way ANOVA. All data are presented as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

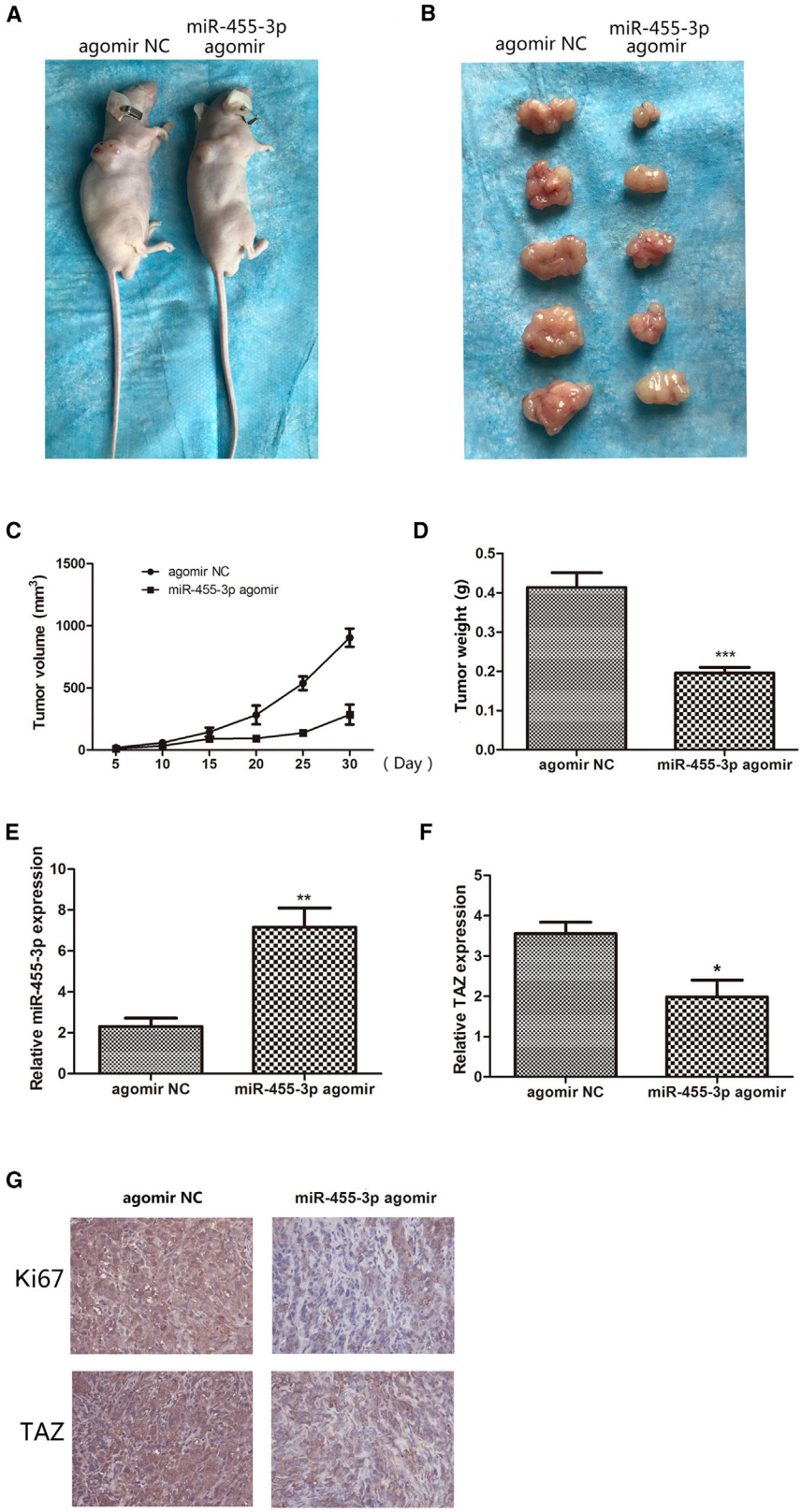

Figure 6. miR-455-3p Functions *In Vivo*

(A–D) miR-455-3p significantly inhibited the growth of xenografts formed by PANC1 when compared with the NC group. (A) Representative images of tumor-bearing mice. (B) Representative images of tumors isolated from tumor-bearing mice. (C) Tumor volume in nude mice. (D) Tumor weight in nude mice. Each group contained five mice (n = 5). (E) Expression of miR-455-3p was detected by qPCR. (F) Expression of TAZ mRNA was detected by qPCR. (G) The protein expression of Ki67 and TAZ was detected by IHC. The statistical significance was evaluated using Student's t test. All data are presented as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

CONFLICTS OF INTEREST

The authors declare no competing financial interests.

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