

Mechanism of miR-217 in Suppressing Pancreatic Cancer Progression by Targeting ENO1

Junwei Du

The Affiliated Hospital of Inner Mongolia Medical University

Yang Liu

The Affiliated Hospital of Inner Mongolia Medical University

Bingchen Xu

The Affiliated Hospital of Inner Mongolia Medical University

Yang Liu

Tongliao City Hospital

Hailong Zheng

International Mongolian Hospital of Inner Mongolia

Zhinan Cui

The Affiliated Hospital of Inner Mongolia Medical University

Qiang Fu

Erenhot People's Hospital

Hui Li

Erenhot People's Hospital

Yiyu Fan

University of Birmingham

Fei Wang

wangfei_allen@immu.edu.cn

The Affiliated Hospital of Inner Mongolia Medical University

Research Article

Keywords:

Posted Date: July 8th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9945651/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Objective

This research was designed to investigate the functional impact of miR-217 in modulating proliferative activity and apoptotic processes in pancreatic carcinoma cells and to investigate its associated molecular mechanisms.

Methods

Five matched pairs of pancreatic ductal adenocarcinoma specimens and corresponding adjacent non-neoplastic tissues were obtained. Quantitative real-time PCR (qRT-PCR) was utilized to assess the expression levels of miR-217. In the PANC-1 cellular model, gain-of-function and loss-of-function strategies were implemented through transfection with an miR-217 mimic or an antisense oligonucleotide (ASO), respectively. Plasmid-based overexpression and short hairpin RNA (shRNA)-mediated knockdown were utilized to modulate ENO1 expression. Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) and colony formation assays. Apoptotic rates were quantified using flow cytometric analysis. Western immunoblotting was performed to determine protein expression of ENO1, AKT1, N-cadherin, E-cadherin, and Vimentin. The direct binding of miR-217 to the 3'-untranslated region (3'-UTR) of ENO1 mRNA was confirmed using a dual-luciferase reporter system. Functional rescue studies were conducted to evaluate the regulatory relationship between miR-217 and ENO1.

Results

miR-217 expression was markedly downregulated in pancreatic tumor tissues. Ectopic overexpression of miR-217 suppressed PANC-1 cell proliferation and induced apoptosis. At the mechanistic level, miR-217 upregulation led to reduced expression of ENO1, AKT1, N-cadherin, and Vimentin, accompanied by elevated E-cadherin levels. The dual-luciferase assay confirmed direct binding of miR-217 to the 3'-UTR of ENO1. Restoration of ENO1 expression counteracted the suppressive influence of miR-217 on proliferation and its promotive effect on apoptosis.

Conclusion

miR-217 is underexpressed in pancreatic cancer and functions as a tumor suppressor by directly targeting ENO1. This regulation subsequently affects AKT1 signaling and epithelial–mesenchymal transition (EMT) markers, ultimately inhibiting proliferation and facilitating apoptosis in pancreatic cancer cells.

Introduction

Pancreatic carcinoma ranks among the most aggressive malignancies of the digestive tract and is associated with an exceedingly poor prognosis. The disease's asymptomatic early presentation and limitations in early screening often result in diagnosis at progressed stages, which is associated with a global five-year survival rate of less than 10%, thereby imposing a substantial burden on both affected individuals and society [1, 2]. Although progress has been made in surgical techniques, gemcitabine-based chemotherapeutic regimens, and novel targeted and immunotherapeutic approaches [3, 4], the pervasive occurrence of intrinsic or acquired chemoresistance, coupled with a high propensity for distant metastasis, continues to be major contributors to therapeutic failure and mortality in pancreatic cancer patients [5]. Consequently, there is a pressing need to deepen the understanding of the molecular mechanisms driving pancreatic carcinogenesis and progression, and to identify effective strategies to counteract drug resistance and suppress metastatic spread.

In recent years, epigenetic mechanisms have garnered increasing interest in oncology research. MicroRNAs (miRNAs), which are endogenous non-coding RNA molecules approximately 18–24 nucleotides in length, play pivotal roles in the post-transcriptional regulation of gene expression by modulating mRNA stability or translational efficiency. They are involved in critical biological processes including cellular proliferation, apoptosis, differentiation, motility, and angiogenesis. Dysregulation of miRNA expression has been closely linked to the pathogenesis, progression, invasive behavior, metastatic dissemination, and treatment resistance of multiple cancers [6]. Substantial evidence indicates that specific miRNAs are significantly dysregulated in pancreatic cancer and may serve as promising diagnostic biomarkers or therapeutic targets [7, 8]. Among these, miR-217 has been reported to be downregulated in several solid tumors, such as gastric cancer [9], hepatocellular carcinoma [10], renal cell carcinoma [11], and glioblastoma [12]. It acts by repressing oncogenic targets (e.g., ATAD2, EZH2) thereby inhibiting tumor cell proliferation, migration, and invasion, while promoting apoptosis [12, 13]. Nevertheless, the expression pattern, precise biological functions, and downstream regulatory network of miR-217 in pancreatic cancer remain incompletely elucidated and sometimes inconsistent. Its potential involvement in chemoresistance and metastasis in pancreatic cancer warrants further in-depth investigation.

Metabolic reprogramming, notably the augmentation of aerobic glycolysis (the Warburg effect), represents a hallmark of pancreatic cancer, supplying necessary energy and biosynthetic precursors for rapid tumor growth, invasion, metastasis, and evasion of apoptosis [14]. Alpha-enolase (ENO1), a key glycolytic enzyme [15], not only catalyzes glycolysis to fuel cancer cells but also exhibits multifunctional roles in cell cycle regulation, apoptosis suppression, enhancement of invasive and migratory capacities, stimulation of angiogenesis, and mediation of chemoresistance [16]. Research has shown that ENO1 is highly overexpressed in pancreatic tumor tissues and cell lines, and its expression levels correlate positively with clinical stage, depth of invasion, lymph node metastasis, and unfavorable patient outcomes [17, 18]. Importantly, the AKT signaling pathway, which is centrally involved in regulating cell survival, proliferation, metabolism, and motility, is frequently hyperactivated in pancreatic cancer and represents a validated therapeutic target [19]. Preliminary studies suggest that ENO1 can potentiate proliferation and metastasis in gastric and colorectal cancer cells via the protein kinase B (AKT) pathway

[20, 21]. However, the upstream regulatory mechanisms underlying ENO1 overexpression in pancreatic cancer, particularly whether it is subject to post-transcriptional control by specific miRNAs, have not yet been systematically and thoroughly explored.

Building upon this research background and critical scientific questions, this study proposes the following hypothesis: miR-217 may act as a tumor suppressor and be underexpressed in pancreatic cancer, directly targeting the oncogene ENO1 and negatively regulating its protein expression. Subsequent downregulation of ENO1 would inhibit aberrant activation of the downstream AKT signaling pathway, ultimately restraining the proliferative, invasive, and migratory capacities of pancreatic cancer cells. To test this hypothesis, the present study will integrate bioinformatic analyses with cellular and molecular biology techniques—including miRNA expression profiling, dual-luciferase reporter assays for target validation, and gain- and loss-of-function experiments—to systematically examine the expression and functional roles of miR-217 in pancreatic cancer, as well as its mechanistic regulation of the AKT pathway through targeting ENO1. This investigation aims to furnish novel theoretical insights into the pathogenesis of pancreatic cancer and to provide important experimental evidence and potential molecular targets for the development of innovative treatment strategies centered on restoring miR-217 expression or targeting the ENO1/AKT axis.

Materials and Methods

1.1 Collection of Tissue Samples

Matched pairs of pancreatic cancer tissue and neighboring non-cancerous tissue samples (n = 5 per group) were collected from patients diagnosed at The Affiliated Hospital of Inner Mongolia Medical University. Tissues were promptly preserved at – 80°C for subsequent analysis. Informed written consent was obtained from all subjects. The experimental procedures were approved by the Ethics Committee at The Affiliated Hospital of Inner Mongolia Medical University.

1.2 Cell Culture

The human pancreatic ductal epithelial cell line HPDE6-C7, along with pancreatic carcinoma cell lines including PANC-1, were utilized in this study, SW1990, and Capan-1 were cultured in Dulbecco's Modified Eagle Medium (DMEM) with high glucose, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. All cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

The human normal pancreatic ductal epithelial cell line HPDE6-C7 (Catalog No. HY-A374) was obtained from Cellfactory. Human pancreatic cancer cell lines PANC-1 (Catalog No. JY218) and Capan-1 (Catalog No. JY217) were purchased from Jinyuan Biology. SW1990 human pancreatic cancer cells (Catalog No. BNCC338480) were acquired from BeNa Culture Collection (BNCC). DMEM high-glucose medium (C11995500BT) was obtained from Gibco; FBS (Batch: 20140421) was sourced from Gemini; Trypsin-EDTA solution (T1300) was purchased from Beijing Solarbio Science & Technology Co., Ltd.

1.3 Transfection with miR-217 Mimic and Inhibitor

PANC-1 cells in the logarithmic growth phase were categorized into four experimental groups: miR-217 mimic group (overexpression), negative control mimic group (NC mimic), miR-217 antisense oligonucleotide group (miR-217 ASO), and negative control ASO group (NC ASO). After cell attachment, transfections were carried out using Lipofectamine™ 2000 according to the manufacturer's instructions, with 50 µL of either mimic/ASO or corresponding negative control reagent combined with 50 µL transfection reagent per condition. Cells were harvested 24 hours post-transfection for further analysis. miR-217 mimic, miR-217 ASO, and their negative controls were acquired from GenePharma; Lipofectamine™ 2000 was procured from Invitrogen.

1.4 Plasmid-Mediated ENO1 Overexpression and Knockdown

Log-phase PANC-1 cells were divided into four groups: ENO1 overexpression (PANC-1-OE-ENO1), overexpression negative control (PANC-1-OE-nc), ENO1 knockdown (PANC-1-si-ENO1), and knockdown negative control (PANC-1-si-nc). Following adherence, transfection was performed using Lipofectamine™ 2000 with 50 µL of plasmid (OE-ENO1 or si-ENO1) or negative control reagent along with 50 µL transfection reagent. Cells were collected after 24 hours for subsequent experiments. The human OE-ENO1 plasmid, si-ENO1 plasmid, and corresponding negative controls were supplied by GenePharma; Lipofectamine™ 2000 was from Invitrogen.

1.5 MTT Proliferation Assay

Following transfection, PANC-1 cells were quantified and plated into 96-well plates at a density of 1×10^4 cells per well, with each group comprising three replicate wells. After 48 hours of culture, the supernatant was removed and 110 µL of MTT reagent was introduced into each well. The plates were subjected to gentle agitation for 10 minutes to facilitate complete dissolution of formazan crystals. Optical density (OD) was subsequently determined at a wavelength of 490 nm employing a microplate reader. The MTT assay kit (M1020) utilized in this experiment was sourced from Beijing Solarbio Science & Technology Co., Ltd.

1.6 Colony Formation Assay

Transfected cells were seeded into 6-well plates at a density of 500 cells per well and maintained in culture for a period of 14 days, with the medium being replaced every 48 hours. Following the incubation, cell colonies were fixed using 4% paraformaldehyde and subjected to staining with crystal violet. Colonies containing more than 50 cells were enumerated for quantitative analysis. The colony formation rate was calculated as (number of colonies / number of cells seeded) $\times$ 100%. All experiments were performed in triplicate.

1.7 Apoptosis Analysis by Flow Cytometry

A total of 1×10^6 transfected PANC-1 cells per sample were collected, washed with cold PBS, and resuspended in 500 µL PBS. Cells were incubated with 5 µL of Annexin V-APC and 5 µL of propidium iodide (PI) for 15 minutes at room temperature under light-protected conditions. Apoptotic rates were

quantified with a BD FACSCalibur™ flow cytometer (Model: E97501093). The Annexin V-APC/PI Apoptosis Detection Kit (Catalog No.: AP101-100) was procured from Hangzhou Lianke Biotechnology Co., Ltd.

1.8 Quantitative Real-Time PCR (qRT-PCR)

Total RNA isolation from both tissue samples and cultured cell lines was performed employing Redzol reagent. RNA purity and concentration were assessed spectrophotometrically (Biochrom NanoVue Plus). Reverse transcription was performed using a commercial kit. qPCR was conducted under the following conditions: 95°C for 30 s, 40 cycles of 95°C for 15 s and 60°C for 30 s. GAPDH served as the internal control. Relative expression was calculated via the $2^{-(\Delta\Delta Ct)}$ method. Redzol Reagent (FTR-50) was from Sangon Biotech; the Reverse Transcription Kit (QP056) was from GeneCopoeia; 2× SYBR Green qPCR Master Mix (MPC2203026) was from Servicebio. Primers were synthesized by Sangon Biotech (Shanghai).

1.9 Western Blotting

Cell lysis was carried out using RIPA buffer supplemented with PMSF. Protein quantification was conducted via the BCA method. Protein separation was achieved through SDS-PAGE electrophoresis, followed by transfer onto PVDF membranes. Subsequent to blocking with 5% skimmed milk, the membranes were subjected to overnight incubation at 4°C with primary antibodies targeting ENO1, AKT1, N-cadherin, E-cadherin, Vimentin, and GAPDH (all 1:1000). HRP-conjugated secondary antibodies (1:5000) were applied for 1 hour at room temperature. Blots were visualized using an ECL kit and imaged with a Chemi Scope 6100 system. Band densities were quantified with ImageJ. Antibodies were purchased from Thermo Fisher Scientific and Abcam.

1.10 Dual-Luciferase Reporter Assay

Bioinformatic prediction using TargetScan 8.0 indicated a binding potential between miR-217 and ENO1. Reporter plasmids containing either the wild-type (WT-ENO1) or mutated (MUT-ENO1) 3'-UTR sequences were generated and subsequently co-transfected into PANC-1 cells alongside miR-217 mimic or negative control oligonucleotides. Luciferase activity was assessed 48 hours post-transfection with a dual-luciferase detection system. Normalization of Firefly luciferase luminescence was performed relative to Renilla luciferase activity. The Dual-Luciferase Reporter Assay Kit (RG009) was supplied by Beyotime Biotechnology.

1.11 Statistical Analysis

Data are expressed as mean $\pm$ SD. Comparisons between two groups used Student's t-test; multiple groups were analyzed by one-way ANOVA. Statistical significance was defined as $P < 0.05$. All analyses were performed using GraphPad Prism 9.0.

Results

2.1 Low Expression of miR-217 and High Expression of ENO1 in Pancreatic Cancer Tissues and Cells

To characterize the expression pattern of miR-217 in pancreatic cancer, matched specimens of tumor tissues and adjacent non-cancerous tissues (n = 5 per group) were evaluated. Quantitative real-time PCR analysis demonstrated a significant decrease in miR-217 expression and a concurrent increase in ENO1 mRNA levels in cancerous tissues relative to matched normal samples (Fig. 1A–B). Furthermore, miR-217 expression was assessed in an immortalized human pancreatic duct epithelial cell line (HPDE6-C7) and multiple pancreatic cancer-derived cell lines (PANC-1, SW1990, Capan-1). In agreement with the tissue data, all carcinoma cell lines exhibited substantially lower miR-217 levels compared to HPDE6-C7 cells, with the most notable downregulation detected in PANC-1 cells (Fig. 1C). Based on these observations, PANC-1 was chosen for further functional studies.

2.2 Effects of miR-217 on PANC-1 Cell Proliferation and Apoptosis

MTT assays indicated that transfection with miR-217 mimic significantly suppressed PANC-1 cell proliferation, whereas miR-217 inhibition enhanced proliferative capacity (Fig. 2A). Colony formation assays further demonstrated that miR-217 overexpression markedly reduced the number and size of colonies (Fig. 2B–C). Flow cytometric analysis confirmed that miR-217 mimic promoted apoptosis, while its inhibition exerted an anti-apoptotic effect (Fig. 2D). These findings collectively indicate that miR-217 acts as a tumor suppressor in pancreatic cancer by restraining proliferation and inducing apoptosis.

2.3 miR-217 Directly Targets ENO1 and Modulates Downstream Signaling

Bioinformatic prediction via TargetScan 8.0 identified a putative binding site for miR-217 within the 3'-UTR of ENO1 mRNA, suggesting a direct regulatory interaction (Fig. 3A). Dual-luciferase reporter assays validated that miR-217 mimic significantly reduced luciferase activity in wild-type (WT)-ENO1 transfectants ($P < 0.0001$), but not in mutant (MUT)-ENO1 constructs (Fig. 3B), confirming ENO1 as a direct target of miR-217. Given the established roles of AKT signaling and epithelial-mesenchymal transition (EMT) in pancreatic cancer malignancy, we evaluated their expression in clinical samples and manipulated cells. qRT-PCR analysis indicated elevated expression of AKT, N-cadherin, and Vimentin, alongside reduced E-cadherin, in tumor tissues (Fig. 3C). Western blot analysis in PANC-1 cells showed that miR-217 overexpression downregulated protein levels of ENO1, AKT, N-cadherin, and Vimentin, while upregulating E-cadherin. Conversely, miR-217 inhibition produced opposing effects (Fig. 3D–I). These data suggest that miR-217 modulates pancreatic cancer cell behavior partly through regulating ENO1-mediated AKT signaling and EMT.

2.4 Rescue Experiments Confirm Functional Dependence on ENO1

To further elucidate the mechanistic link between miR-217 and ENO1, rescue experiments were conducted. ENO1 was either overexpressed or knocked down via plasmid transfection in PANC-1 cells, with efficiency confirmed by qRT-PCR (Fig. 4A). Western blot analysis revealed that ENO1 overexpression partially reversed the suppressive effects of miR-217 mimic on ENO1, AKT, N-cadherin, and Vimentin expression, and attenuated the induction of E-cadherin (Fig. 4B–G). Functional assays including MTT (Fig. 4H), colony formation (Fig. 4I–J), and flow cytometry (Fig. 4K) consistently demonstrated that ENO1 overexpression rescued the miR-217-mediated suppression of proliferation and induction of apoptosis.

Discussion

Accumulating evidence has established miR-217 as a key tumor suppressor in various cancers, with its downregulation frequently associated with advanced disease stage, metastasis, therapeutic resistance, and poor survival [10, 22, 23]. Functioning as a post-transcriptional regulator, miR-217 modulates oncogenic processes including proliferation, apoptosis, and EMT through targeting multiple cancer-driving genes [24, 25]. In line with studies in gastric and hepatic cancers [9, 10], the present study confirmed significant underexpression of miR-217 in pancreatic tumor tissues and cell lines. Restoring miR-217 expression effectively inhibited proliferation and promoted apoptosis in PANC-1 cells. Notably, we identified ENO1 as a direct functional target of miR-217 in pancreatic cancer, revealing a novel mechanism underlying its tumor-suppressive effects. Previous reports also indicate that miR-217 enhances gemcitabine sensitivity via cell cycle modulation [26], further supporting its central role in pancreatic cancer pathogenesis and treatment response.

ENO1, a glycolytic enzyme involved in metabolic reprogramming, is overexpressed in multiple malignancies including pancreatic cancer and contributes to invasion, metastasis, chemoresistance, and immune evasion [27–29]. It facilitates tumor progression through upregulation of oncoproteins such as YAP1 and MYC [30, 31], whereas its knockdown impedes cancer cell growth and motility [32]. This study confirmed a direct regulatory interaction between miR-217 and ENO1 via dual-luciferase assay. Rescue experiments established that ENO1 overexpression counteracts the inhibitory effects of miR-217 on AKT signaling, EMT marker expression, proliferation, and apoptosis, identifying ENO1 as a critical downstream mediator. AKT hyperactivation is a hallmark of pancreatic cancer, driving survival, proliferation, and resistance [33, 34], and its inhibition represents a promising therapeutic strategy [35]. Moreover, ENO1 promotes metastasis and drug resistance through EMT induction [36, 37]. We observed that ENO1 upregulation increased N-cadherin and Vimentin while decreasing E-cadherin—a pattern indicative of the “cadherin switch” characteristic of EMT [38]. By integrating miR-217 deficiency, ENO1 overexpression, AKT activation, and EMT progression, we propose a coherent signaling axis (miR-

217/ENO1/AKT/EMT) that links non-coding RNA regulation, metabolic alteration, oncogenic signaling, and malignant phenotype.

In conclusion, through clinical correlation analysis and functional in vitro experiments, this study demonstrates that miR-217 acts as a tumor suppressor in pancreatic cancer by directly targeting ENO1, leading to inhibition of AKT signaling and EMT, thereby suppressing proliferation, inducing apoptosis, and impairing metastatic potential. The miR-217/ENO1 axis holds promise as a diagnostic and prognostic biomarker. Therapeutic strategies aimed at restoring miR-217 function (e.g., utilizing miRNA mimics or stable delivery systems) or inhibiting ENO1 (e.g., via small molecules targeting its oncogenic functions) may offer novel avenues for pancreatic cancer treatment.

Study Limitations and Future Directions

Although this work systematically delineates the tumor-suppressive role of miR-217 through targeting ENO1, several limitations remain. The clinical sample size was relatively small, and the role of the miR-217/ENO1 axis in invasion and metastasis was not assessed in advanced in vivo models (e.g., orthotopic or metastatic models). Additionally, while we focused on AKT signaling and EMT, the potential contributions of non-glycolytic functions of ENO1 (e.g., as a plasminogen receptor or immune modulator) remain unexplored. Future studies should validate the anti-metastatic efficacy of targeting this axis in vivo, investigate non-glycolytic mechanisms, and evaluate combinatorial effects with chemotherapy or immunotherapy. Exploring the role of the miR-217/ENO1 axis in shaping the immunosuppressive tumor microenvironment may also provide innovative insights for combination therapy.

Conclusion

This study demonstrates that miR-217 is downregulated in pancreatic cancer and directly targets ENO1, leading to inhibition of PI3K/AKT signaling and suppression of EMT. These effects collectively contribute to reduced proliferation, increased apoptosis, and impaired aggressiveness of pancreatic cancer cells. The miR-217/ENO1 axis represents a valuable biomarker and a promising therapeutic target for future intervention strategies.

Ethics

This study was approved by the Ethics Committee of The Affiliated Hospital of Inner Mongolia Medical University. Ethics approval No.YKD202201230.All procedures performed in studies involving human participants were in accordance with the ethical standards of the 2024 updated version Declaration of Helsinki and its later amendments or comparable ethical standards. All patients provided written informed consent.This study is an in vitro cellular and retrospective pathological specimen-based research without prospective clinical intervention on human subjects, hence no clinical trial registration number was obtained.

Declarations

Ethics: This study was approved by the Ethics Committee of The Affiliated Hospital of Inner Mongolia Medical University. Ethics approval No.YKD202201230.All procedures performed in studies involving human participants were in accordance with the ethical standards of the 2024 updated version Declaration of Helsinki and its later amendments or comparable ethical standards. All patients provided written informed consent.This study is an in vitro cellular and retrospective pathological specimen-based research without prospective clinical intervention on human subjects, hence no clinical trial registration number was obtained.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author via email request.

Consent to Publish

The corresponding author confirms on behalf of all co-authors that:

1. This manuscript has not been published previously in any form (except conference abstract, academic thesis or invited lecture summary) and is not under simultaneous consideration by any other journal.
2. All co-authors have fully read and approved the final version of the manuscript, agree to the author sequence and submission to Discover Oncology.
3. The host institution where this research was performed has approved the publication of this work explicitly or implicitly.
4. The work is original research; all cited third-party materials, figures and tables have obtained valid reproduction permissions from copyright holders, and relevant permission documents are retained for editorial inspection upon request.
5. All authors agree to transfer the copyright of the accepted manuscript to Springer Nature for exclusive global reproduction, distribution, translation, online/offline electronic release and reprinting rights in all formats.

No changes to author list or author order will be accepted after this consent is submitted.

Competing interests: None of the authors have any relevant conflict of interest or other financial disclosures.

Funding: No funding was used in this study.

Authors' contributions: Fei Wang, Junwei Du and Yiyu Fan conceived and designed the study. Junwei Du and Yang Liu collected and organized the clinical data. Yang Liu, Hailong Zheng,Bingchen Xu and Zhinan Cui conducted statistical analysis and interpreted results. Junwei Du and Yang Liu assisted with

literature review and drafting the manuscript. Yiyu Fan, Hui Li, and Qiang Fu critically revised the manuscript for intellectual content. All authors reviewed and approved the final version.

Acknowledgements: Not applicable.

References

1. Pergolizzi RG, Brower ST. Molecular Targets for the Diagnosis and Treatment of Pancreatic Cancer. *Int J Mol Sci* 2024, 25(19).
2. McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. *World J Gastroenterol*. 2018;24(43):4846–61.
3. Beutel AK, Halbrook CJ. Barriers and opportunities for gemcitabine in pancreatic cancer therapy. *Am J Physiol Cell Physiol*. 2023;324(2):C540–52.
4. Bear AS, Vonderheide RH, O'Hara MH. Challenges and Opportunities for Pancreatic Cancer Immunotherapy. *Cancer Cell*. 2020;38(6):788–802.
5. Zeng S, Pottler M, Lan B, Grutzmann R, Pilarsky C, Yang H. Chemoresistance in Pancreatic Cancer. *Int J Mol Sci* 2019, 20(18).
6. Hussen BM, Hidayat HJ, Salihi A, Sabir DK, Taheri M, Ghafouri-Fard S. MicroRNA: A signature for cancer progression. *Biomed Pharmacother*. 2021;138:111528.
7. Gahlawat AW, Fahed L, Witte T, Schott S. Total circulating microRNA level as an independent prognostic marker for risk stratification in breast cancer. *Br J Cancer*. 2022;127(1):156–62.
8. Wang J, Wang Q, Guan Y, Sun Y, Wang X, Lively K, Wang Y, Luo M, Kim JA, Murphy EA et al. Breast cancer cell-derived microRNA-155 suppresses tumor progression via enhancing immune cell recruitment and antitumor function. *J Clin Invest* 2022, 132(19).
9. Chen DL, Zhang DS, Lu YX, Chen LZ, Zeng ZL, He MM, Wang FH, Li YH, Zhang HZ, Pelicano H, et al. microRNA-217 inhibits tumor progression and metastasis by downregulating EZH2 and predicts favorable prognosis in gastric cancer. *Oncotarget*. 2015;6(13):10868–79.
10. Zhang M, Li M, Li N, Zhang Z, Liu N, Han X, Liu Q, Liao C. miR-217 suppresses proliferation, migration, and invasion promoting apoptosis via targeting MTDH in hepatocellular carcinoma. *Oncol Rep*. 2017;37(3):1772–8.
11. Li H, Zhao J, Zhang JW, Huang QY, Huang JZ, Chi LS, Tang HJ, Liu GQ, Zhu DJ, Ma WM. MicroRNA-217, down-regulated in clear cell renal cell carcinoma and associated with lower survival, suppresses cell proliferation and migration. *Neoplasma*. 2013;60(5):511–5.
12. Korleski J, Sudhir S, Rui Y, Caputo CA, Sall S, Johnson AL, Khela HS, Madhvacharyula T, Rasamsetty A, Li Y et al. miR-217-5p NanomiRs Inhibit Glioblastoma Growth and Enhance Effects of Ionizing Radiation via EZH2 Inhibition and Epigenetic Reprogramming. *Cancers (Basel)* 2024, 17(1).
13. Dutta M, Das B, Mohapatra D, Behera P, Senapati S, Roychowdhury A. MicroRNA-217 modulates pancreatic cancer progression via targeting ATAD2. *Life Sci*. 2022;301:120592.

14. Zheng JH, Zhu YH, Yang J, Ji PX, Zhao RK, Duan ZH, Yao HF, Jia QY, Yin YF, Hu LP, et al. A CLIC1 network coordinates matrix stiffness and the Warburg effect to promote tumor growth in pancreatic cancer. *Cell Rep.* 2024;43(8):114633.
15. Huppertz I, Perez-Perri JI, Mantas P, Sekaran T, Schwarzl T, Russo F, Ferring-Appel D, Koskova Z, Dimitrova-Paternoga L, Kafkia E, et al. Riboregulation of Enolase 1 activity controls glycolysis and embryonic stem cell differentiation. *Mol Cell.* 2022;82(14):2666–80. e2611.
16. Zhu Q, Li J, Sun H, Fan Z, Hu J, Chai S, Lin B, Wu L, Qin W, Wang Y, et al. O-GlcNAcylation of enolase 1 serves as a dual regulator of aerobic glycolysis and immune evasion in colorectal cancer. *Proc Natl Acad Sci U S A.* 2024;121(44):e2408354121.
17. Huang CK, Lv L, Chen H, Sun Y, Ping Y. ENO1 promotes immunosuppression and tumor growth in pancreatic cancer. *Clin Transl Oncol.* 2023;25(7):2250–64.
18. Principe M, Borgoni S, Cascione M, Chattaragada MS, Ferri-Borgogno S, Capello M, Bulfamante S, Chapelle J, Di Modugno F, Defilippi P, et al. Alpha-enolase (ENO1) controls alpha v/beta 3 integrin expression and regulates pancreatic cancer adhesion, invasion, and metastasis. *J Hematol Oncol.* 2017;10(1):16.
19. Stanciu S, Ionita-Radu F, Stefani C, Miricescu D, Stanescu S, Il, Greabu M, Ripszky Totan A, Jinga M. Targeting PI3K/AKT/mTOR Signaling Pathway in Pancreatic Cancer: From Molecular to Clinical Aspects. *Int J Mol Sci* 2022, 23(17).
20. Liu Y, Hou Y, Zhang F, Wang X. ENO1 deletion potentiates ferroptosis and decreases glycolysis in colorectal cancer cells via AKT/STAT3 signaling. *Exp Ther Med.* 2024;27(4):127.
21. Sun L, Lu T, Tian K, Zhou D, Yuan J, Wang X, Zhu Z, Wan D, Yao Y, Zhu X, et al. Alpha-enolase promotes gastric cancer cell proliferation and metastasis via regulating AKT signaling pathway. *Eur J Pharmacol.* 2019;845:8–15.
22. Chen G, Yang Z, Feng M, Wang Z. microRNA-217 suppressed epithelial-to-mesenchymal transition through targeting PTPN14 in gastric cancer. *Biosci Rep* 2020, 40(1).
23. Yan J, Wu G, Chen J, Xiong L, Chen G, Li P. Downregulated miR-217 expression predicts a poor outcome in acute myeloid leukemia. *Cancer Biomark.* 2018;22(1):73–8.
24. He S, Wang Z, Tang H, Dong J, Qu Y, Lv J. MiR-217 Inhibits Proliferation, Migration, and Invasion by Targeting SIRT1 in Osteosarcoma. *Cancer Biother Radiopharm.* 2019;34(4):264–70.
25. Zhao W, Wang X, Jiang Y, Jia X, Guo Y. miR-217-5p Inhibits Invasion and Metastasis of Prostate Cancer by Targeting Clusterin. *Mamm Genome.* 2021;32(5):371–80.
26. Panebianco C, Trivieri N, Villani A, Terracciano F, Latiano TP, Potenza A, Perri F, Binda E, Pazienza V. Improving Gemcitabine Sensitivity in Pancreatic Cancer Cells by Restoring miRNA-217 Levels. *Biomolecules* 2021, 11(5).
27. Wan W, Li Y, Sun W, Cheng Z, Ma F, Shen S, Liu H, Zhang J. The DCDC2/ENO1 axis promotes tumor progression and immune evasion in intrahepatic cholangiocarcinoma via activating FGL1-LAG3 checkpoint. *J Exp Clin Cancer Res.* 2025;44(1):177.

28. Xie F, Zhang H, Dai X, Tu M, Yu C, Liu L, Guo Y, Sun H, Gao Q, Wang J et al. HPD is an RNA-Binding Protein Sustaining Ovarian Cancer Cell Glycolysis, Tumor Growth, and Drug Resistance. *Adv Sci (Weinh)* 2025:e03999.
29. Yajima S, Ito M, Suzuki T, Oshima Y, Sumazaki M, Shiratori F, Takizawa H, Li SY, Zhang BS, Yoshida Y, et al. Application of serum anti-ENO1 and anti-SSNA1 antibody biomarkers in predicting the prognosis of gastric cancer. *Oncol Lett*. 2025;30(1):360.
30. Ma H, Kong L, Liu L, Du Y, Zhu X, Wang J, Zhao W. ENO1 contributes to the gemcitabine resistance of pancreatic cancer through the YAP1 signaling pathway. *Mol Carcinog*. 2024;63(7):1221–34.
31. Lu Y, Ma H, Xiong X, Du Y, Liu L, Wang J, Zhao W. Deletion of ENO1 sensitizes pancreatic cancer cells to gemcitabine via MYC/RRM1-mediated glycolysis. *Sci Rep*. 2025;15(1):9941.
32. Song Q, Zhang K, Sun T, Xu C, Zhao W, Zhang Z. Knockout of ENO1 leads to metabolism reprogramming and tumor retardation in pancreatic cancer. *Front Oncol*. 2023;13:1119886.
33. Ebrahimi S, Hosseini M, Shahidsales S, Maftouh M, Ferns GA, Ghayour-Mobarhan M, Hassanian SM, Avan A. Targeting the Akt/PI3K Signaling Pathway as a Potential Therapeutic Strategy for the Treatment of Pancreatic Cancer. *Curr Med Chem*. 2017;24(13):1321–31.
34. Zhang Z, Yu H, Yao W, Zhu N, Miao R, Liu Z, Song X, Xue C, Cai C, Cheng M, et al. RRP9 promotes gemcitabine resistance in pancreatic cancer via activating AKT signaling pathway. *Cell Commun Signal*. 2022;20(1):188.
35. He Y, Sun MM, Zhang GG, Yang J, Chen KS, Xu WW, Li B. Targeting PI3K/Akt signal transduction for cancer therapy. *Signal Transduct Target Ther*. 2021;6(1):425.
36. Li HJ, Ke FY, Lin CC, Lu MY, Kuo YH, Wang YP, Liang KH, Lin SC, Chang YH, Chen HY, et al. ENO1 Promotes Lung Cancer Metastasis via HGFR and WNT Signaling-Driven Epithelial-to-Mesenchymal Transition. *Cancer Res*. 2021;81(15):4094–109.
37. Gu J, Zhong K, Wang L, Ni H, Zhao Y, Wang X, Yao Y, Jiang L, Wang B, Zhu X. ENO1 contributes to 5-fluorouracil resistance in colorectal cancer cells via EMT pathway. *Front Oncol*. 2022;12:1013035.
38. Loh CY, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, Chong PP, Looi CY. The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges. *Cells* 2019, 8(10).

Figures

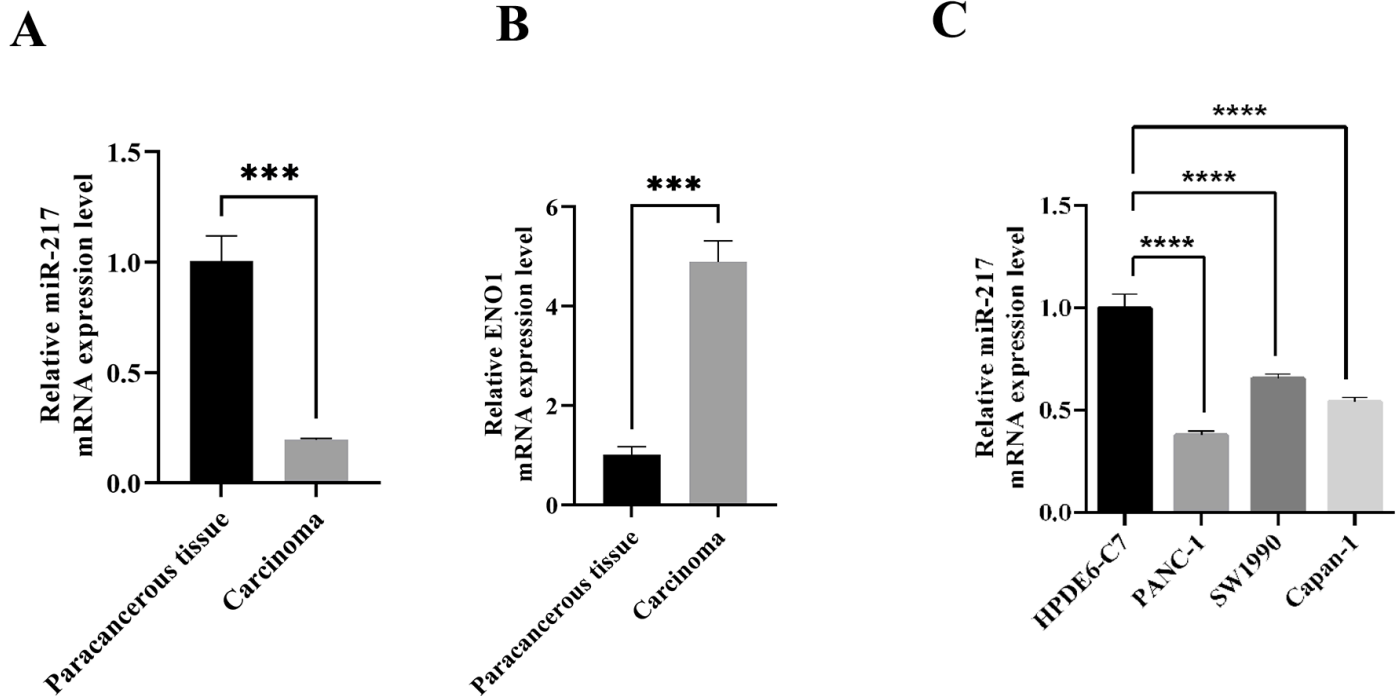


Figure 1

Expression of miR-217 and ENO1 in Pancreatic Carcinoma Tissues and Cell Lines

A–B: Relative expression levels of miR-217 and ENO1 mRNA in pancreatic ductal adenocarcinoma tissues (n = 5) and matched adjacent non-neoplastic tissues (n = 5) as determined by RT-qPCR. **C:** Expression profiles of miR-217 and ENO1 in the normal human pancreatic ductal epithelial cell line HPDE6-C7 and pancreatic cancer cell lines (PANC-1, SW1990, Capan-1) assessed via RT-qPCR. *** P < 0.001; **** P < 0.0001.

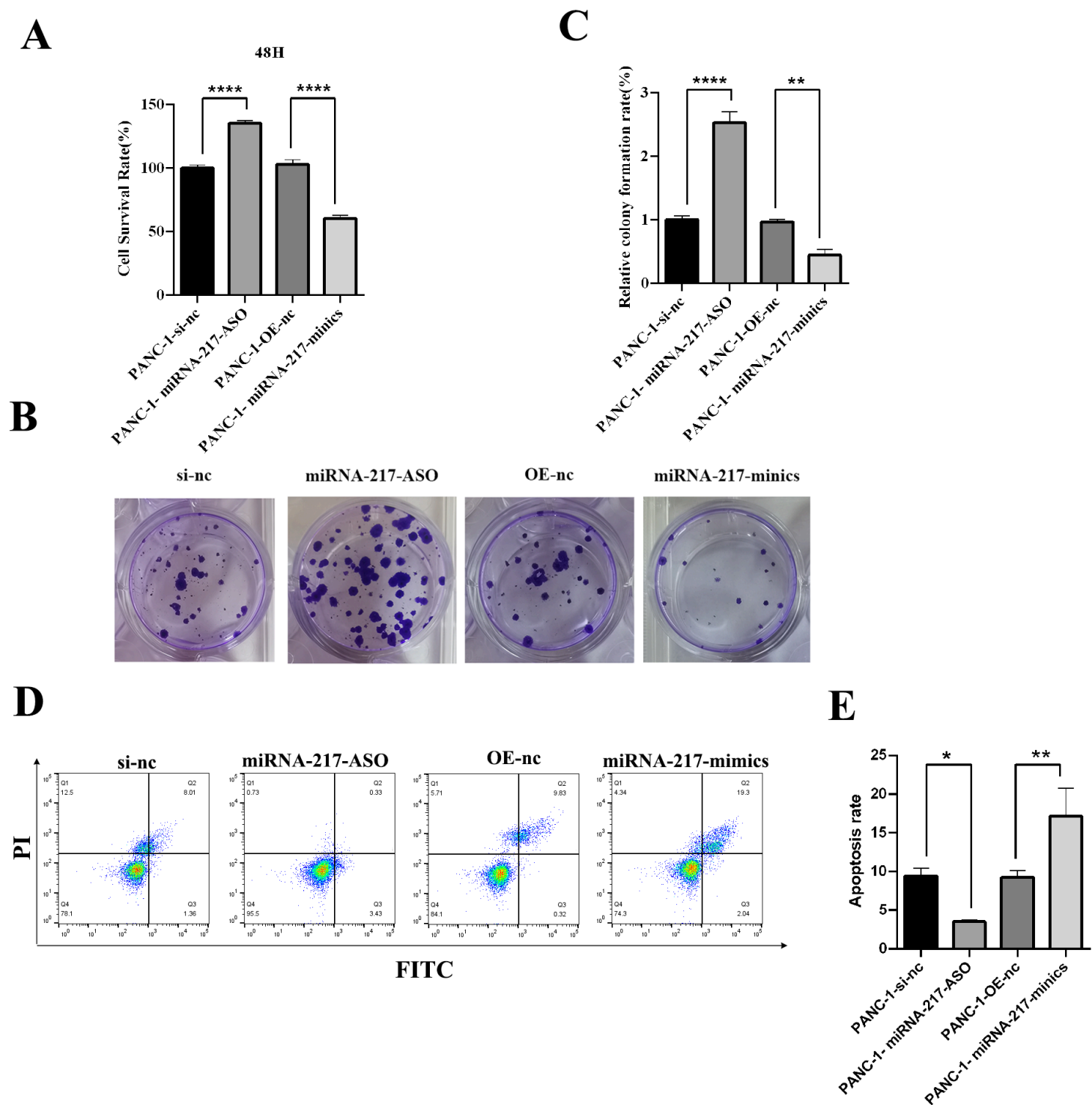


Figure 2

Influence of miR-217 on Proliferation and Apoptosis in PANC-1 Cells

A: Viability of PANC-1 cells following transfection with miR-217 mimic or miR-217 antisense oligonucleotide (ASO), evaluated by MTT assay. **B–C:** Colony formation assay illustrating the impact of miR-217 mimic and miR-217 ASO on the clonogenic capacity of PANC-1 cells. **D–E:** Apoptotic rates measured via flow cytometry after Annexin V-APC/PI staining.

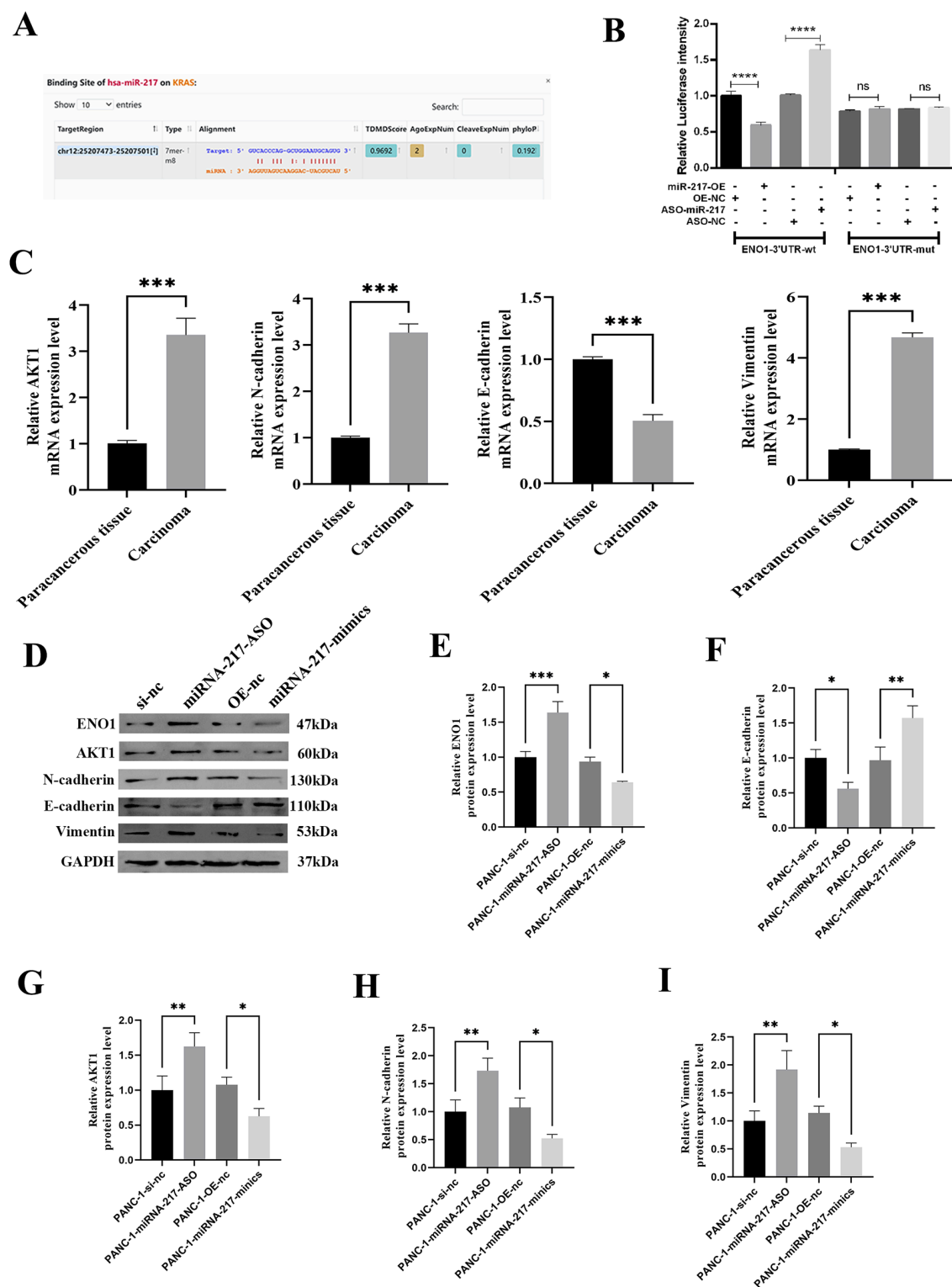


Figure 3

Regulatory Effects of miR-217 on ENO1 and Downstream Signaling Pathways in PANC-1 Cells

A: Predicted binding site between miR-217 and the 3'-UTR of ENO1 mRNA, as identified using TargetScan 8.0. **B:** Relative luciferase activity in PANC-1 cells co-transfected with wild-type (WT) or mutant (MUT) ENO1 3'-UTR reporter constructs and either miR-217 mimic, miR-217 inhibitor, or their respective negative controls (NC). **C:** mRNA expression levels of AKT1, N-cadherin, E-cadherin, and Vimentin in

tumor tissues versus adjacent normal tissues quantified by RT-qPCR. **D–I**: Western blot analysis of ENO1, AKT1, N-cadherin, E-cadherin, and Vimentin protein expression in PANC-1 cells transfected with miR-217 mimic or miR-217 ASO. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant.

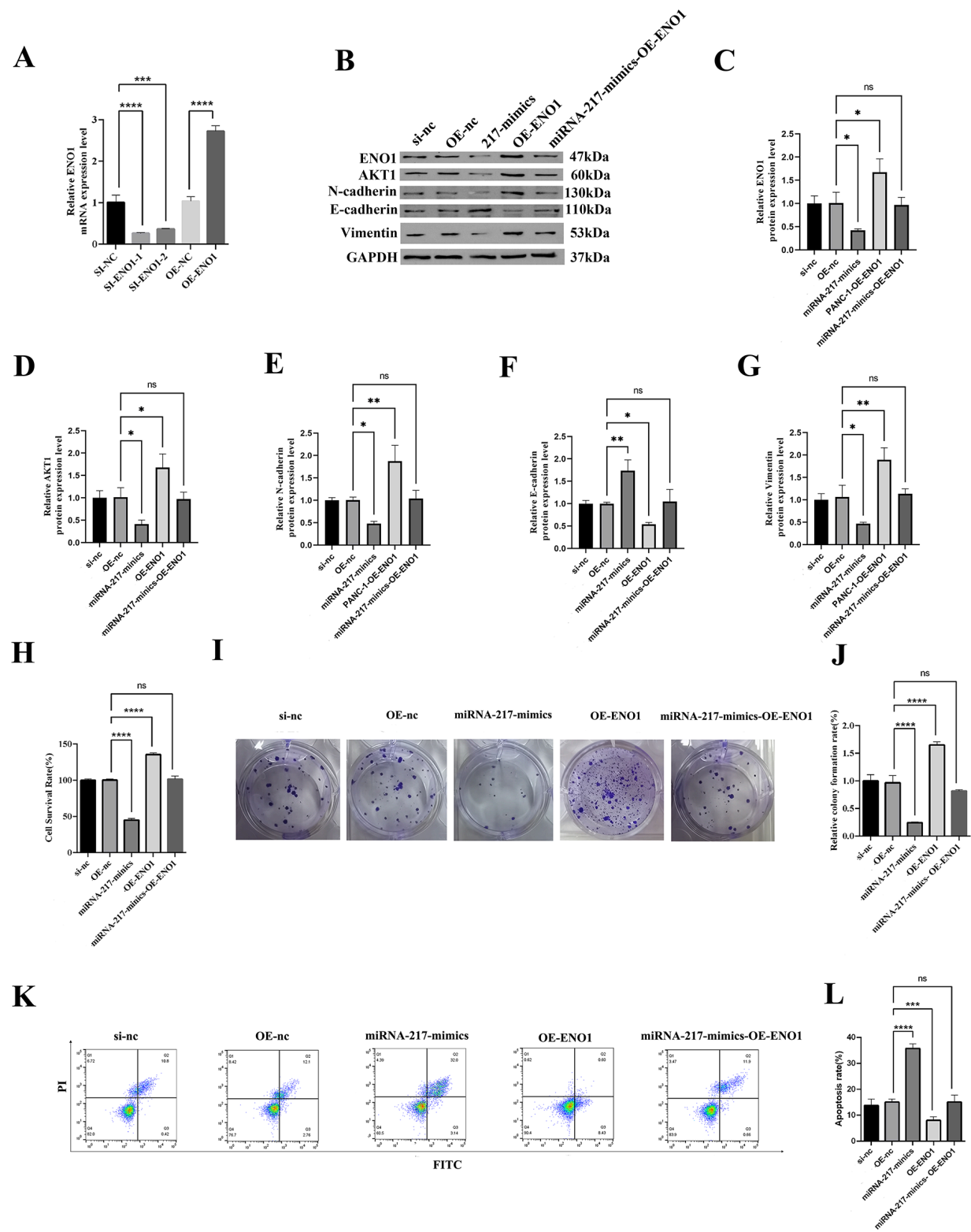


Figure 4

miR-217 Modulates Cell Proliferation and Apoptosis via Suppression of ENO1

A: Transfection efficiency of ENO1 overexpression plasmid (OE-ENO1) and short hairpin RNA targeting ENO1 (sh-ENO1) assessed by RT-qPCR. **B–G:** Protein expression levels of ENO1, AKT1, N-cadherin, E-cadherin, and Vimentin evaluated by Western blot under different transfection conditions. **H:** Cell proliferation measured by MTT assay. **I–J:** Colony formation ability analyzed via clonogenic assay. **K–L:** Apoptosis rates determined by flow cytometry. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GAPDH2.tif](#)
- [GAPDH3.tif](#)
- [GAPDH1.tif](#)
- [ENO11.tif](#)
- [ENO13.tif](#)
- [ENO12.tif](#)
- [AKT11.tif](#)
- [ATK13.tif](#)
- [AKT12.tif](#)
- [Ncadherin1.tif](#)
- [Ncadherin2.tif](#)
- [Ncadherin3.tif](#)
- [Ecadherin1.tif](#)
- [Ecadherin2.tif](#)
- [Ecadherin3.tif](#)
- [Vimentin1.tif](#)
- [Vimentin3.tif](#)
- [Vimentin2.tif](#)
- [GAPDH3.tif](#)
- [GAPDH1.tif](#)
- [GAPDH2.tif](#)
- [ENO12.tif](#)
- [ENO11.tif](#)
- [ENO13.tif](#)

- AKT12.tif
- AKT11.tif
- AKT13.tif
- Ncadherin1.tif
- Ncadherin3.tif
- Ncadherin2.tif
- Ecadherin1.tif
- Ecadherin3.tif
- Ecadherin2.tif
- Vimentin1.tif
- Vimentin2.tif
- Vimentin3.tif