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DNMT3A p.R882C driven proliferation and anti-apoptotic effects in pancreatic cancer cells

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Pancreatic cancer, one of the most lethal malignancies, is characterized by insidious onset, frequent late-stage diagnosis, rapid progression, and limited surgical opportunities. Pancreatic ductal adenocarcinoma (PDAC), accounting for over 90% of pancreatic cancer cases, remains a therapeutic challenge due to the scarcity of effective targeted therapies. In this study, we collected formalin-fixed paraffin-embedded (FFPE) specimens from three patients with moderately to poorly differentiated PDAC, extracted genomic DNA, and performed whole-exome sequencing. Through bioinformatics analysis, we identified 68 high-risk deleterious variants, including the DNMT3A p.R882C mutation. This mutation exhibits low frequency in public databases (e.g., esp6500si, GnomAD) and is consistently predicted as deleterious by multiple algorithms (SIFT, Polyphen, LRT, CADD). To investigate its functional impact, wild-type and mutant DNMT3A constructs were cloned into p3xflag-CMV-10 plasmids and transfected into the pancreatic cancer cell lines PANC-1 and PaTu 8988t. In vitro cellular experiments demonstrated that the DNMT3A p.R882C mutation does not alter DNMT3A expression at mRNA or protein levels but significantly promotes cancer cell proliferation and migration while inhibiting apoptosis. These findings suggest that the DNMT3A p.R882C mutation may play a critical role in PDAC pathogenesis. This study not only provides essential laboratory evidence for elucidating PDAC development but also offers new insights for potential targeted therapeutic strategies in pancreatic cancer.

Keywords PDAC, DNMT3A, Mutation, Whole-exome sequencing

Pancreatic cancer remains one of the most lethal malignancies worldwide, with pancreatic ductal adenocarcinoma (PDAC) constituting over 90% of pancreatic cancer cases and demonstrating a 5-year survival rate below 13%[1]. Characterized by late diagnosis, aggressive progression, and profound therapeutic resistance, PDAC is projected to become the second-leading cause of cancer-related deaths by 2030[2]. This dismal prognosis underscores the urgent need to decipher PDAC's complex molecular landscape and develop precision therapeutic strategies.

The genomic landscape of PDAC is dominated by four hallmark driver genes: KRAS (94.7%), TP53 (81.5%), CDKN2A (25.2%), and SMAD4 (33.8%)[3]. KRAS mutations initiate tumorigenesis by constitutively activating MAPK and PI3K-AKT pathways, while later TP53 loss enables genomic instability and metastatic dissemination[4]. The CDKN2A gene encodes two proteins, $p16^{INK4a}$ and $p14^{ARF}$. $p16^{INK4a}$ inhibits CDK4 activity, is a regulator of cell cycle progression, and a tumor suppressor gene[5]. As for SMAD4, it plays a role in activating transforming growth factor-β (TGF-β) signaling pathway and translocating to the nucleus as a transcriptional cofactor to facilitate the transcription of TGF-β-responsive genes in cancer cells, thereby regulating a variety of critical processes, including tumor development and immunotherapy[6]. Furthermore, emerging evidence highlights the role of epigenetic dysregulation, particularly enhancer reprogramming mediated by FOXA1 and super-enhancer complexes, in maintaining oncogenic transcriptional programs[7]. Although numerous studies have been conducted on the driver genes of pancreatic cancer, the development of targeted therapies for PDAC has still seen limited progress[8].

DNA (cytosine-5)-methyltransferase 3 A (DNMT3A) is involved in de novo establishment of DNA methylation and plays a vital role in tumorigenesis[9,10]. Missense mutations in the DNMT3A gene occur frequently at the early stages of tumor development and are often observed in hematologic malignances, especially in acute myeloid leukemia (AML), with a prevalence of the p.R882H mutation[11,12]. In PDAC, previous studies have demonstrated that DNMT3A is highly expressed and associated with poor prognosis in PDAC patients. DNMT3A regulates

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PDAC cell proliferation, partly by modulating extracellular IL8 levels to accelerate the G1-S phase transition of the cell cycle, thereby activating the STAT3 signaling pathway and ultimately inducing cyclin D1 expression. Furthermore, DNMT3A inhibits apoptosis in PDAC cells[13]. Recent findings indicate that aberrant upregulation of DNMT1/3A promotes hypermethylation of promoter regions, leading to suppression of the tumor suppressor gene CLDN1 and ultimately contributing to pancreatic carcinogenesis[14]. In summary, the overexpression of DNMT3A facilitates the initiation and progression of pancreatic cancer and is strongly correlated with unfavorable clinical outcomes in PDAC patients. However, whether the DNMT3A p.R882 hotspot mutation site also exerts a crucial function in the development and progression of PDAC remains unclear.

In this study, we performed whole-exome sequencing on tumor tissues and paired adjacent non-tumorous tissues from patients with moderately to poorly differentiated PDAC. Germline single nucleotide polymorphisms (SNPs) were filtered using adjacent normal tissues as reference controls. Subsequent bioinformatics analysis identified the DNMT3A p.R882C variant as a pathogenic mutation. Functional validation in the human pancreatic cancer cell lines PANC-1 and PaTu 8988t demonstrated that overexpression of the DNMT3A p.R882C mutant, compared to wild-type DNMT3A, significantly promoted cellular proliferation and migration while suppressing apoptosis. These findings suggest that the DNMT3A p.R882C mutation may play a pivotal role in PDAC tumorigenesis and progression. Our results provide novel experimental evidence elucidating the molecular mechanisms underlying PDAC development and offer potential therapeutic targets for precision treatment strategies in this malignancy.

Materials and methods

PDAC specimens

We collected three pairs of paraffin-embedded specimens (cancer and adjacent tissues) from pancreatic cancer patients in the Department of Pathology at Jining No.1 People's Hospital. These specimens had been confirmed by two pathologists as moderately to poorly differentiated pancreatic ductal adenocarcinoma. Appropriate amounts of wax shavings from the paraffin-embedded specimens were dissected for DNA extraction using a DNA extraction kit. The extracted DNA was subsequently sent to Novogene Bioinformatics Technology Co., Ltd. for whole-exome sequencing (WES) analysis.

The study was approved by the Institutional Research Ethics Committee of Jining No.1 People's Hospital and conducted according to the Declaration of Helsinki.

Bioinformatics analysis of Whole-Exome sequencing

Raw sequencing data underwent quality control (FastQC) and adapter trimming (Trimmomatic). Clean reads were aligned to the human reference genome (GRCh38) using BWA-MEM, followed by duplicate removal (Picard). Variant calling (SNVs/indels) was performed using GATK HaplotypeCaller with base quality recalibration. Annotation and prioritization were conducted via ANNOVAR and SnpEff, incorporating population databases (gnomAD) and clinical databases (ClinVar, OMIM). Variants were filtered by minor allele frequency (< 1%), predicted pathogenicity (ACMG guidelines), and phenotypic relevance. The sequencing data of matched cancer tissues were filtered using those from adjacent normal tissues to exclude patient-specific single nucleotide polymorphisms.

Cell culture

The PANC-1 (Catalog number: CL-0184) and PaTu 8988t (Catalog number: CL-0579) cell lines were purchased from Wuhan Procell Life Science & Technology Co., Ltd. PANC-1 and PaTu 8988t human pancreatic cancer cells were cultured in DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C under 5% CO_2. Cells were passaged at 80–90% confluence using 0.25% trypsin-EDTA and subcultured every 3–4 days.

Western blot analysis

Proteins were extracted using RIPA lysis buffer (Thermo Fisher), quantified via BCA assay, and separated on 10% SDS-PAGE gels. After transfer to PVDF membranes (Millipore), blocking was performed with 5% non-fat milk. The membrane was incubated with the primary antibody overnight at 4 °C (DYKDDDDK tag Monoclonal antibody, Proteintech, Cat No. 66008-4-Ig; Beta Actin Monoclonal antibody, Proteintech, Cat No. 66009-1-Ig), followed by incubation with the secondary antibody at room temperature for 2 h. Signals were detected using ECL substrate (Bio-Rad) and quantified with ImageJ. Beta ACTIN served as the loading control.

RT-PCR analysis

Total RNA was isolated with TRIzol reagent (Invitrogen), reverse-transcribed using the PrimeScript RT Kit (Takara), and amplified with SYBR Green Master Mix (Roche) on a ABI7900 system. Cycling conditions: 95 °C for 30 s, 40 cycles of 95 °C/5 sec and 60 °C/30 sec. β-Actin was used for normalization. The primers were:

β-Actin: F: 5′-CCTGAACCCCAAGGCCAACC-3′.
R: 5′- CAGGGATAGCACAGCCTGGA-3′.
DNMT3A: F: 5′-GCCCAAGGTCAAGGAGATTATT – 3′.
R: 5′-GAGATGCAGATGTCCTCAATGT-3′.

Colony formation assay

PANC-1 and PaTu 8988t cells (500/well) were seeded in 6-well plates and cultured for 14 days. Colonies were fixed with 4% paraformaldehyde, stained with 0.5% crystal violet, and quantified using ImageJ. Clusters with ≥ 50 cells were counted as colonies.

Mutation tyep	Patient 1	Patient 2	Patient 3	Total
SNV	219	245	102	566
Indel	3	4	9	16
CNV	30	93	56	179

Table 1. Statistical analysis of mutation counts from WES results. SNV: Single Nucleotide Variant; Indel: Insertion-Deletion; CNV: Copy Number Variation.

Mutaiton type	H	H*	H+	H-	M	L	L*	L+	L-
P1-SNV	21	2	0	0	2	182	0	2	10
P1-InDel	0	0	0	0	0	3	0	0	0
P2-SNV	30	0	1	2	2	199	2	1	8
P2-InDel	2	0	0	0	0	2	0	0	0
P3-SNV	9	1	0	0	1	89	1	0	1
P3-InDel	0	0	0	0	0	7	1	0	1

Table 2. Classify and statistically analyze the hazard levels of mutations. H: High risk; M: Medium risk; L: Low risk; *: Completely lethal gene; +: Potentially lethal gene; -: non-lethal gene; P: Patient; SNV: Single Nucleotide Variant; InDel: Insert or Delete.

CCK-8 proliferation assay
Cells (3×10^3/well) were seeded in 96-well plates. At 0 and 48 h, 10 µL CCK-8 (Dojindo) was added, incubated for 1.5 h, and absorbance was measured at 450 nm. Triplicates were analyzed for each timepoint.

Wound healing assay
The confluent monolayers of PANC-1 and PaTu 8988t cells were scratched using a 200-µL pipette tip. Debris was removed by washing with PBS, and the medium was replaced with serum-free medium. Wound closure was then imaged (Nikon Eclipse TS2) at 0, 24, and 48 h. Finally, the area of the migration region was measured using ImageJ, and the migration rate was analyzed.

Trans-well assay
For migration, 1×10^5 PANC-1 and PaTu 8988t cells in serum-free DMEM were seeded into 8-µm upper chambers (Corning). For invasion, chambers were coated with Matrigel (1:8 dilution). Complete medium was used as chemoattractant. Migrated/invaded cells were fixed (methanol), stained (0.1% crystal violet), and counted after 24 h.

Apoptosis analysis
PANC-1 and PaTu 8988t cells were harvested, washed, and stained with Annexin V-FITC/PI (BD Biosciences) for 15 min. Apoptotic populations were quantified using a CytoFLEX flow cytometer (Beckman Coulter). Data were analyzed with CytExpert software (v2.4).

Statistical analysis
Data are presented as mean ± SD. Student's t-test (two-tailed, unpaired) was performed using GraphPad Prism 9.0. Differences with $^*p < 0.05$, $^{**}p < 0.01$, or $^{***}p < 0.001$ were considered statistically significant. All experiments included ≥ 3 biological replicates.

Results
Whole-Exome sequencing analysis
Since moderately to poorly differentiated PDAC constitutes the majority of histologically diagnosed pancreatic cancers, we selected formalin-fixed, paraffin-embedded (FFPE) specimens from three PDAC patients who underwent surgery at Jining No.1 People's Hospital in 2023. The H&E staining results indicated a moderately to poorly differentiated pancreatic ductal adenocarcinoma. DNA was extracted from paired tumor and adjacent non-cancerous tissues for whole-exome sequencing. Germline single nucleotide polymorphisms were filtered using sequencing data from adjacent non-cancerous tissues. As shown in Table 1, a total of 566 somatic single nucleotide variants (SNVs), 16 insertion-deletion mutations (Indels), and 179 copy number variations (CNVs) were identified across the three patients.

Subsequently, these variants were categorized through bioinformatics analysis into high-risk (H), moderate-risk (M), and low-risk (L) variants. The WES results revealed that Patient 1 harbored 23 high-risk variants, Patient 2 exhibited 35 high-risk variants, and Patient 3 carried 10 high-risk variants. The statistical results are summarized in Table 2, and the information on the 68 high-risk variants is comprehensively detailed in Supplementary Material Table S1.

DNMT3A p.R882C variant as a potential pathogenic mutation

We first performed whole-exome sequencing on FFPE samples from three paired patients. To identify somatic driver mutations, we applied a rigorous bioinformatics filtering pipeline. First, variants with extremely low (rare) allele frequencies in population databases such as ESP6500 and gnomAD were retained to exclude common polymorphisms. Second, the functional impact of the remaining variants was predicted using multiple algorithms, including SIFT, PolyPhen-2, LRT, MutationTaster, FATHMM-MKL, REVEL, and CADD. Among these, the DNMT3A p.R882C variant was unanimously predicted as deleterious by all tools. Although the NTRK1 p.W299R and RNF43 p.L65F variants were predicted to have a mild or tolerated impact by FATHMM-MKL and REVEL, they were retained based on their rarity in populations and the deleterious predictions from other algorithms. Ultimately, considering their combined rarity and potential deleterious effects, we identified these three variants as potential driver mutations (Table 3).

Among these high-risk variants, DNMT3A p.R882C heterozygous mutation (NM_022552: c.2644 C > T, p.R882C) exhibits an extremely low frequency in population databases such as ESP6500 and gnomAD. Bioinformatics tools, including SIFT, PolyPhen-2, LRT, CADD, FATHMM-MKL and REVEL, consistently predicted it as deleterious.

DNMT3A consists of 912 amino acids. The p.R882C variant is located within the SAM-dependent MTase C5-type domain of DNMT3A, suggesting that this mutation may impair the enzymatic activity of DNMT3A (Fig. 1A). To determine whether this mutation alters the three-dimensional protein structure or hydrogen bonding, we analyzed the mutant protein using PyMol. Structural modeling revealed no significant conformational changes or disruptions in hydrogen bond networks in the mutated DNMT3A protein (Fig. 1B, C).

The p.R882C mutation does not affect DNMT3A expression

To investigate whether the DNMT3A p.R882C mutation alters its expression, we overexpressed p3xflag-CMV-10 empty vector, p3xflag-CMV-10-DNMT3A (wild-type) and p3xflag-CMV-10-DNMT3A^{R882C} (mutant) in PANC-1 and PaTu 8988t cell lines. mRNA and protein were subsequently isolated for analysis via RT-PCR and Western blot. Results demonstrated that the p.R882C mutation had no significant impact on DNMT3A expression at either the mRNA or protein level (Fig. 2A, B, C).

DNMT3A p.R882C promotes proliferation and migration of pancreatic cancer cells

To further validate the physiological relevance of the DNMT3A p.R882C variant, we performed a series of cellular assays. First, we overexpressed p3xflag-CMV-10 empty vector, p3xflag-CMV-10-DNMT3A (wild-type) and p3xflag-CMV-10-DNMT3A^{R882C} (mutant) in PANC-1 and PaTu 8988t cells and evaluated the impact of this mutation on pancreatic cancer cell proliferation using colony formation and CCK-8 assays. Results demonstrated a significant increase in colony numbers in both cells expressing the mutant DNMT3A compared to wild-type controls (Fig. 3A), with statistical analysis corroborating this observation (Fig. 3B). To further validate these

Gene	Analysis tool	Mutation rate	Score	Risk prediction
DNMT3A	esp6500si_all	0.0003	/	/
	GnomAD_AF_ALL	0.0001	/	/
	SIFT	/	0	Deleterious
	Polyphen-2_HDIV	/	1.0	Probably_damaging
	Polyphen-2_HVAR	/	0.936	Probably_damaging
	LRT	/	0	Deleterious
	Mutation Taster	/	1	Deleterious
	CADD	/	34	Deleterious
	FATHMM_MKL	/	0.991	Deleterious
	REVEL	/	0.890	Deleterious
NTRK1	esp6500si_all	0	/	/
	GnomAD_AF_ALL	0	/	/
	SIFT	/	0.042	Deleterious
	Polyphen-2_HDIV	/	1.0	Deleterious
	Polyphen-2_HVAR	/	0.998	Deleterious
	LRT	/	0.000028	Deleterious
	Mutation Taster	/	1.0	Deleterious
	CADD	/	27.1	Deleterious
	FATHMM_MKL	/	1.6	Tolerated
	REVEL	/	0.489	Tolerated
RNF43	esp6500si_all	0	/	/
	GnomAD_AF_ALL	0	/	/
	SIFT	/	0.013	Deleterious
	Polyphen-2_HDIV	/	1.0	Deleterious
	Polyphen-2_HVAR	/	0.999	Deleterious
	LRT	/	0.015698	Normal
	Mutation Taster	/	1.0	Deleterious
	CADD	/	25.3	Deleterious
	FATHMM_MKL	/	2.67	Tolerated
	REVEL	/	0.219	Tolerated

Table 3. Pathogenicity prediction of DNMT3A c. c.2644 C > T (p.R882C) mutation.

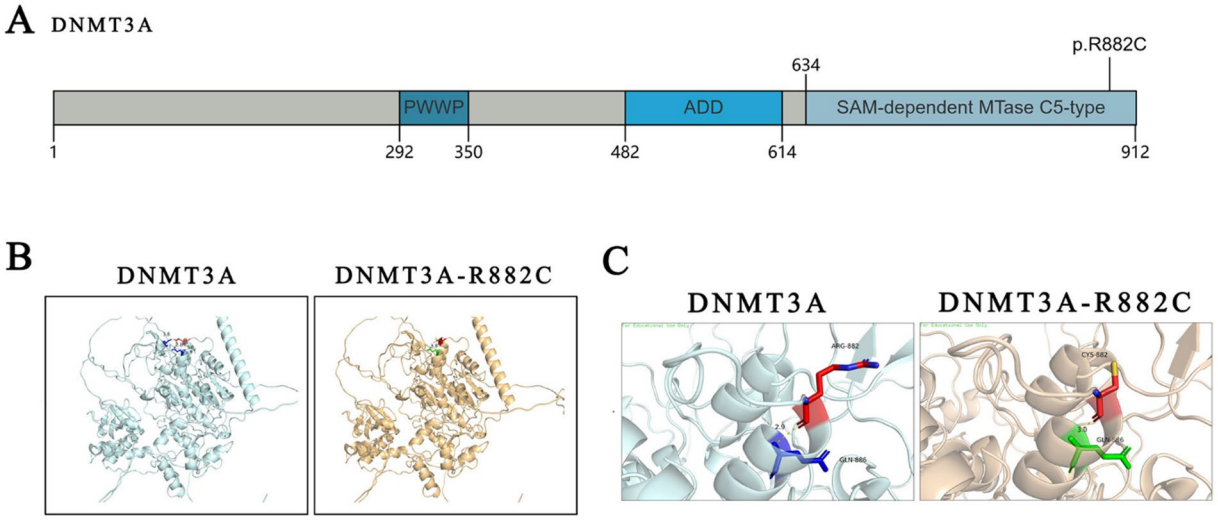

Fig. 1. Schematic diagrams of the 2D and 3D structures of DNMT3A. (**A**) Schematic representation of the DNMT3A amino acid sequence and protein domains, with the p.R882C mutation site highlighted in this study; (**B**) Three-dimensional (3D) structural comparison of wild-type DNMT3A and mutant DNMT3A proteins; (C) Hydrogen bonding network at the p.R882C mutation site in DNMT3A.

findings, we conducted CCK-8 proliferation assays, which revealed a marked increase in OD450 absorbance in PANC-1 and PaTu 8988t cells harboring the DNMT3A p.R882C mutation (Fig. 3C). These results collectively indicate that the DNMT3A p.R882C mutation enhances pancreatic cancer cell proliferation.

To determine whether the DNMT3A p.R882C mutation influences cell migration, we overexpressed wild-type and mutant constructs in PANC-1 and PaTu 8988t cells and performed wound healing and Trans-well migration assays. The wound healing assay showed that the p.R882C mutant group exhibited faster wound closure rates compared to the wild-type group (Fig. 4A, C). Similarly, Trans-well migration assays demonstrated a significant increase in migratory cell numbers in the mutant group (Fig. 4B, D). These findings suggest that the DNMT3A p.R882C mutation promotes pancreatic cancer cell migration.

DNMT3A p.R882C mutation suppresses apoptosis in pancreatic cancer cells

To assess the effect of DNMT3A p.R882C on apoptosis, PANC-1 and PaTu 8988t cells overexpressing FLAG empty vector, wild-type or mutant DNMT3A were subjected to Annexin V-FITC/propidium iodide (PI) dual staining followed by flow cytometry. The results demonstrated that cells expressing the DNMT3A p.R882C mutant exhibited a significantly reduced apoptosis rate compared to the wild-type control group (Fig. 5A). Statistical analysis revealed that the mutant group showed markedly lower percentages of apoptotic cells, whether in early apoptosis, late apoptosis, or total apoptosis (Fig. 5B). These findings indicate that the DNMT3A p.R882C mutation inhibits apoptosis in pancreatic cancer cells.

Discussion

In this study, we collected three paired PDAC specimens and performed whole-exome sequencing. Through bioinformatics analysis, we identified 68 high-risk deleterious variants. Among these, DNMT3A was found to be highly expressed in PDAC and promoted pancreatic cancer proliferation[13]. The DNMT3A p.R882C mutation exhibited low mutation frequencies in databases such as esp6500si and GnomAD, and was predicted to be a pathogenic variant by multiple bioinformatics tools, including SIFT, Polyphen, LRT, and CADD. Therefore, we overexpressed p3xflag-CMV-10, p3xflag-CMV-10-DNMT3A (wild-type) and p3xflag-CMV-10-DNMT3A ^{R882C} (mutant) in the PANC-1 and PaTu 8988t cell line to investigate the functional impact of this mutation. RT-PCR and Western blot results demonstrated that the DNMT3A p.R882C mutation did not alter its expression at either mRNA or protein levels. However, cellular functional assays revealed that this mutation significantly enhanced the proliferation and migration of PANC-1 cells while suppressing apoptosis. Collectively, these findings suggest that the DNMT3A p.R882C mutation may play a critical role in the pathogenesis and progression of pancreatic cancer.

Our whole-exome sequencing analysis identified 68 high-risk deleterious variants. Based on current research data, three of these genes—NTRK1, DNMT3A, and RNF43 —are strongly associated with the pathogenesis and progression of pancreatic cancer[13,15,16]. In functional cellular assays, the NTRK1 p.W299R and RNF43 p.L65F variants yielded negative results (data not shown), highlighting that bioinformatics predictions may not always align with experimental outcomes.

DNMT3A is widely recognized as being independently associated with inferior prognosis in AML patients[17,18]. Approximately 60% of AML patients carry the DNMT3A p.R882 mutation[19]. The DNMT3A p.R882 mutation primarily includes four subtypes: p.R882C, p.R882H, p.R882S, and p.R882P, with p.R882H being the most prevalent among these missense mutations[20,21]. Current research suggests that the DNMT3A

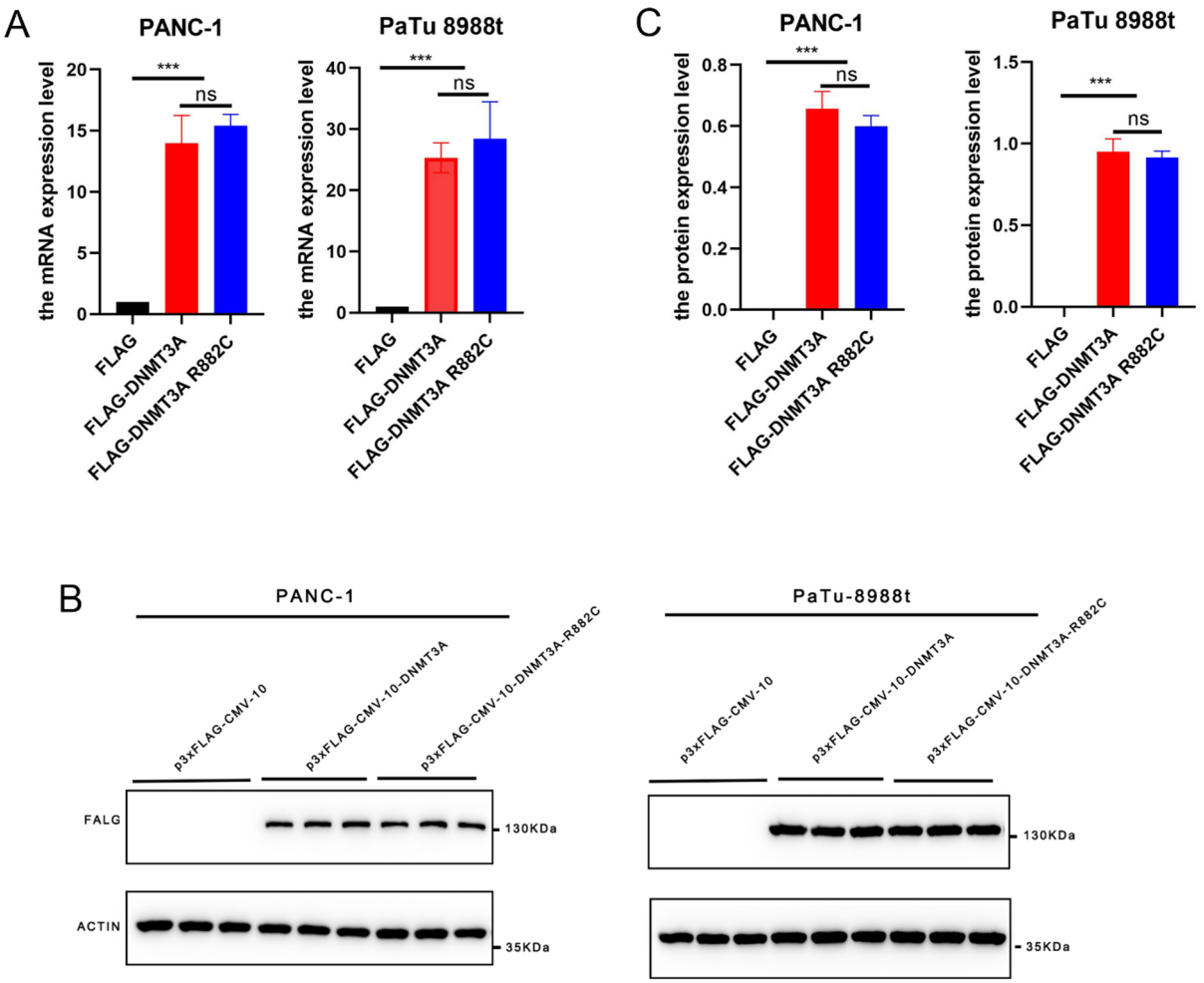

Fig. 2. DNMT3A p.R882C mutation does not affect its expression levels. (**A**) RT-PCR analysis of FLAG empty vector, wild-type and mutant DNMT3A mRNA expression levels; (**B**) WB detection of FLAG empty vector, wild-type and mutant DNMT3A protein expression levels; (**C**) Quantitative analysis of protein expression levels.

p.R882 mutation disrupts the formation of DNMT3A homotetramers, ultimately reducing its methyltransferase activity by approximately 80%, which contributes to leukemogenesis in AML[22–24]. Changes in the expression of many genes may not be a direct result of methylation by DNMT3A, but rather secondary effects arising from the silencing of its direct targets (such as transcription factors). For example, DNMT3A may silence a repressor of a transcription factor that inhibits apoptosis, ultimately leading to the suppression of apoptosis. This represents a complex regulatory network. In AML, the DNMT3A R882H mutation is a common hotspot mutation. This dominant-negative mutation interferes with the function of wild-type DNMT3A, resulting in genome-wide hypomethylation. Consequently, in this mutational context, some targets that should normally be methylated by DNMT3A remain in a hypomethylated state, leading to dysregulated gene expression. Currently, in AML, reported downstream targets of DNMT3A include genes such as CDKN2B, CDKN1A, and CEBPA[25–27]. However, while DNMT3A p.R882 mutations have been predominantly reported in AML, they have not been previously documented in pancreatic cancer. Through whole-exome sequencing, we identified this mutation in PDAC. Several critical questions remain to be explored: whether this mutation plays an equally crucial role in PDAC, whether it could serve as a biomarker for early screening in PDAC, and whether it represents a potential therapeutic target for future PDAC treatments. Additionally, it remains unclear whether the DNMT3A p.R882C mutation in PDAC similarly leads to reduced methyltransferase activity through impaired homotetramer formation, ultimately driving PDAC progression, or whether functional alterations arise from methylation modifications or ADP-ribosylation at the arginine residue of DNMT3A p.R882. These mechanisms constitute the primary focus of our ongoing research.

In cytological experiments, Western blot results indicate that the DNMT3A p.R882C variant does not affect the protein stability of DNMT3A. Since the mutation site is located within the SAM-dependent MTase C5-type domain, it is highly likely that the DNMT3A p.R882C variant impairs the enzymatic activity of DNMT3A, which aligns with its pathogenic mechanism in acute myeloid leukemia[22]. Multiple cytological studies have shown that the DNMT3A p.R882C variant promotes cell proliferation and migration while inhibiting apoptosis. Although

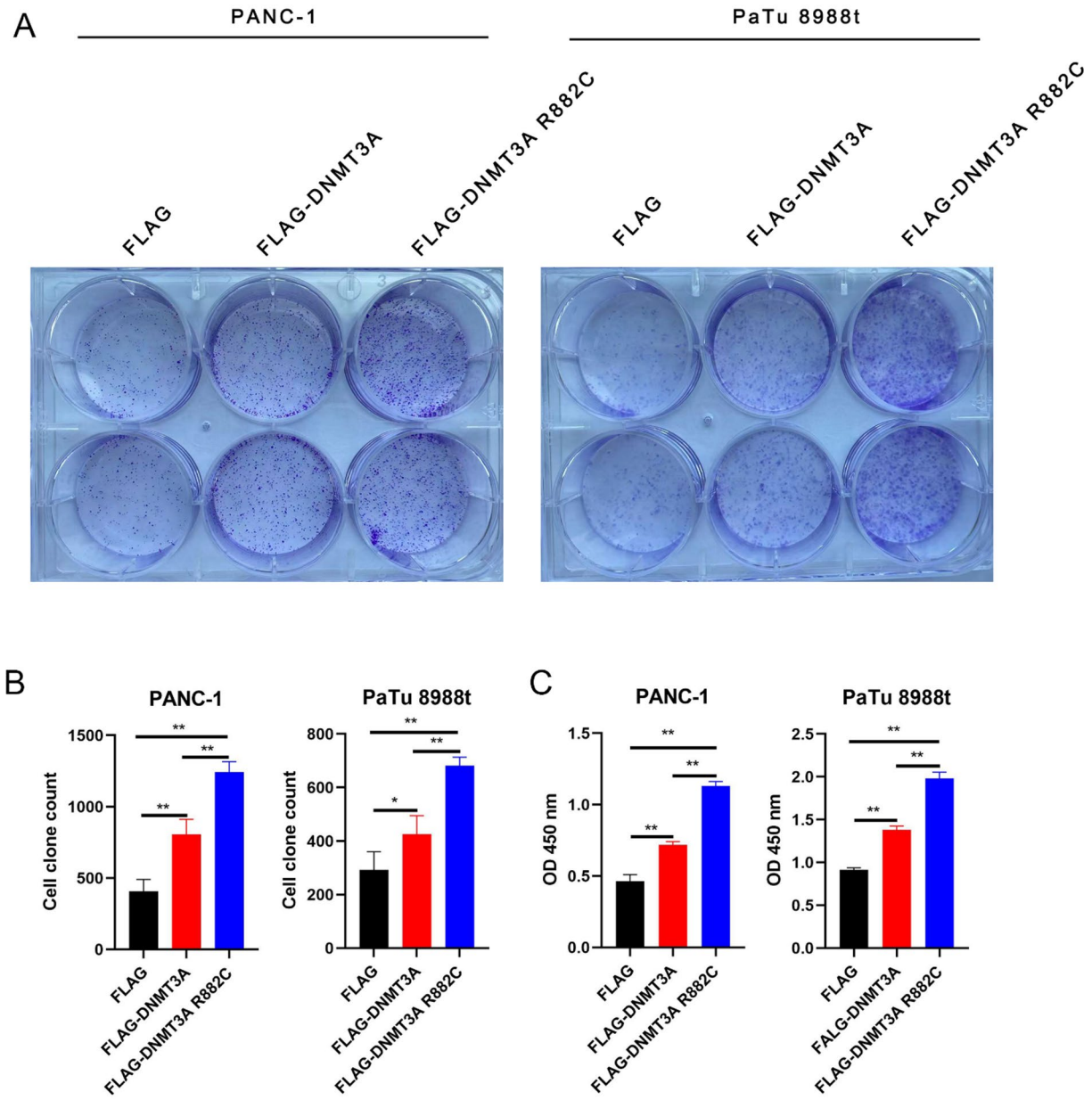

Fig. 3. DNMT3A p.R882C promotes proliferation of pancreatic cancer cell line PANC-1 and PaTu 8988t. (**A**) Colony formation assay assessing FLAG empty vector, wild-type and mutant DNMT3A in PANC-1 and PaTu 8988t cells; (**B**) Quantitative analysis of colony formation results; (**C**) CCK-8 assay evaluating FLAG empty vector, wild-type and mutant DNMT3A in PANC-1 and PaTu 8988t cells.

the role of the DNMT3A p.R882 variant in pancreatic cancer remains to be explored, its mechanism in AML has been extensively studied. It is widely accepted that the DNMT3A p.R882C variant disrupts the formation of DNMT3A homotetramers, leading to reduced enzymatic activity and subsequent global hypomethylation, particularly in intergenic regions and repetitive sequences, thereby inducing genomic instability[12]. In terms of epigenetic regulation, the mutation compromises chromatin binding capacity, resulting in aberrant methylation of target genes[28]. For example, tumor suppressor genes may be silenced due to hypermethylation, while proto-oncogenes may be activated via hypomethylation. In summary, the DNMT3A p.R882C mutation promotes tumorigenesis—especially in AML—by reducing enzymatic activity, disrupting normal methylation patterns, impairing cellular differentiation, and synergizing with other genetic abnormalities. The core mechanism lies in the disruption of the epigenetic regulatory network, which drives genomic instability and oncogene activation.

Additionally, we systematically analyzed the prognostic value of the DNMT3A p.R882 variant in pancreatic ductal adenocarcinoma (PDAC) using the COSMIC (https://cancer.sanger.ac.uk/cosmic) and cBioPortal (https://www.cbioportal.org/) databases. Regrettably, no statistically significant results were obtained from either database. Our critical assessment suggests that this outcome may primarily stem from the following methodological constraints:

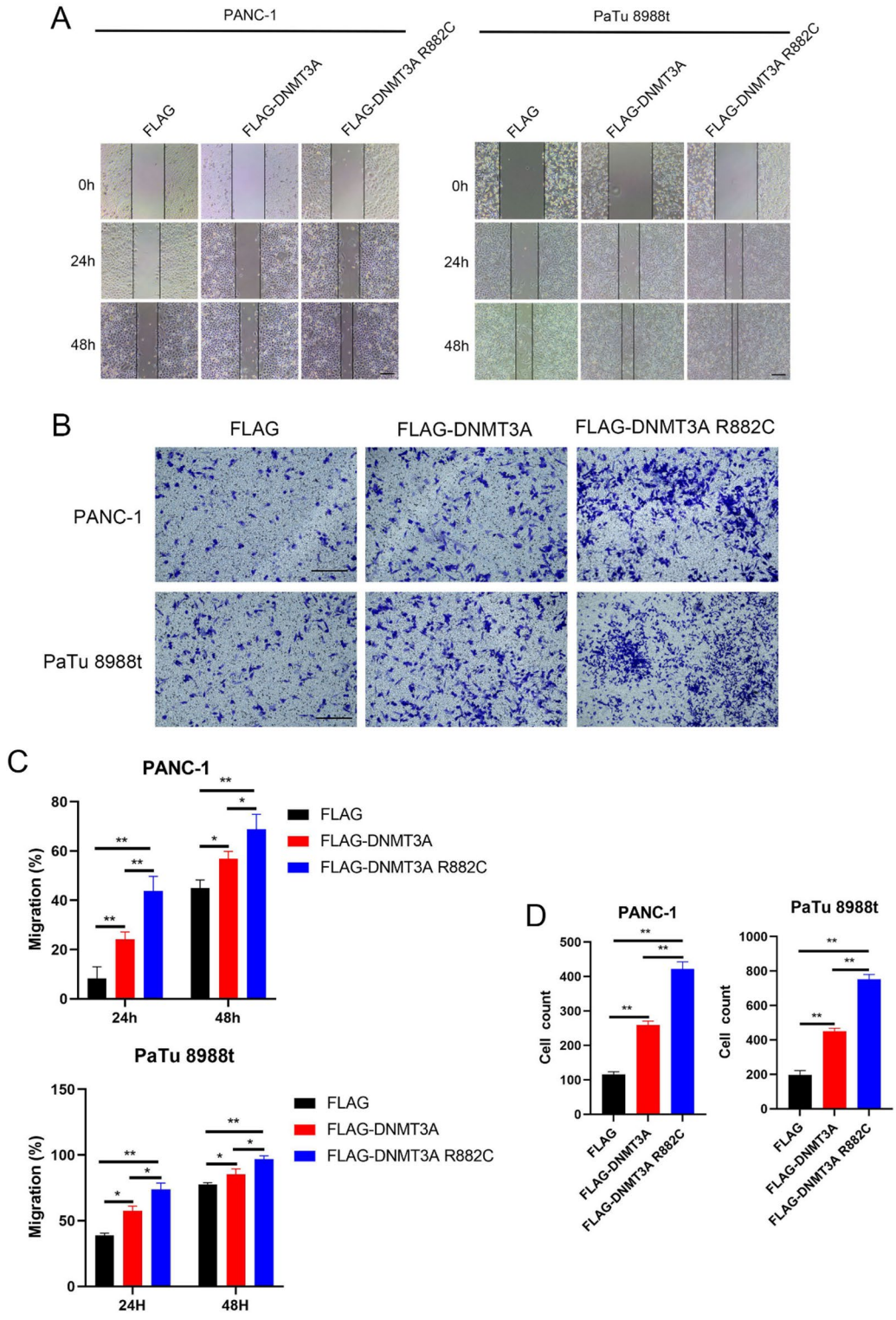

Fig. 4. DNMT3A p.R882C promotes migration of pancreatic cancer cell lines. (**A**) Wound healing assay assessing FLAG empty vector, wild-type and mutant DNMT3A in PANC-1 and PaTu 8988t cells; (**B**) Transwell migration assay evaluating FLAG empty vector, wild-type and mutant DNMT3A in PANC-1 and PaTu 8988t cells; (**C**) Quantitative analysis of wound healing assay results; (**D**) Quantitative analysis of Transwell migration assay results. scale bar = 100 μm.

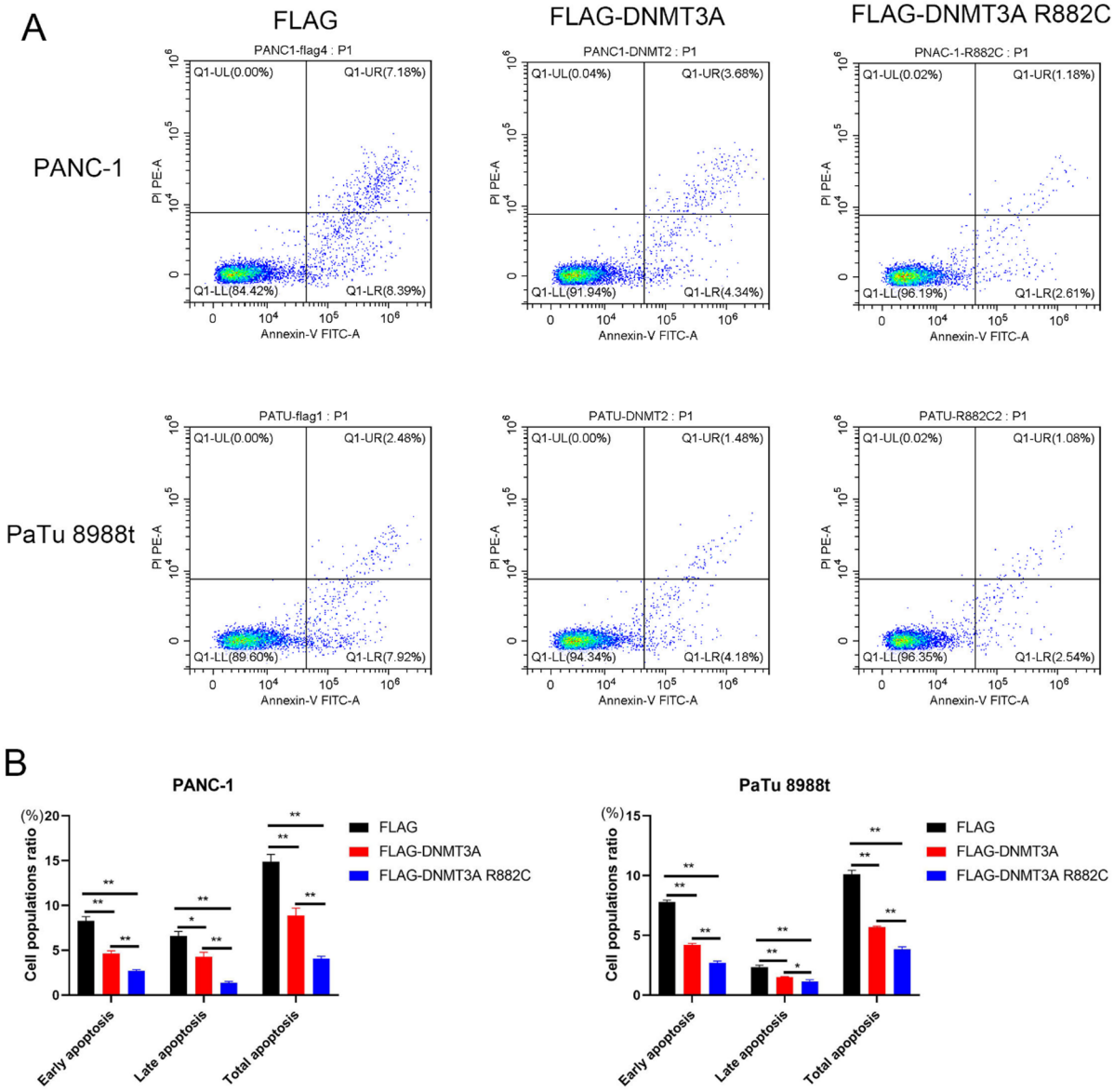

Fig. 5. DNMT3A p.R882C suppresses apoptosis in pancreatic cancer cells. (**A**) Flow cytometry analysis of apoptosis in FLAG empty vector, wild-type and mutant DNMT3A-expressing PANC-1 and PaTu 8988t cells; (**B**) Quantitative analysis of early-stage apoptotic cells, late-stage apoptotic cells and total apoptotic cells.

1)Gene panel limitations: The PDAC-specific gene panels currently available in cBioPortal lack coverage of DNMT3A, thereby precluding evaluation of its association with PDAC patient prognosis.

2)Low mutation prevalence and insufficient cohort size: The TCGA PDAC cohort exhibits a relatively small sample size compared to other cancer types. Furthermore, the DNMT3A p.R882C variant does not represent a recurrent mutational hotspot in PDAC, collectively explaining the absence of statistically significant findings in the COSMIC database.

Regarding the absence of DNMT3A knockdown in this study, the primary reasons are as follows: (1) The main focus of this study is the DNMT3A p.R882C point mutation. We introduced the DNMT3A protein carrying this mutation into pancreatic cancer cell lines by transfecting mutant plasmids to investigate the role of this mutation in tumorigenesis, rather than studying the effects of altered DNMT3A expression levels, such as overexpression or interference, on tumor progression. (2) Both our RT-PCR and Western blot experiments demonstrated that the DNMT3A p.R882C point mutation does not affect DNMT3A expression levels. (3) In AML research, existing studies have shown that the DNMT3A p.R882C mutation promotes tumorigenesis independently of changes in its expression levels. (4) We assessed the baseline expression levels of DNMT3A in multiple cell lines via Western blot and found that, compared to other cell lines such as H1299, PANC-1 exhibits relatively low baseline DNMT3A expression, making knockdown impractical. In summary, we believe that performing DNMT3A knockdown in pancreatic cancer cell lines may not be highly meaningful and is also not highly feasible.

Data availability

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Author contributions

Zhen Qu was responsible for research design, experimental work, and manuscript writing; Jinglin Mao handled experimental work and material collection; Yang Qian conducted experimental work and material collection; Jingyu Cao provided guidance for the research; Shulong Jiang oversaw research design and overall supervision of the project.

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Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of Jining No. 1 People's Hospital. The research protocols

were conformed to the tenets of the Declaration of Helsinki. Informed consents were taken from patients for publication of this study.

Competing interests
The authors declare no competing interests.

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