Interferon regulatory factor 5-induced upregulation of zinc-finger protein 217 promotes pancreatic carcinoma progression

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Abstract. The aim of the present study was to investigate the molecular mechanisms of zinc-finger protein 217 (ZNF217) in pancreatic carcinoma (PC) progression. ZNF217-associated expression and survival data from patients with PC were retrieved from the Gene Expression Profiling Interactive Analysis server. The mRNA expression level of ZNF217 was detected by reverse transcription-quantitative PCR. Cell Counting Kit-8, colony formation, wound-healing and Transwell assays were conducted to assess cellular proliferation, migratory and invasive abilities. Proliferation was also examined by immunofluorescence detection of Ki67 expression, and chromatin immunoprecipitation (ChIP) and luciferase reporter assays were performed to detect the interaction between ZNF217 and interferon regulatory factor 5 (IRF5). ZNF217 was found to be significantly upregulated in tumor tissues and cancer cell lines, which was associated with a poor survival rate in patients with PC. ZNF217 silencing markedly suppressed cellular proliferation and migratory and invasive abilities, as well as decreased the expression of Ki67. IRF5 was also upregulated in PC tumor tissues and was shown to positively regulate the activity of the ZNF217 promoter and its mRNA expression levels. Furthermore, ChIP assays demonstrated that IRF5 bound to the promoter region of ZNF217 *in vitro*. In conclusion, ZNF217 silencing exerted notable inhibitory effects on the progression of PC. Thus, ZNF217 may serve as a potential target for developing novel therapeutic strategies for PC.

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Introduction

Pancreatic carcinoma (PC) is one of the most aggressive solid malignant tumors, seriously threatening human health and life. The incidence of PC has risen in recent years, and 60,430 new PC cases and 48,220 PC-related deaths were estimated in 2021 worldwide (1). Despite considerable improvements in surgical techniques, chemotherapeutic regimens and neoadjuvant chemoradiotherapies in recent years, the 5-year survival rate of patients with PC remains <5% on account of early local invasion and distant metastasis (2). Thus, to improve clinical diagnosis and treatment, identification of the molecular mechanisms associated PC tumorigenesis is of great importance.

Zinc-finger protein 217 (ZNF217), a member of the Krüppel-like family of transcriptional factors, is an oncogene that coordinates complex cellular progresses, controls the early and final stages of tumor development, and is considered to be a powerful biomarker of tumorigenesis (3). ZNF217 expression is frequently amplified in malignant tumors, such as breast cancer, hepatocellular carcinoma and colorectal cancer; high expression levels of ZNF217 are associated with a poor clinical prognosis and the development of metastases (4-6). However, whether ZNF217 is involved in the progression of PC remains unclear.

Transcription factor interferon regulatory factor 5 (IRF5), a member of the IRF family, has been widely recognized as a key regulator of inflammation, providing novel avenues for the development of therapeutic agents for inflammatory diseases (7). Accumulating evidence has demonstrated that IRF5 is involved in the regulation of genes induced by oncogenesis. For example, IRF5 was reported to be highly expressed in human thyroid cancer cells and to promote cancer cell proliferation, accelerating the development of thyroid cancer (8). Conversely, IRF5 also acts as a tumor suppressor in both gastric and renal cancer, where its expression is downregulated (9,10). Thus, the true role of IRF5 appears to depend on the cancer type in question. Until now, the role of IRF5 in PC has not been investigated.

According to predictions from the AnimalTFDB3.0 website (http://bioinfo.life.hust.edu.cn/AnimalTFDB/), an association was observed between IRF5 and ZNF217, suggesting that IRF5 may be a transcription factor of ZNF217. However, until now, there is no evidence demonstrating the link between IRF5 and ZNF217 expression in PC. Therefore, the aim of the present study was to determine the role of ZNF217 and its connection with IRF5 in PC, and to investigate its effects on cellular proliferation, migration and invasiveness, as well as its potential molecular mechanism.

Materials and methods

Bioinformatics analysis. Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn) is an interactive web server for the analysis of RNA sequencing expression data from The Cancer Genome Atlas and the Genotype Tissue Expression projects (11). The expression of ZNF217 and IRF5 was analyzed using the GEPIA database.

Cell culture. The HPDE6c7 normal human pancreatic ductal epithelial cell line and human PC cell lines (BxPC-3, SW1990, PANC-1 and CFPAC-1) were obtained from the Shanghai Advanced Research Institute, Chinese Academy of Sciences. Cells were cultured in DMEM (Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin, and maintained at 37˚C (5% CO_2) in a humidified atmosphere.

Transfection. cDNA encoding ZNF217 or IRF5 was amplified and inserted into the pcDNA3.1 vector (Shanghai GenePharma Co., Ltd) to overexpress ZNF217 (Oe-ZNF217) or IRF5 (Oe-IRF5), respectively. An empty vector was used as the negative control (Oe-NC) for Oe-ZNF217 and Oe-IRF5. Short hairpin (sh)RNAs (pGPU6) targeting ZNF217 (shRNA-ZNF217#1 and shRNA-ZNF217#2), shRNA-IRF5#1, shRNA-IRF5#2 were obtained from Shanghai GenePharma Co., Ltd., and an empty pGPU6 vector was considered as the negative control (shRNA-NC). The shRNA sequences were as follows: shRNA-ZNF217#1 forward, 5'-CCGGCAAGGTATACTCTTCAAATAACTCGAGTTATTTGAAGAGTATACCTTGTTTTTG-3' and reverse, 5'-AATTCAAAAACAAGGTATACTCTTCAAATAACTCGAGTTATTTGAAGAGTATACCTTG-3'; shRNA-ZNF217#2 forward, 5'-CCGGCATGGTGATGAGGGCATTTAACTCGAGTTAAATGCCCTCATCACCATGTTTTTG-3' and reverse, 5'-AATTCAAAAACATGGTGATGAGGGCATTTAACTCGAGTTAAATGCCCTCATCACCATG-3'; shRNA-IRF5#1 forward, 5'-CCGGGGGTGCACACCCATGTTATAACTCGAGTTATAACATGGGTGTGCACCCTTTTTG-3' and reverse, 5'-AATTCAAAAAGGGTGCACACCCATGTTATAACTCGAGTTATAACATGGGTGTGCACCC-3'; and shRNA-IRF5#2 forward, 5'-CCGGATGCTAGATATCTGCATATTTCTCGAGAAATATGCAGATATCTAGCATTTTTTG-3' and reverse, 5'-AATTCAAAAAATGCTAGATATCTGCATATTTCTCGAGAAATATGCAGATATCTAGCAT-3'. Cells were seeded into 6-well plates ($2x10^5$ cells/well) 24 h before transfection, and then transfected with a final concentration of 50 nM shRNA and/or 15 nM overexpression vectors using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions at 37˚C for 48 h. Subsequently, cells were harvested for subsequent experiments.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. A UV spectrophotometer was used to determine the RNA A280/A260 value. Total RNA was reverse transcribed into cDNA using the RT Reagent Kit (Takara Biotechnology Co., Ltd.) in accordance with the manufacturer's protocols. qPCR was performed using the Real-time PCR Sequence Detection system (Bio-Rad Laboratories, Inc.) with the SYBR Green PCR Master mix (Thermo Fisher Scientific, Inc.) per the manufacturer's protocols. The thermocycling conditions were as follows: 50˚C for 2 min and 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 sec and 60˚C for 30 sec. The primer sequences were as follows: ZNF217 forward, 5'-GAGAAGCGAATGGTGAAAGC-3' and reverse, 5'-CAGCGCTCAAGTATGCAAAA-3'; IRF5 forward, 5'-CATTACTGTACAGGTGGTGC-3' and reverse, 5'-AGATGTGATGGAGCTCCTTG-3'; and β-actin forward, 5'-CTCCATCCTGGCCTCGCTGT-3' and reverse, 5'-GCTGTCACCTTCACCGTTCC-3'. β-actin was used as the internal reference, and the relative expression level of each gene was calculated using the $2^{-\Delta\Delta Cq}$ method (12).

Cell Counting Kit-8 (CCK-8) assay. Cell viability was assessed using the CCK-8 assay (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. Briefly, cells were incubated in 96-well plates ($5x10^3$ cells/well) at 37˚C. CCK-8 solution was then added to each well at 24, 48 and 72 h, and the cells were incubated for another 3 h at 37˚C. The absorption value of each well at 450 nm was detected using an ELx808 microplate reader (BioTek Instruments, Inc.).

Colony formation assay. Cells were resuspended in DMEM and seeded into 6-well plates (500 cells/well) and incubated for 2 weeks. During this period, the culture media were replaced every 2-3 days. Finally, the cells were washed with PBS, fixed with 100% methanol for 10 min at room temperature and stained with 0.5% crystal violet for 5 min at room temperature. The number of cell colonies (>50 cells) was observed and counted using an inverted microscope (Olympus Corporation).

Immunofluorescence assay. Cells were seeded in 24-well plates ($2x10^4$ cells/well). Following a 24-h incubation at 37˚C, cells were fixed in 4% paraformaldehyde at 4˚C for 30 min, washed three times with PBS and then permeabilized with 0.5% Triton X-100 for 15 min at room temperature. Subsequently, the cells were blocked with 5% bovine serum albumin (Thermo Fisher Scientific, Inc.) at room temperature for 60 min, followed by incubation with a primary antibody against Ki67 (1:200; cat. no. ab15580; Abcam) overnight at 4˚C. A mouse anti-rabbit immunoglobulin G (IgG)-fluorescein isothiocyanate (FITC) secondary antibody (1:100; cat. no. sc-2359; Santa Cruz Biotechnology, Inc.) was added and the cells were incubated at room temperature for 1 h in the dark, followed by the addition of DAPI at room temperature for 3 min (also in the dark). Immunofluorescence images were acquired using

a confocal laser microscope (magnification, x100; Leica Microsystems GmbH).

Wound-healing assay. Cells were cultured to 100% confluence and the monolayers were scratched using a 200-μl pipette tip to create a wound. Non-adherent cells were removed by washing three times with PBS, and cells were then cultured in fresh serum-free DMEM for 48 h. Images were captured at 0 and 48 h using an inverted light microscope (magnification, x100; Olympus Corporation).

Transwell assay. Cells were resuspended in 200 μl serum-free DMEM and then seeded into the upper chambers of 24-well Transwell inserts ($5x10^4$ cells/well), which were pre-coated with Matrigel (BD Biosciences) overnight at 37˚C; 600 μl DMEM supplemented with 10% FBS was added to the lower chambers. After a 24-h incubation period, the cells on the upper surface of the chamber were removed using a cotton swab, and the invasive cells were fixed with 100% methanol at room temperature for 10 min and stained with 0.1% crystal violet for 5 min at room temperature. The images were captured using an inverted light microscope (magnification, x100; Olympus Corporation).

Chromatin immunoprecipitation (ChIP) assay. It was predicted from JASPAR database (https://jaspar.genereg.net/) that there were three potential IRF5 responsive elements binding to the ZNF217 promoter region. To verify this binding relationship at the E2 region, ChIP assay was conducted. Cells were cross-linked by incubation with 1% formaldehyde for 10 min at 37˚C, followed by quenching at room temperature for 5 min with glycine (125 mM; Sigma-Aldrich; Merck KGaA). Subsequently, cells were harvested by centrifugation at 300 x g for 3 min at room temperature, washed with PBS, and lysed in SDS lysis buffer (Upstate Biotechnology, Inc.), and the chromatin from the cell lysates was sonicated with a 10-sec on and 10-sec off mode for 12 cycles on ice to shear the DNA into fragments at 20 kHz. Following sonication, the samples were centrifuged at 13,000 x g for 10 min at 4˚C, and the supernatant was pre-adsorbed with 80 μl salmon sperm DNA/protein A-agarose (MilliporeSigma). The 100 μl lysates were incubated with 5 μg anti-IgG (cat. no. ab109489; Abcam) and anti-IRF5 (cat. no. ab181553; Abcam) antibodies for 2 h at 4˚C. The immune complexes were recovered using a salmon sperm DNA-saturated protein A agarose gel (MilliporeSigma) according to the manufacturer's protocol. Following immunoprecipitation and elution, the eluent was heated to 65˚C to reverse the cross-link. The immunoprecipitated DNA was purified using the ChIP DNA Clean & Concentrator Kits (Zymo Research) according to the manufacturer's instructions and was quantified by RT-qPCR as aforementioned. The ChIP primer sequences used for the detection of ZNF217 enrichment on E2 promoter were as follows: Forward, 5'-TCTTCA TGCCTCTACCCATCC-3', and reverse, 5'-ATGGCTCTG CCCTAATCCTCT-3'.

Dual-luciferase reporter assay. Three deletion mutants (E1 Del, E2 Del and E3 Del) from potential IRF5 responsive elements in the ZNF217 promoter region (E1, E2, and E3) were obtained from Genloci Biotechnologies, Inc. The IRF5-binding motif and ZNF217 promoter full length or the deletion mutants were ligated into pGL3-based plasmids (Promega Corporation). When cell growth reached 70%, cells were transfected with 15 nM pGL3-based reporter constructs and pRL-SV40 (which was used as the internal reference) using Lipofectamine 2000. After 48 h incubation at 37˚C, the relative luciferase activity of each group was determined using a Dual-Luciferase Reporter Assay kit (Promega Corporation); luciferase activity was normalized to *Renilla*.

Statistical analysis. All data were analyzed using SPSS software (version 20; IBM Corp.) and are presented as the mean ± SD from at least three repeats. A paired Student's t-test (comparisons between two groups) or one-way ANOVA with Tukey's post hoc test (comparisons among ≥3 groups) were used to compare differences. For the Kaplan-Meier survival curve analysis, Mantel-Cox (log-rank) test was performed. To determine the correlation between ZNF217 and IRF5, the Pearson's correlation coefficient was calculated using the non-log scale and visualized using the log-scale axis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

ZNF217 expression is upregulated in patients with PC and is associated with a poor outcome. Data from GEPIA were analyzed to investigate ZNF217 expression in PC. Significantly higher expression levels of ZNF217 were observed in the tumor tissues of patients with PC compared with the normal tissues (Fig. 1A). The expression of ZNF217 was also found to vary with PC tumor stage (Fig. 1B). The overall trend of ZNF217 expression was upward from stage I to IV, with the exception of at stage III. The abnormally low expression of ZNF217 expression at stage III may be because relatively few samples were used. In addition, Kaplan-Meier curve analysis revealed that high ZNF217 expression was significantly associated with poor overall survival rate ($P<0.05$), but was not significantly associated with disease-free survival rate ($P=0.079$) (cut-off value=50% used to separate high and low groups) (Fig. 1C and D). These findings demonstrated that ZNF217 is highly expressed in the tumor tissues of patients with PC and suggested that ZNF217 may be associated with poor survival rate.

ZNF217 silencing suppresses PC cell proliferation. RT-qPCR was performed to detect the mRNA expression levels of ZNF217 in different PC cell lines (BxPC-3, SW1990, PANC-1 and CFPAC-1) compared with the normal human pancreatic ductal epithelial cell line (HPDE6c7). The results revealed that the ZNF217 mRNA expression was significantly increased in PC cell lines, but particularly in SW1990 and CFPAC-1 cells (Fig. 2A), thus SW1990 and CFPAC-1 cells were used for subsequent experiments. To investigate the specific role of ZNF217 in PC, ZNF217 expression was silenced by transfection with shRNA-ZNF217#1 or shRNA-ZNF217#2 (Fig. 2B). Owing to a higher transfection efficacy, shRNA-ZNF217#2 was used for further investigation. CCK-8 assays revealed that cellular viability was inhibited by silencing ZNF217 expression, and that the inhibitory effects became more significant

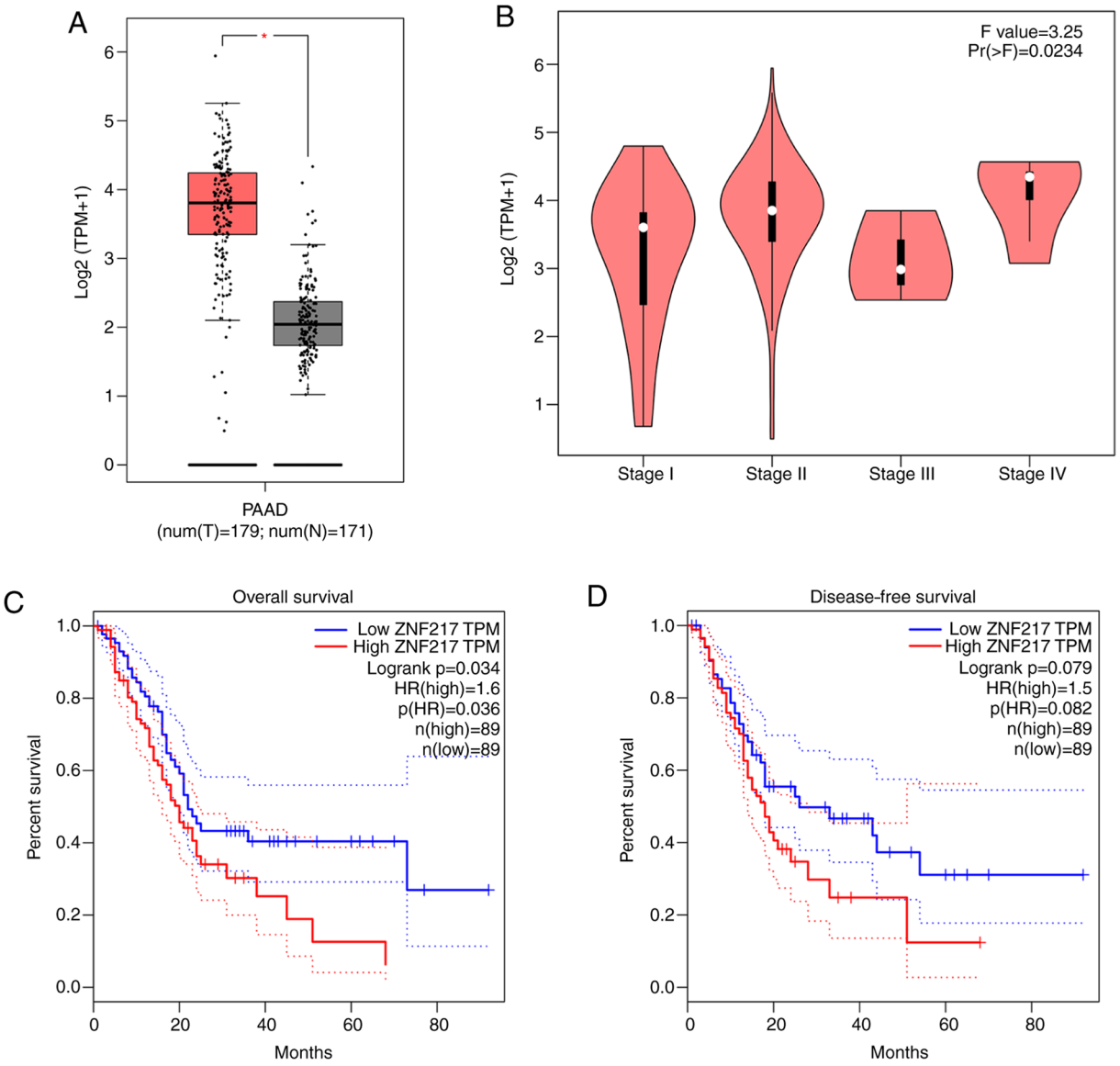

Figure 1. ZNF217 expression is increased and is associated with a poor outcome in patients with PC. (A) GEPIA data from The Cancer Genome Atlas and the Genotype Tissue Expression databases were used to analyze ZNF217 expression in normal samples (grey box) and tumor samples (red box) from patients with PC. Analysis of the association between ZNF217 expression and (B) tumor stage, (C) overall survival and (D) disease-free survival rate in patients with PC was conducted using GEPIA. *P<0.05. GEPIA, Gene Expression Profiling Interactive Analysis; HR, hazard ratio; N, normal; PAAD, pancreatic adenocarcinoma; PC, pancreatic carcinoma; T, tumor; TPM, transcripts per million; ZNF217, zinc-finger protein 217.

with prolonged incubation times (Fig. 2C and D). The colony formation assay results showed that ZNF217 knockdown significantly decreased the number of SW1990 and CFPAC-1 cell colonies formed (Fig. 2E and F). In addition, immunofluorescence detection revealed markedly decreased expression of Ki67 upon ZNF217 silencing in both SW1990 and CFPAC-1 cells (Fig. 2G). These findings suggested that ZNF217 silencing suppressed cellular proliferation in PC.

ZNF217 silencing suppresses PC cell migration and invasion. Wound-healing and Matrigel assays were performed to evaluate PC cell migratory and invasive abilities, respectively. As shown in Fig. 3A and B, the distance of cell migration was reduced in CFPAC-1 and SW1990 cells when ZNF217 expression was knocked down, indicating that ZNF217 silencing inhibited cellular migratory ability. In addition, the number of invasive cells was decreased when ZNF217 was silenced (Fig. 3C and D), indicating that ZNF217 silencing inhibited cellular invasive ability.

IRF5 is upregulated in PC and regulates ZNF217 expression by promoting ZNF217 transcription. To further verify the aforementioned results, the expression of IRF5 in PC was assessed using bioinformatics analysis. The GEPIA database revealed high expression levels of IRF5 in PC tumor tissues compared with normal tissue (Fig. 4A). In addition, ZNF217 expression showed a moderate positive linear correlation with that of IRF5 in PC tissue samples (Fig. 4B). Notably, three putative IRF5 responsive elements (E1, E2 and E3) were identified in the ZNF217 promoter region (Fig. 4C). Through gain- and loss-of-expression experiments, the effects of IRF5 on ZNF217 in PC were investigated. RT-qPCR analysis

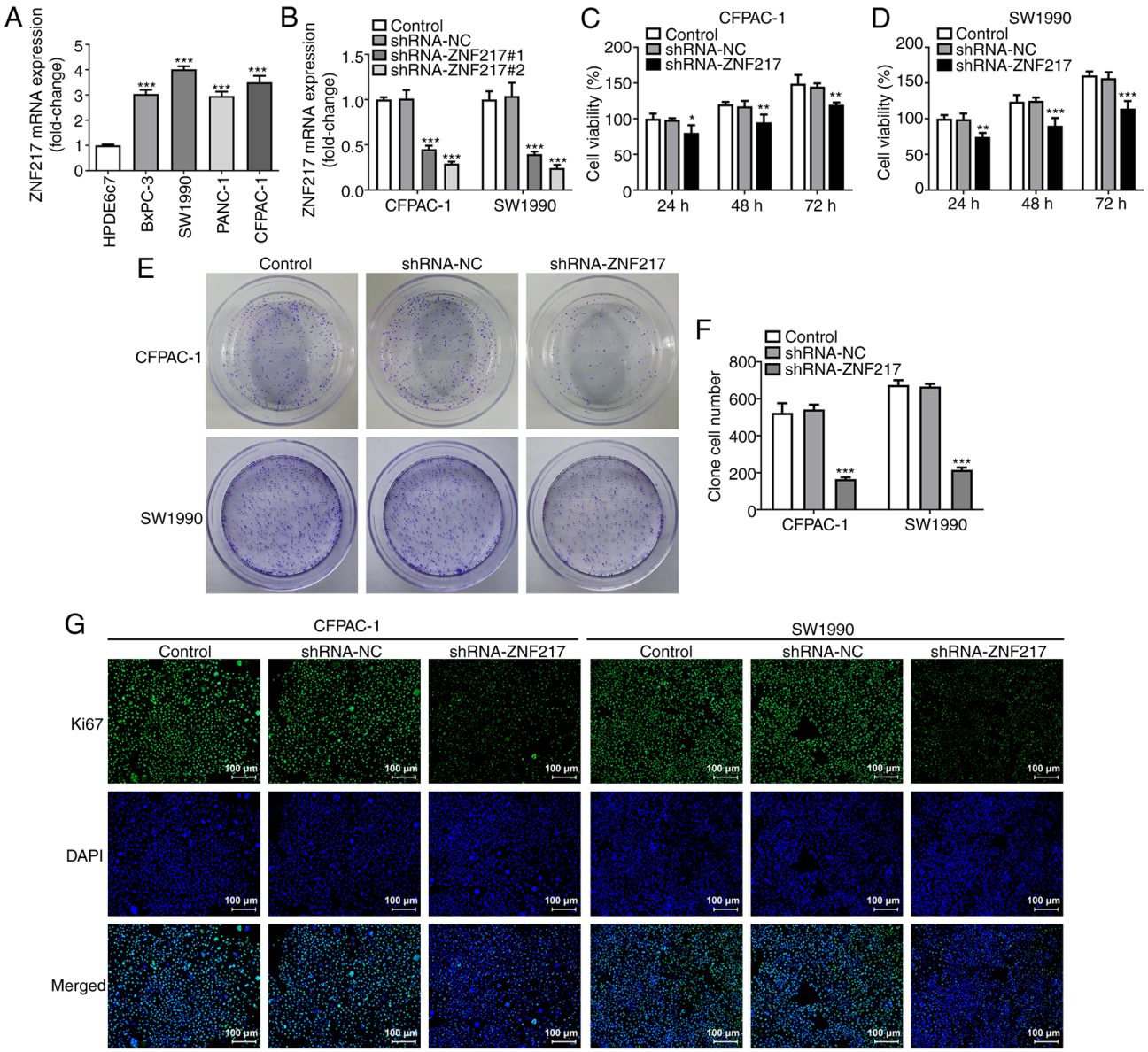

Figure 2. ZNF217 silencing suppresses cellular proliferation in PC. (A) ZNF217 mRNA expression was detected in different PC cell lines (BxPC-3, SW1990, PANC-1 and CFPAC-1) and normal human pancreatic ductal epithelial cells (HPDE6c7). ***P<0.001 vs. HPDE6c7 cells. (B) ZNF217 mRNA expression after transfection with shRNA-ZNF217#1, #2 or the shRNA-NC in CFPAC-1 and SW1990 cells. Viability of (C) CFPAC-1 and (D) SW1990 cells at 24, 48 and 72 h post-transfection was detected using the Cell Counting Kit-8 assay. (E) Colony formation assay and (F) number of colonies formed following transfection. (G) Expression of Ki67 in both CFPAC-1 and SW1990 cells was detected using immunofluorescence analysis. *P<0.05, **P<0.01 and ***P<0.001 vs. shRNA-NC. NC, negative control; PC, pancreatic carcinoma; shRNA, short hairpin RNA; ZNF217, zinc-finger protein 217.

demonstrated successful transfection of the shRNA-IRF5#2 plasmid used for IRF5 silencing and the Oe-IRF5 plasmid used for IRF5 overexpression (Fig. 4D). IRF5 knockdown significantly decreased ZNF217 mRNA levels and transcriptional activity (Fig. 4E and F). To determine which element was primarily responsible for the regulatory association between IFR5 and ZNF217, the three putative IRF5 binding sites were then individually deleted, and named E1-Del, E2-Del and E3-Del. Upon IRF5 overexpression in CFPAC-1 and SW1990 cells, a limited increase in ZNF217 transcriptional activity was observed when the E2 element was deleted (Fig. 4G and H), indicating that this element may be the primary responsible agent for IRF5-induced activation of ZNF217 transcription. ChIP analysis subsequently revealed that IRF5 was enriched at the ZNF217 promoter within the E2 region (Fig. 4I), demonstrating the binding association between IRF5 and the ZNF217 promoter.

IRF5 silencing suppresses PC cell proliferation, migration and invasiveness by regulating ZNF217. The role of IRF5 in PC was evaluated. Compared with HPDE6c7 cells, the mRNA expression levels of IRF5 were significantly upregulated in SW1990 and CFPAC-1 PC cells (Fig. 5A). Subsequently, SW1990 cells were transfected with Oe-NC or Oe-ZNF217, and the mRNA expression of ZNF217 was significantly increased in the Oe-ZNF217 group compared with the control (Fig. 5B). SW1990 cells were transfected with shRNA-IRF5 or co-transfected with either Oe-NC or Oe-ZNF217, and a series of functional experiments including CCK-8, colony formation, wound-healing and Matrigel

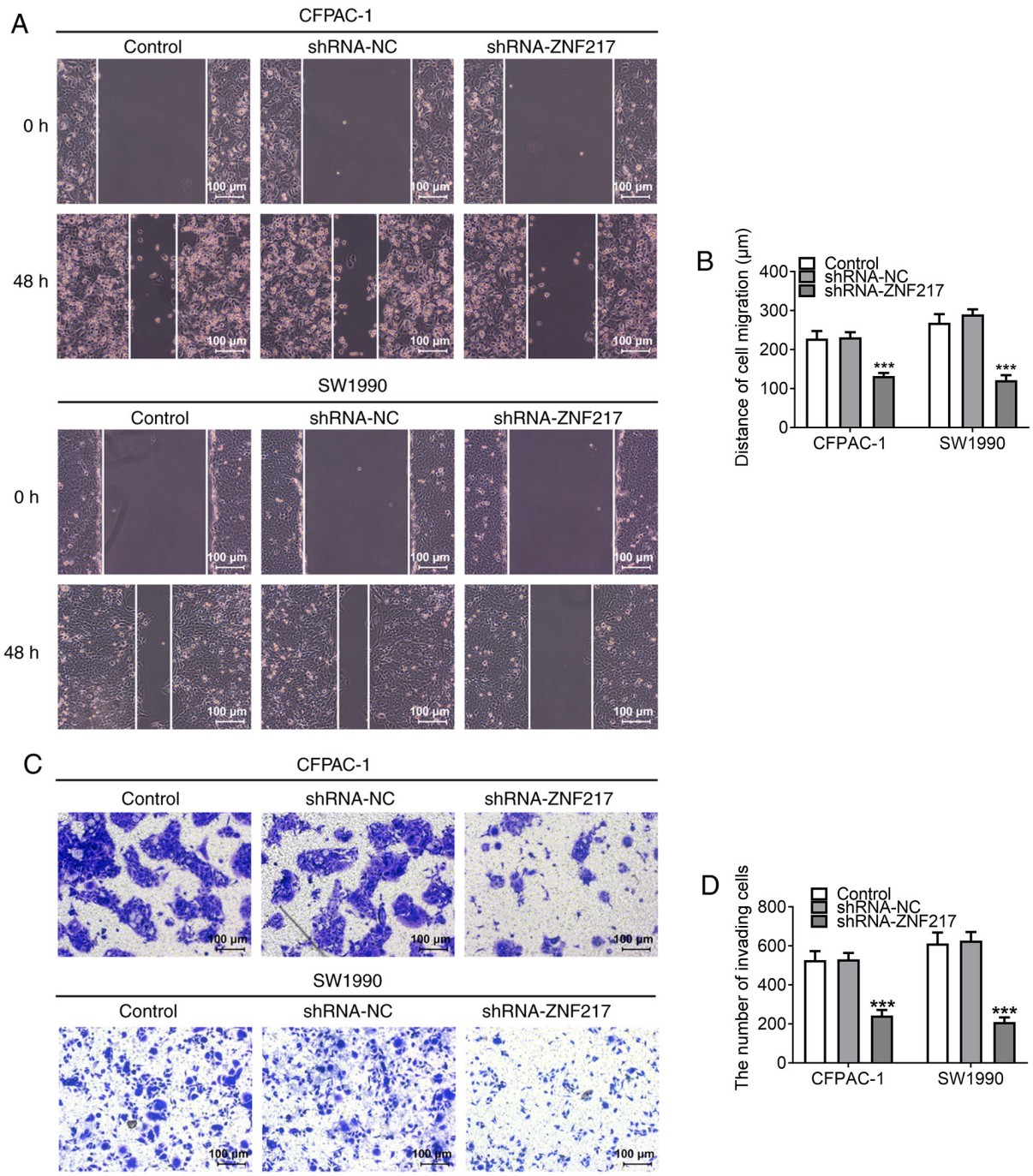

Figure 3. ZNF217 silencing suppresses cellular migration and invasiveness in pancreatic carcinoma. (A) After transfection, a wound-healing assay was performed to assess CFPAC-1 and SW1990 cell migration. (B) Quantification of distance of cell migration. (C) Matrigel assays were performed to assess CFPAC-1 and SW1990 cell invasiveness. (D) Quantification of the number of invading cells. ***P<0.001 vs. shRNA-NC. NC, negative control; shRNA, short hairpin RNA; ZNF217, zinc-finger protein 217.

assays were conducted to assess cellular proliferation, migration and invasiveness upon IRF5 silencing. IRF5 silencing significantly decreased cell viability (Fig. 5C), clone cell number (Fig. 5D and E), migration (Fig. 5F and G) and invasiveness (Fig. 5H and I), indicating a potential antitumor activity of IRF5 knockdown. Furthermore, the inhibitory effects of IRF5 silencing on cellular proliferation, migration and invasiveness were partly abolished by ZNF217 overexpression, suggesting that silencing IRF5 expression had effects on PC cell proliferation, migration and invasiveness through the regulation of ZNF217.

Discussion

In the past decades, several risk factors associated with the occurrence of PC have been identified (13); however, owing to its unsatisfactory 5-year survival rate, there is an urgent requirement for the identification of novel biomarkers to accurately monitor PC development and progression. To the best of our knowledge, the present study was the first to investigate the role and potential regulatory mechanisms of ZNF217 in PC, the findings of which may contribute to the early diagnosis, effective treatment and prognosis of patients with PC.

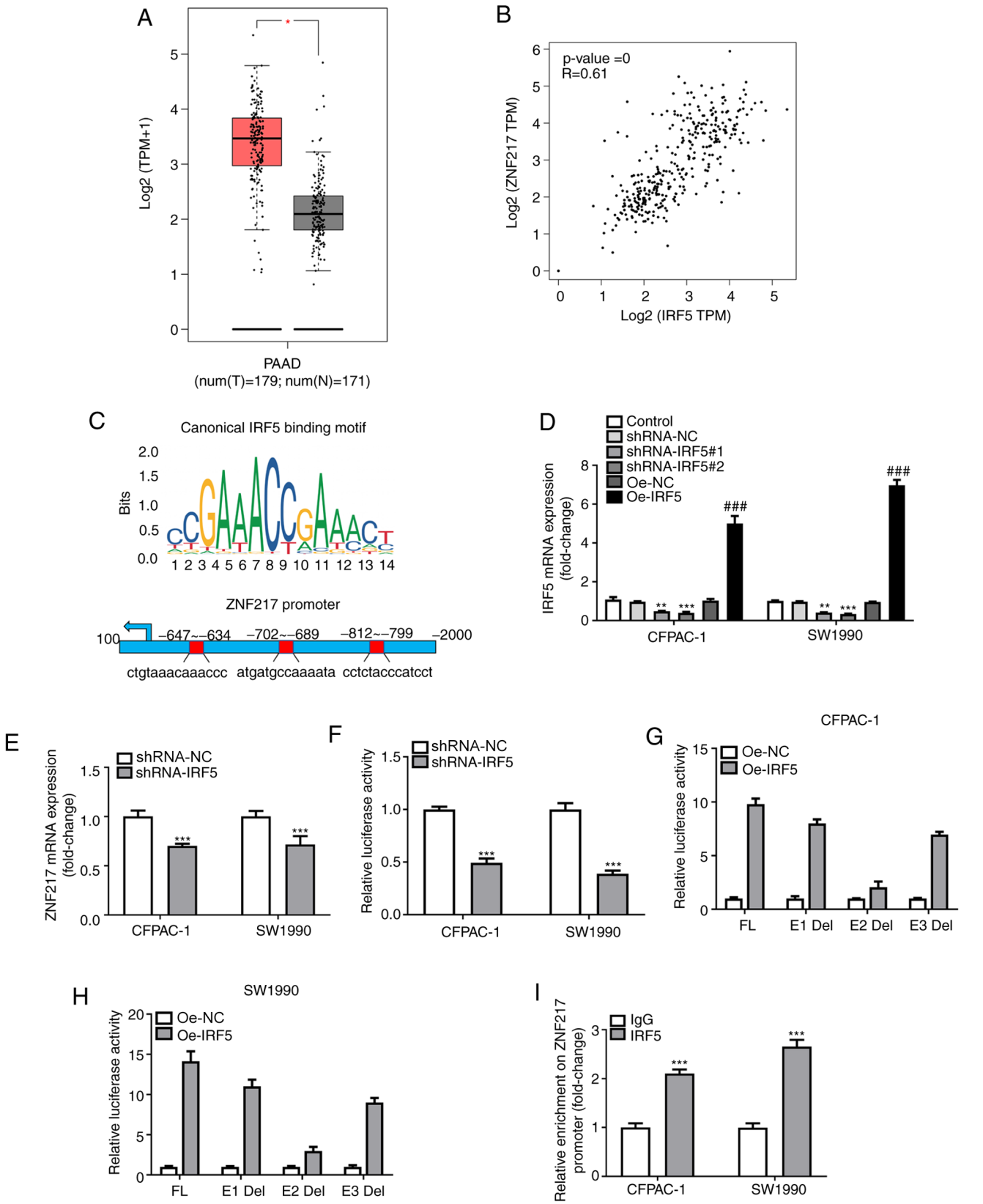

Figure 4. IRF5 is increased in pancreatic carcinoma and regulates ZNF217 expression. (A) Gene Expression Profiling Interactive Analysis was used for IRF5 expression analysis. The red box indicates the tumor samples, and the grey box indicates the normal samples. *P<0.05. (B) Correlation between ZNF217 and IRF5 expression. (C) Schematic diagram of the IRF5 binding motif (JASPAR database) and three potential IRF5 responsive elements (E1, E2 and E3) in the ZNF217 promoter region. (D) IRF5 mRNA expression following knockdown or overexpression of IRF5 was determined by RT-qPCR. **P<0.01, ***P<0.001 vs. shRNA-NC; $^{###}$P<0.001 vs. Oe-NC. (E) ZNF217 mRNA expression following knockdown of overexpression of IRF5 was determined by RT-qPCR. (F) Luciferase readout of ZNF217 promoter transcriptional activity upon IFR5 silencing. ***P<0.001 vs. control. Luciferase assay of three ZNF217 promoter deletion mutants upon IRF5 overexpression in (G) CFPAC-1 and (H) SW1990 cells. (I) Immunoprecipitated chromatin fragments obtained from the chromatin immunoprecipitation assay were analyzed by RT-qPCR. ***P<0.001 vs. IgG. Del, deletion; E, element; FL, full-length; HR, hazard ratio; IRF5, interferon regulatory factor 5; N, normal; NC, negative control; Oe, overexpression; PAAD, pancreatic adenocarcinoma; RT-qPCR, reverse transcription-quantitative PCR; shRNA, short hairpin RNA; T, tumor; TPM, transcripts per million; TSS, transcriptional start; ZNF217, zinc-finger protein 217.

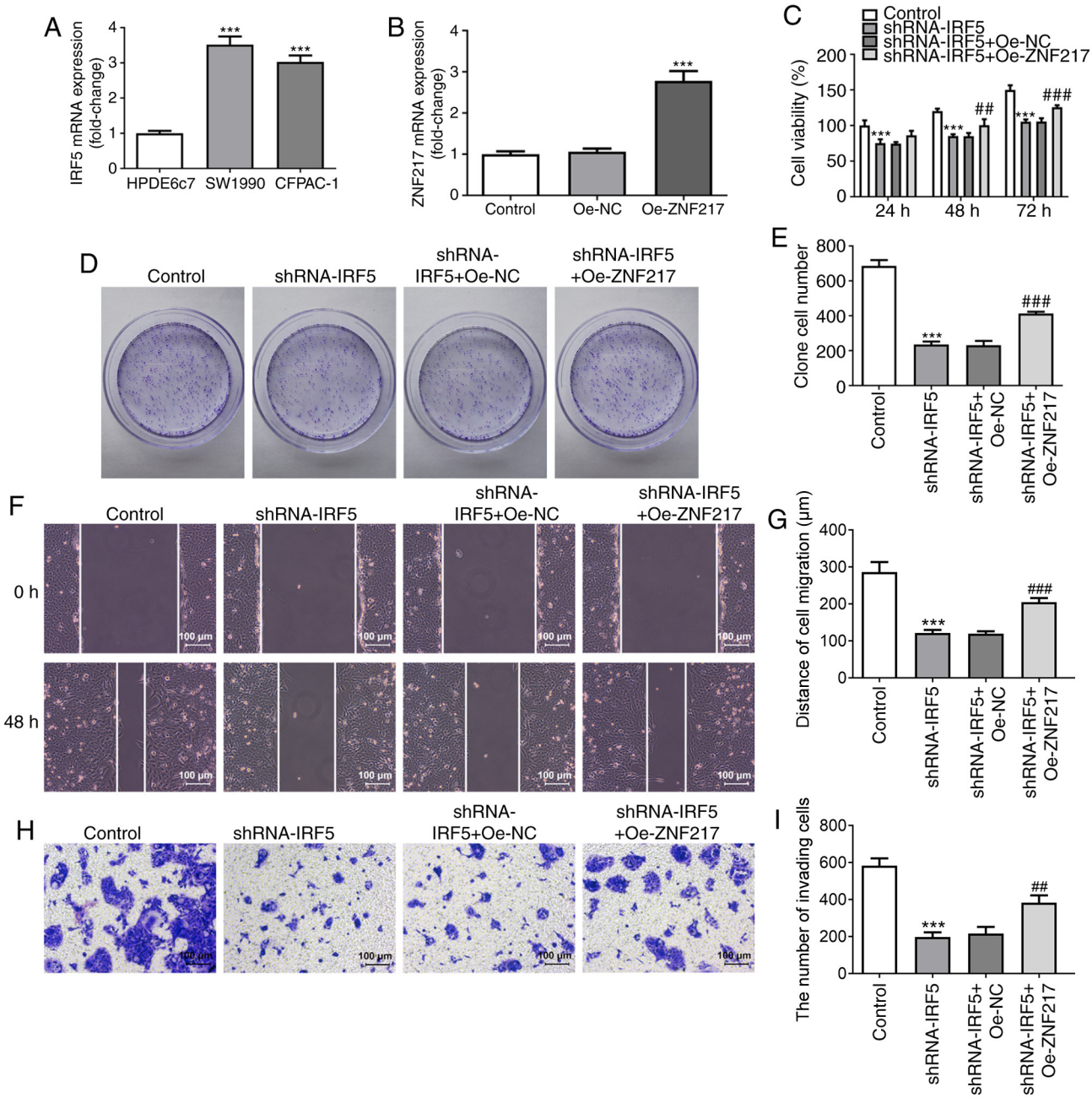

Figure 5. IRF5 silencing suppresses PC cell proliferation, migration and invasion by regulating ZNF217. (A) IRF5 mRNA expression levels were detected in PC cell lines (SW1990 and CFPAC-1) and normal human pancreatic ductal epithelial cells (HPDE6c7). ***P<0.001 vs. HPDE6c7 cells. (B) SW1990 cells were transfected with Oe-NC or Oe-ZNF217, and ZNF217 mRNA expression was detected. (C) SW1990 cells were transfected with shRNA-IRF5 alone or co-transfected with either Oe-NC or Oe-ZNF217, and cell viability at 24, 48 and 72 h post-transfection was detected using a Cell Counting Kit-8 assay. (D) Colony formation and (E) the number of colonies formed following transfection. (F) Wound-healing assays were performed to assess SW1990 cell migration. (G) Quantification of distance of cell migration. (H) Matrigel assay was performed to assess SW1990 cell invasiveness. (I) Quantification of the number of invading cells. ***P<0.001 vs. control; ##P<0.01, ###P<0.001 vs. shRNA-IRF5 + Oe-NC. IRF5, interferon regulatory factor 5; NC, negative control; Oe, overexpression; PC, pancreatic carcinoma; shRNA, short hairpin RNA; ZNF217, zinc-finger protein 217.

ZNF217 has been found to be a crucial oncogene in several types of human cancer. For example, ZNF217 was reported to promote cellular migration, invasiveness and chemotaxis towards the bone environment in breast cancer cells, accelerating breast cancer metastasis to the bone (5). In addition, elevated expression of ZNF217 promoted prostate cancer growth by restraining ferroportin-mediated iron egress (14). Li *et al* (15) revealed that ZNF217 knockdown attenuated the proliferation, migration and invasiveness of colorectal cancer cells, confirming the oncogenic role of ZNF217 in this malignancy. As the oncogenic activity of ZNF217 has been reported, the inhibition of ZNF217 may present an important approach for the treatment of malignant tumors. In the present study, the GEPIA database revealed high expression levels of ZNF217 in the tumoral tissues compared with the normal tissues of patients with PC, and this high expression level was associated with a poor survival rate. A series of *in vitro* experiments revealed that ZNF217 silencing significantly suppressed cell proliferation, as well as migratory and invasive abilities, which was consistent with the aforementioned previous reports, thus demonstrating an important regulatory role for

ZNF217 in PC, and a potential target for PC diagnosis, treatment and prognosis.

In human cancers, epigenetic changes represent a vital mechanism for the activation of oncogenes or the repression of tumor suppressor genes (6). Accumulating evidence has suggested that IRF5 works as a transcription factor to influence and regulate gene transcription in various diseases. For example, Guo *et al* (16) reported that IRF5 was able to enhance the transcription of matrix metalloproteinase 3 by binding to its promoter, thus regulating its expression in human chondrocytes. Pimenta *et al* (17) reported that IRF5 regulated mammary epithelial cell migration by binding to α6-tubulin, thus altering filamentous actin bundling and promoting breast cancer cell migration. In the present study, an interaction between ZNF217 promoter (E2 region) and IRF5 was identified. IRF5 was found to regulate the transcriptional activity and, thus, the mRNA expression of ZNF217 by directly occupying its promoter region. Therefore, high expression levels of IRF5 may account for the upregulation of ZNF217 in PC.

There are, however, some limitations to the present study. This study only focused on the roles of ZNF217 and IRF5 at the cellular level *in vitro;* thus, further validation of the roles of ZNF217 and IRF5 *in vivo* and at the clinical level is required in future work.

In conclusion, the findings of the present study revealed that ZNF217 was upregulated in PC and was associated with poor patient survival rates. Furthermore, ZNF217 silencing exerted notable inhibitory effects on the proliferation, migration and invasion of PC cells. Additionally, IRF5 was demonstrated to positively regulate ZNF217 expression by binding to the promoter-specific region in PC. Thus, ZNF217 may be a potential target for developing novel therapeutic strategies for PC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XQ and SL wrote the paper. XQ, SL, YQ and FW performed the experiments. XQ, SL and YQ analyzed the data. LM designed the experiments and revised the manuscript. All authors read and approved the final manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved. XQ and LM confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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