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Mechanism of lncRNA PLACT1 in regulating the proliferation of pancreatic adenocarcinoma cells through the KLF2/KIAA1522 axis

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Summary. Background. Pancreatic adenocarcinoma (PAAD) is among the most common cancers worldwide. This study aims to investigate the role of long noncoding RNA pancreatic cancer-associated transcript 1 (LncRNA PLACT1) in PAAD cell proliferation.

Methods. PAAD and normal cells were cultured. The levels of PLACT1, Krüppel-like factor 2 (KLF2), and KIAA1522 were detected. After PLACT1 expression was interfered with, cell proliferation was detected using the cell counting kit-8, clone formation assay, and 5-ethynyl-2-deoxyuridine (EdU) staining. The binding of PLACT1 to euchromatic histone lysine methyltransferase 2 (EHMT2) was analyzed. The enrichment of EHMT2 and histone H3 lysine 9 dimethylation (H3K9me2) on the KLF2 promoter was analyzed by chromatin immunoprecipitation. KLF2 expression was detected after EHMT2 intervention. The binding of KLF2 to the KIAA1522 promoter was analyzed. The nude mouse xenograft model was constructed to detect the role of PLACT1 *in vivo*.

Results. PLACT1 and KIAA1522 were highly expressed, and KLF2 was poorly expressed in PAAD cells. Silencing PLACT1 decreased cell proliferation, the number of cell clones, and EdU-positive cells. Mechanistically, PLACT1 inhibited KLF2 expression by recruiting EHMT2 to induce H3K9me2 in the KLF2 promoter region, resulting in reduced KLF2 enrichment at the KIAA1522 promoter and increased KIAA1522 expression. KLF2 downregulation or KIAA1522 overexpression alleviated the inhibitory effect of PLACT1 silencing on PAAD cell proliferation. PLACT1 silencing prevented PAAD tumorigenesis by regulating the KLF2/KIAA1522 pathway.

Conclusion. PLACT1 silencing inhibited PAAD by inhibiting KLF2 and promoting KIAA1522 expression, suggesting the therapeutic effect of PLACT1 silencing on PAAD.

Keywords: Pancreatic Adenocarcinoma, LncRNA PLACT1, KLF2, KIAA1522, EHMT2

Introduction

Pancreatic adenocarcinoma (PAAD) is a highly lethal disease, with poor five-year survival rates (2-9%) due to its typically late-stage diagnosis (McGuigan et al., 2018). PAAD is characterized by rapid progression, high rates of metastasis and recurrence, and fast development of resistance (Rong et al., 2021). In the past few decades, the global burden of PAAD has been increasing sharply due to the increasing prevalence of its risk factors, like smoking, obesity, alcohol consumption, long-term diabetes, and pancreatitis (Klein, 2021). Currently, although the combination of surgical resection and systemic chemotherapy has improved patient outcomes, the high overall recurrence rates and low five-year survival rates after surgery remain a formidable challenge for PAAD treatment (McGuigan et al., 2018). Therefore, elucidating the potential mechanisms of PAAD progression is crucial for its diagnosis and treatment.

Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nt that do not encode proteins and are involved in epigenetic modifications, protein/RNA stability, translation, and post-translational modifications (Bridges et al., 2021). LncRNAs participate in the

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development of malignant tumors and drug resistance through transcriptional processing and chromatin remodeling and have potential as biomarkers and therapeutic targets for cancer management (McCabe and Rasmussen, 2021). Epigenetics mainly involves various covalent modifications of histones and nucleic acids, regulating chromatin structure and gene expression (Hogg et al., 2020). Importantly, mounting evidence suggests that lncRNAs regulate PAAD progression through epigenetic mechanisms (Terashima et al., 2018; Bravo-Vazquez et al., 2023). A recent study has reported that PLACT1 is a newly discovered lncRNA and is highly expressed in PAAD (Ren et al., 2020). However, no reports have detailed the mechanism by which PLACT1 participates in PAAD through epigenetic modifications.

Krüppel-like factor 2 (KLF2) belongs to the KLF protein family, a family of developmental transcription factors involved in regulating cell growth and differentiation, and it possesses tumor-suppressive or promotive functions (Taghehchian et al., 2023). A previous study has shown that KLF2 is typically downregulated in PAAD and has tumor-suppressive effects (Yuedi et al., 2020). Another report has found the presence of histone H3 lysine 9 dimethylation (H3K9me2) on *KLF2*, an important epigenetic modification that can suppress specific gene transcription activity (Ohguchi et al., 2016). Notably, H3K27 trimethylation or H3K4 demethylation on the *KLF2* promoter suppresses KLF2 expression, leading to PAAD malignant progression (Lian et al., 2018). LncRNA TRERNA1 suppresses CDH1 expression by inducing H3K9me2 on the *CDH1* promoter through the recruitment of euchromatic histone lysine methyltransferase 2 (EHMT2) (Song et al., 2019). Therefore, we speculate that KLF2 is a downstream mechanism of PLACT1-mediated epigenetic modifications.

In the preliminary phase of the study, the JASPAR database predicted that KLF2 has binding sites on the *KIAA1522* promoter. *KIAA1522* is a newly cloned gene that shows high expression in various cancers, such as colorectal carcinoma and hepatocellular carcinoma (Xu et al., 2020; Yi et al., 2022). Extensive reports support the role of KIAA1522 in promoting cancer cell proliferation and survival, and its association with a poor prognosis (Xie et al., 2017; Shi et al., 2023). Moreover, overexpression of KIAA1522 promotes the proliferation and migration of PAAD cells (Lin et al., 2021). Based on the above evidence, KIAA1522 may be regulated by KLF2 and thus participate in the malignant proliferation of PAAD cells.

In this study, we investigated the specific mechanisms of PLACT1 in PAAD cell proliferation, aiming to provide new theoretical foundations for PAAD treatment.

Materials and methods

Ethics statement

Animal experiments were conducted under the guidance of the Animal Care and Use Ethics Committee of Yangzhou University Medical College (Approval number: 202402111). All procedures involving the use, care, and handling of animals complied with the Guide for the Care and Use of Laboratory Animals of National Institutes of Health (National Research Council (US), 2011).

Cell culture

PAAD cell lines (Panc 03.27, SW1990, AsPC-1, and BxPC-3) and normal human pancreatic ductal epithelial cell line (HPDE-C7), purchased from ATCC (Manassas, VA, USA), were cultured in Dulbecco's modified Eagle medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone; GE Healthcare Life Sciences, Logan, UT, USA) and maintained in a humidified incubator at 37°C with 5% CO_2 and 95% air.

Cell transfection

PAAD cells were seeded in six-well plates at a density of 5×10^5 cells per well and then transfected the next day with Lipofectamine 3000 reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. The cells were collected for further experimentation 48 hours after transfection. PLACT1 small interfering RNA (siRNA) (si-PLACT1), negative control (si-NC), KLF2 siRNA (si-KLF2), EHMT2 siRNA (si-EHMT2), control vectors (oe-NC), and KIAA1522 overexpression vectors (oe-KIAA1522) were synthesized by Geneseed Biotechnology (Guangzhou, Guangdong, China).

Cell counting kit-8 (CCK-8) assay

Cell proliferation was evaluated using the CCK-8 assay. Briefly, cells were seeded in 96-well plates (3000 cells per well). At 24, 48, 72, and 96 hours, 10 µL of CCK-8 solution (Dojindo, Tokyo, Japan) was added to each well. The absorbance was measured at 450 nm using an enzyme-linked immunosorbent assay reader (Thermo Fisher Scientific).

Colony formation assay

Cells were seeded in six-well plates at a density of 600 cells per well and maintained in a medium supplemented with 10% FBS. The medium was replenished every three days. After 14 days of culture, the cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The formed visible colonies were counted to determine the number of stained colonies. A cluster consisting of 50 or more cells was considered a colony.

5-Ethynyl-2-deoxyuridine (EdU) staining assay

According to the manufacturer's instructions, the

BeyoClick™ EdU cell proliferation kit (Beyotime, Shanghai, China) was used for this experiment. Briefly, 1×10^4 cells were placed in a 96-well plate and treated with the EdU reagent for 2 hours at room temperature. The cells were then stained with 4',6-diamidino-2-phenylindole for 5 minutes. Finally, the cells were observed under a fluorescence microscope (Olympus, Tokyo, Japan).

Nuclear and cytoplasmic fractionation assay

Nuclear and cytoplasmic fractionation was performed using NE-PER™ nuclear and cytoplasmic extraction reagents according to the protocol. Briefly, for cytoplasmic fractionation, 1×10^7 cells were collected and washed with phosphate-buffered saline (PBS). Cold cytoplasmic extraction reagents (CER) I and CER II were added, and the cells were incubated on ice for 10 minutes. After centrifugation at 16000g for 5 minutes, the supernatant was retained. The pellet was resuspended in cold nuclear extraction reagent, rotating for 15 seconds every 10 minutes for a total of 40 minutes. After centrifugation at 16000g for 10 minutes, the supernatant was collected as the nuclear fraction. Both the cytoplasmic extract and the nuclear extract were stored at -80°C until use. PLACT1 content was analyzed by reverse transcription quantitative polymerase chain reaction (RT-qPCR). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were used as controls.

RNA immunoprecipitation (RIP) assay

The EZMagna RIP kit (Millipore, Billerica, MA, USA) was used to investigate whether PLACT1 can interact with or bind to EHMT2. A total of 1×10^7 cells were lysed in complete RIP lysis buffer. The cell lysate was incubated with magnetic beads conjugated to EHMT2 antibody (ab229455, Abcam, Cambridge, MA, USA) or immunoglobulin G (IgG) antibody (ab172730, Abcam) at 4°C for 6 hours. Afterwards, the beads were washed and incubated with proteinase K to digest the proteins. Finally, purified RNA was subjected to RT-qPCR.

Chromatin immunoprecipitation (ChIP) assay

According to the manufacturer's instructions, the EZ-Magna ChIP™ G immunoprecipitation kit (Millipore) was used. Cells (6×10^6) were fixed with formaldehyde for 10 minutes, and 1.0 M glycine was added to a final concentration of 125 mM to stop the cross-linking, followed by incubation at room temperature for 5 minutes. Then, the cells were sonicated to fragment the DNA. The supernatant was collected after centrifugation (4°C, 12000g, 10 min) and divided into two tubes. One tube received the IgG antibody (ab172730, Abcam) as the negative control. The other tube received the KLF2 antibody (23384-1-AP, Proteintech, Rosemont, IL, USA), EHMT2 antibody (ab229455, Abcam), or H3K9me2 antibody (ab176882, Abcam). After mixing, the samples were incubated overnight at 4°C. DNA-protein complexes were precipitated with Protein A-agarose/salmon sperm DNA, followed by centrifugation at 12000 g for 5 minutes. Next, the supernatant was discarded, and non-specific complexes were washed. The complexes were incubated overnight at 65°C to reverse the cross-links, and DNA fragments were purified and recovered using the phenol/chloroform extraction method.

Dual-luciferase reporter assay

The JASPAR website (http://jaspar.genereg.net/) predicted the binding sites of KLF2 on the *KIAA1522* promoter (Rauluseviciute et al., 2024). Recombinant luciferase reporter gene vectors with truncated or mutated binding sites were co-transfected with KLF2 expression vectors into cells (1.2×10^5 cells). At 48 hours post-transfection, the cells were collected and lysed. Luciferase activity was then measured using the dual-luciferase reporter system (Promega, Madison, WI, USA) and a luciferase assay kit (Biovision, San Francisco, CA, USA).

Xenograft experiment in nude mice

A total of 24 BALB/c nude mice (four-week-old, male) were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, Hunan, China) and housed under specific pathogen-free conditions in laminar flow cabinets. SW1990 cells were infected with lentivirus containing PLACT1 shRNA (sh-PLACT1) and negative control lentivirus (sh-NC) (GenePharma, Shanghai, China) at a multiplicity of infection of 20. Next, the cells were collected, washed with PBS, and resuspended at a concentration of 2×10^7 cells/mL. Each mouse (12 mice per group) received 100 μL of cell suspension subcutaneously on the right flank. Tumor growth was examined every four days, and tumor length (L) and width (W) were measured with calipers to calculate tumor volume (V) using the formula $V = (L\times W^2) \times 0.5$. On day 27 post-tumor cell injection, all mice were euthanized by intraperitoneal injection of 150 mg/kg sodium pentobarbital, and the tumors were excised. Six mice per group were used for immunohistochemistry, and the remaining six for other experiments, such as RT-qPCR.

Immunohistochemistry (IHC) for Ki67

Xenograft tumor tissues were fixed in 4% paraformaldehyde for 12 hours, embedded in paraffin, deparaffinized with xylene, rehydrated with graded ethanol, immersed in 0.01 M citrate buffer for 15-20 minutes, cooled to room temperature, and washed with PBS. Next, the tissues were blocked with goat serum and incubated at room temperature for 20 minutes, after which excess liquid was removed. The tissues were then

immunostained with primary anti-Ki67 antibody (ab15580, Abcam) at room temperature for 1 hour, washed with PBS, and incubated with goat anti-rabbit IgG (ab6721, Abcam) at room temperature for 1 hour. Afterward, the tissues were incubated with 3,3'-diaminobenzidine tetrahydrochloride for 5-10 minutes, counterstained with hematoxylin for 2 minutes, differentiated with ethanol in hydrochloric acid, and washed with water for 10 minutes. Finally, the tissues were dehydrated, cleared, sealed, and observed under a microscope. Under a microscope (20× magnification), five fields of view were randomly selected, and Ki67-positive cells (brown-colored) were counted. The Ki67 positive rate was calculated as: (number of Ki67-positive cells / total number of cells) × 100%.

RT-qPCR

Total RNA was extracted from cells in each group using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). According to the manufacturer's instructions, a reverse transcription kit (Promega) was used to synthesize complementary DNA from the isolated RNA. Real-time PCR was conducted using the Stratagene MAXP3000 PCR System and Brilliant Q-PCR Master Mix. GAPDH was employed as an internal reference. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Primers are listed in Table 1.

Western blot assay

Total protein was extracted from cells in each group using radioimmunoprecipitation assay lysis buffer (Beyotime) and quantified using a bicinchoninic acid assay kit (Thermo Fisher Scientific). Next, 30 μg of protein samples were separated using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (Amersham, GE Healthcare, Chicago, IL, USA). The membranes were blocked with 5% skim milk and then incubated with primary antibodies against KLF2 (23384-1-AP, 1:1000, Proteintech), KIAA1522 (MBS8577874, 1:1000, MyBioSource, San Diego, CA, USA), EHMT2 (ab229455, 1:1000, Abcam), and β-actin (ab5694, 1:1000, Abcam) overnight at 4°C. The membranes were then incubated with secondary antibodies (ab205718, 1:2000, Abcam) for 1 hour at room temperature. The proteins were visualized using enhanced chemiluminescence reagents and imaged using a luminescent imaging system (GE Healthcare, Chicago, IL, USA). Relative protein expression was analyzed using Image-Pro Plus 6.0 software (Media Cybernetics, Los Angeles, CA, USA).

Statistical analysis

All data were analyzed and plotted using SPSS 21.0 statistical software (IBM SPSS Statistics, Armonk, NY, USA) and GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA). Normality and homogeneity of variance tests were first performed, which showed that the data conformed to a normal distribution with homogeneous variances. Comparisons between two groups were analyzed by a *t*-test. Comparisons among multiple groups were analyzed by one-way or two-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. p-values were two-tailed, with $p<0.05$ indicating statistically significant differences and $p<0.01$ indicating highly statistically significant differences.

Results

PLACT1 is increased in PAAD and promotes cell proliferation

RT-qPCR results showed that lncRNA PLACT1 was upregulated in PAAD cell lines ($p<0.05$, Fig. 1A). However, the effect of PLACT1 on the proliferation of PAAD cells was not fully understood. Therefore, we knocked down PLACT1 expression in Panc 03.27 and SW1990 cells ($p<0.05$, Fig. 1B) and observed the changes in cell proliferation. Compared with the si-NC group, the proliferative viability of cells in the si-PLACT1 group was significantly reduced ($p<0.05$, Fig. 1C). Additionally, PLACT1 knockdown significantly decreased the number of colonies and EdU-positive cells ($p<0.05$, Fig. 1D,E). The above evidence confirmed that PLACT1 is increased in PAAD and promotes cell proliferation.

PLACT1 induces H3K9me2 by recruiting EHMT2 to suppress KLF2 expression

The nuclear and cytoplasmic fractionation assay found that PLACT1 was primarily located in the nucleus (Fig. 2A). TRERNA1 induces H3K9me2 by recruiting

Table 1. PCR primer sequences.

Genes	Sequences (5'-3')
LncRNA PLACT1	F: CACGGGAGACTATCTGGCAC R: GGCTTTCGGGAGGCTGATTA
KLF2	F: GAAGCCCTACCACTGCAACT R: GAAGGCACGATCGCACAGAT
KIAA1522	F: GGGCTGTTTAAGAAGAAGGGC R: AAGACGTTGTCCTGGTGCTC
EHMT2	F: CGAGAGGGAGGGGGTTGAT R: GGGCCTACCTCTTCCTCTGT
KIAA1522 promoter	F: CGGGTTCAAGCAATTCGCCT R: GTCAGGAGTTTAAGACCAGCC
KLF2 promoter	F: GCGCTGAGTGAACCCATCCTG R: CCGCAGCCCACGTTCTACTAC
GAPDH	F: GATGCTGGCGCTGAGTACG R: GCTAAGCAGTTGGTGGTGC

EHMT2 to suppress CDH1 expression (Song et al., 2019). KLF2 is lowly expressed in PAAD (Zhang et al., 2016; Lian et al., 2018; Yuedi et al., 2020). H3K9me2 exists on KLF2 (Ohguchi et al., 2016). We found that KLF2 expression was increased as PLACT1 expression was decreased ($p<0.05$, Fig. 2B,C), leading us to speculate that KLF2 was downstream of PLACT1. The RIP experiment revealed the interaction between PLACT1 and EHMT2 ($p<0.05$, Fig. 2D). Moreover, after reducing PLACT1 expression, the enrichment of EHMT2 and H3K9me2 on the *KLF2* promoter was remarkably decreased ($p<0.05$, Fig. 2E). After solely reducing EHMT2 expression, KLF2 expression was increased ($p<0.05$, Fig. 2F,G). In addition, KLF2 expression was significantly reduced in PAAD cells ($p<0.05$, Fig. 2H,I). In summary, PLACT1 induces H3K9me2 by recruiting EHMT2 to the *KLF2* promoter, thereby suppressing KLF2 expression.

Reduced KLF2 expression alleviates the inhibitory effect of PLACT1 knockdown on PAAD cell proliferation

Subsequently, we downregulated KLF2 expression in SW1990 cells and conducted combined experiments with si-PLACT1#2 ($p<0.05$, Fig. 3A-C). After downregulating KLF2, SW1990 cells showed markedly increased proliferative viability ($p<0.05$, Fig. 3D) and an increased number of colonies and EdU-positive cells ($p<0.05$, Fig. 3E,F), partially reversing the inhibitory effect of PLACT1 knockdown on PAAD cell proliferation.

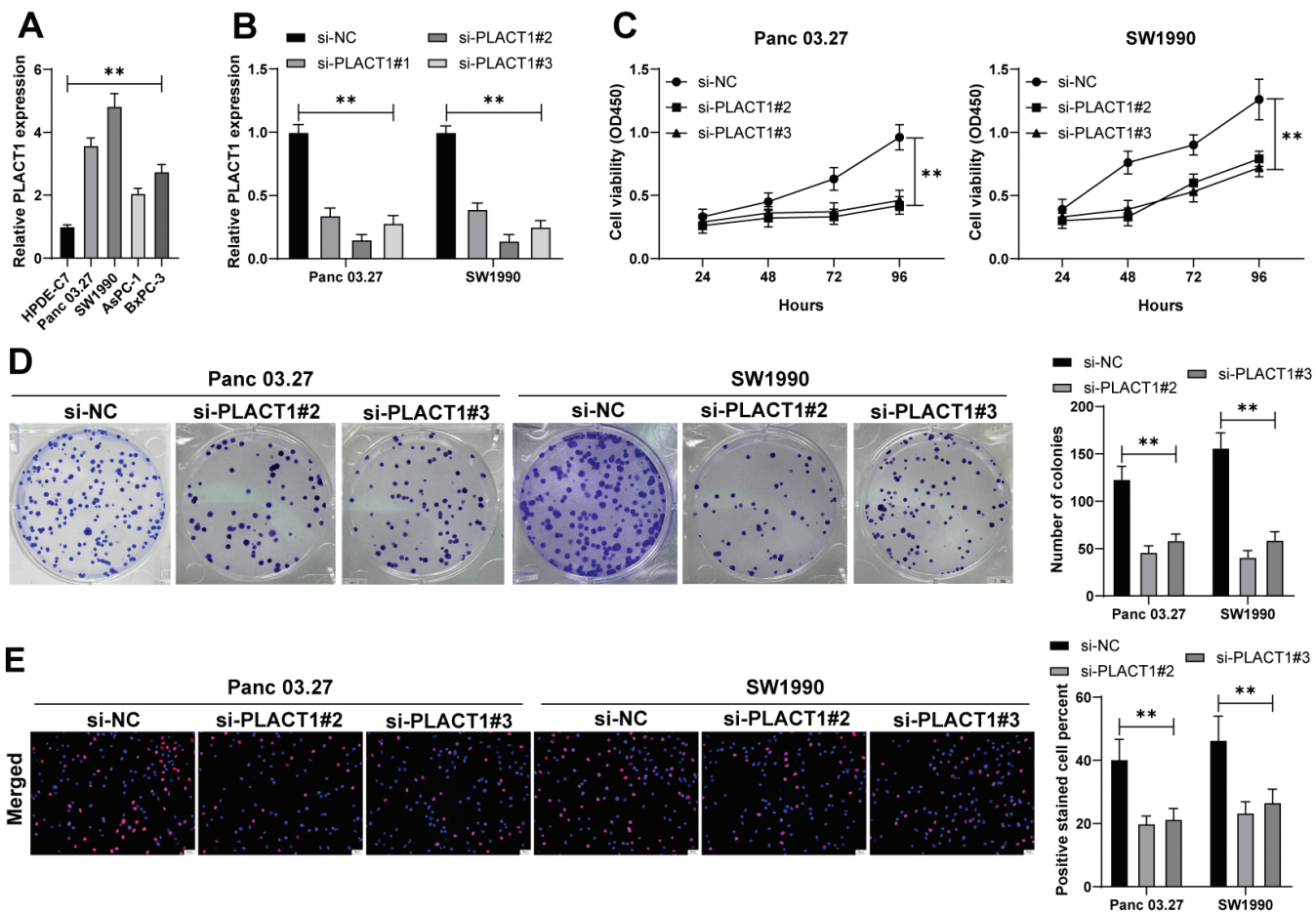


Fig. 1. PLACT1 is increased in PAAD and promotes cell proliferation. **A.** PLACT1 expression in HPDE-C7 and PAAD cell lines was detected by RT-qPCR; Panc 03.27 and SW1990 cells were transfected with PLACT1 small interfering RNA (si-PLACT1), with si-NC transfection as the negative control. **B.** PLACT1 expression in PAAD cells was detected by RT-qPCR. **C.** PAAD cell proliferation was detected by the CCK-8 assay. **D.** Colony formation of PAAD cells (representative images) was detected by colony formation assay, and the number was counted. **E.** The number of EdU-positive cells in PAAD (representative images) was detected by EdU staining assay and counted. Three independent replicate detections were performed, and the data are presented as mean ± standard deviation. Comparisons among multiple groups in panel A were analyzed by one-way ANOVA; comparisons among multiple groups in panels B-E were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons test. $^{**}p<0.01$.

KLF2 transcriptionally suppresses KIAA1522 expression

KLF2, as a transcription factor, targets WEE1 and negatively regulates its expression (Li et al., 2021). The JASPAR database predicted that KLF2 has binding sites on the *KIAA1522* promoter (Fig. 4A). Overexpression of KIAA1522 can promote the progression of PAAD (Lin et al., 2021). ChIP results showed that KLF2 was enriched on the *KIAA1522* promoter, and this enrichment was increased after PLACT1 knockdown but was significantly decreased after *KLF2* silencing (p<0.05, Fig. 4B). The dual-luciferase reporter assay further confirmed the binding of KLF2 to the *KIAA1522* promoter (p<0.05, Fig. 4C). Additionally, KIAA1522 expression was greatly increased in PAAD cells (p<0.05, Fig. 4D,E), decreased after PLACT1 knockdown, and increased after *KLF2* silencing (p<0.05, Fig. 4F,G). In summary, KLF2 transcriptionally suppresses KIAA1522 expression.

Overexpression of KIAA1522 alleviates the inhibitory effect of PLACT1 knockdown on PAAD cell proliferation

Subsequently, we upregulated KIAA1522 expression

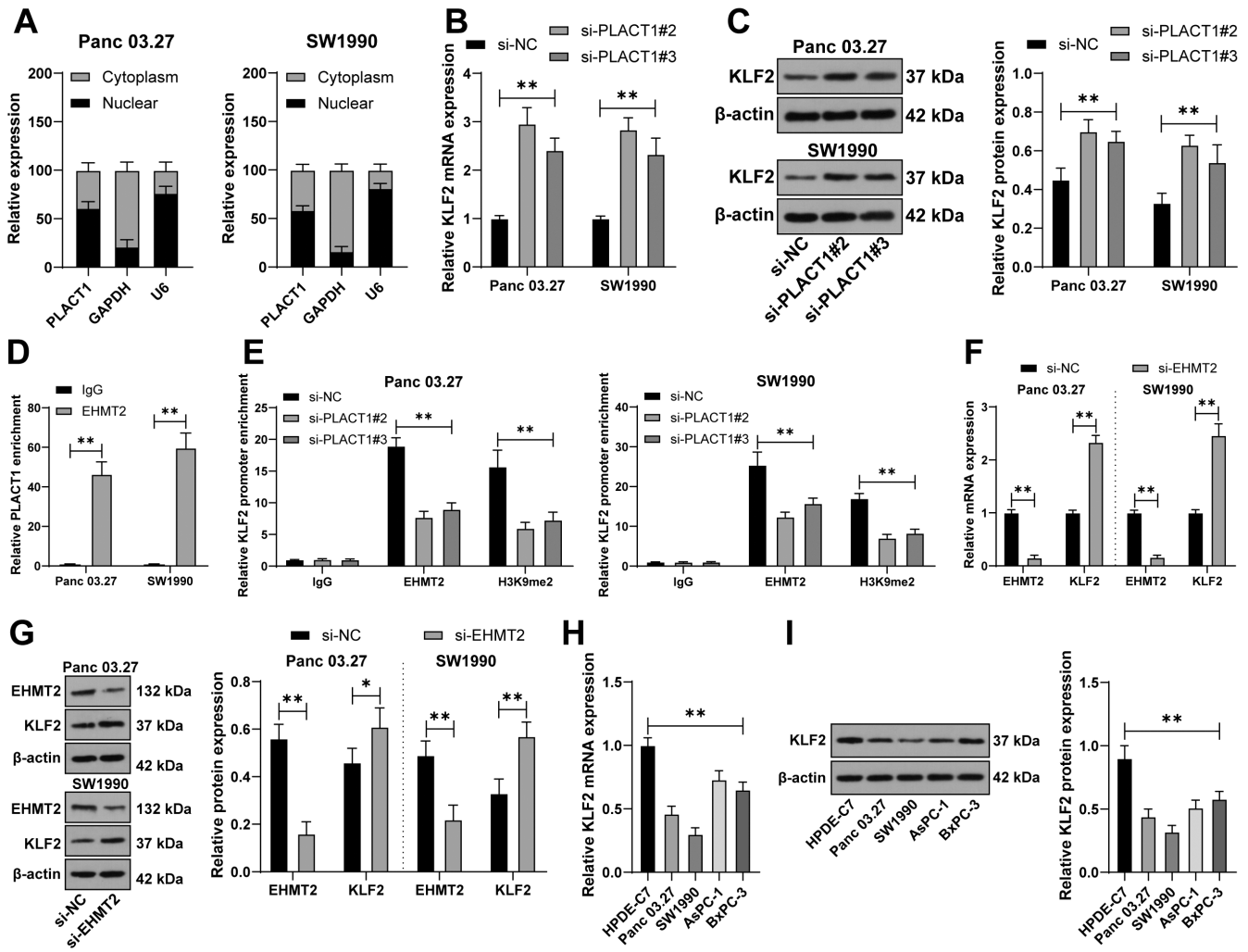


Fig. 2. PLACT1 induces H3K9me2 by recruiting EHMT2 to suppress KLF2 expression. **A.** The localization of PLACT1 in PAAD cells was detected by nuclear and cytoplasmic fractionation assay. **B.** KLF2 mRNA expression in PAAD cell lines was detected by RT-qPCR. **C.** KLF2 protein expression (representative bands) was detected by Western blot. **D.** The binding between EHMT2 and PLACT1 in PAAD cells was analyzed by RIP analysis. **E.** The enrichment of EHMT2 and H3K9me2 on the *KLF2* promoter in PAAD cells was analyzed by ChIP analysis. **F.** EHMT2 and KLF2 expression in PAAD cells after transfection with si-EHMT2 was detected by RT-qPCR. **G.** EHMT2 and KLF2 protein expression (representative bands) in PAAD cells after transfection with si-EHMT2 was detected by western blot. **H.** KLF2 mRNA expression in HPDE-C7 and PAAD cell lines was detected by RT-qPCR. **I.** KLF2 protein expression (representative bands) in HPDE-C7 and PAAD cell lines was detected by western blot. Three independent replicate detections were performed, and the data are presented as mean ± standard deviation. Comparisons among multiple groups in panels H-I were analyzed by one-way ANOVA; comparisons among multiple groups in panels B-G were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons test. *p<0.05, **p<0.01.

in SW1990 cells and conducted combined experiments with si-PLACT1#2 ($p<0.05$, Fig. 5A-C). Upregulation of KIAA1522 substantially increased the proliferative viability, the number of colonies, and EdU-positive cells in SW1990 cells ($p<0.05$, Fig. 5D-F), partially reversing the inhibitory effect of PLACT1 knockdown on PAAD cell proliferation.

Downregulation of PLACT1 inhibits tumor growth via the KLF2/KIAA1522 axis

Finally, we established a xenograft model in nude mice using SW1990 cells infected with lentivirus to verify the role and mechanism of PLACT1 *in vivo*. The results showed that the volume and weight of tumors in the sh-PLACT1 group were lower than in the sh-NC group ($p<0.05$, Fig. 6A,B). Additionally, the positive rate of Ki67 in tumor tissues of the sh-PLACT1 group was significantly lower than that of the sh-NC group ($p<0.05$, Fig. 6C). Furthermore, after PLACT1 knockdown, PLACT1 and KIAA1522 in tumor tissues were decreased, while KLF2 was increased ($p<0.05$, Fig. 6D,E). In summary, downregulation of PLACT1 inhibits tumor growth via the KLF2/KIAA1522 axis.

Discussion

Aberrant expression of lncRNAs plays a crucial role in PAAD development (Ghafouri-Fard et al., 2021). In this study, we found that PLACT1 promoted PAAD cell proliferation. Further mechanistic studies revealed that PLACT1 suppressed KLF2 expression and reduced KLF2 enrichment on the *KIAA1522* promoter, thereby promoting KIAA1522 expression. PLACT1 has the potential to become a novel biomarker and therapeutic target for PAAD.

Abnormally high expression of lncRNAs has been widely reported in the malignant proliferation of PAAD.

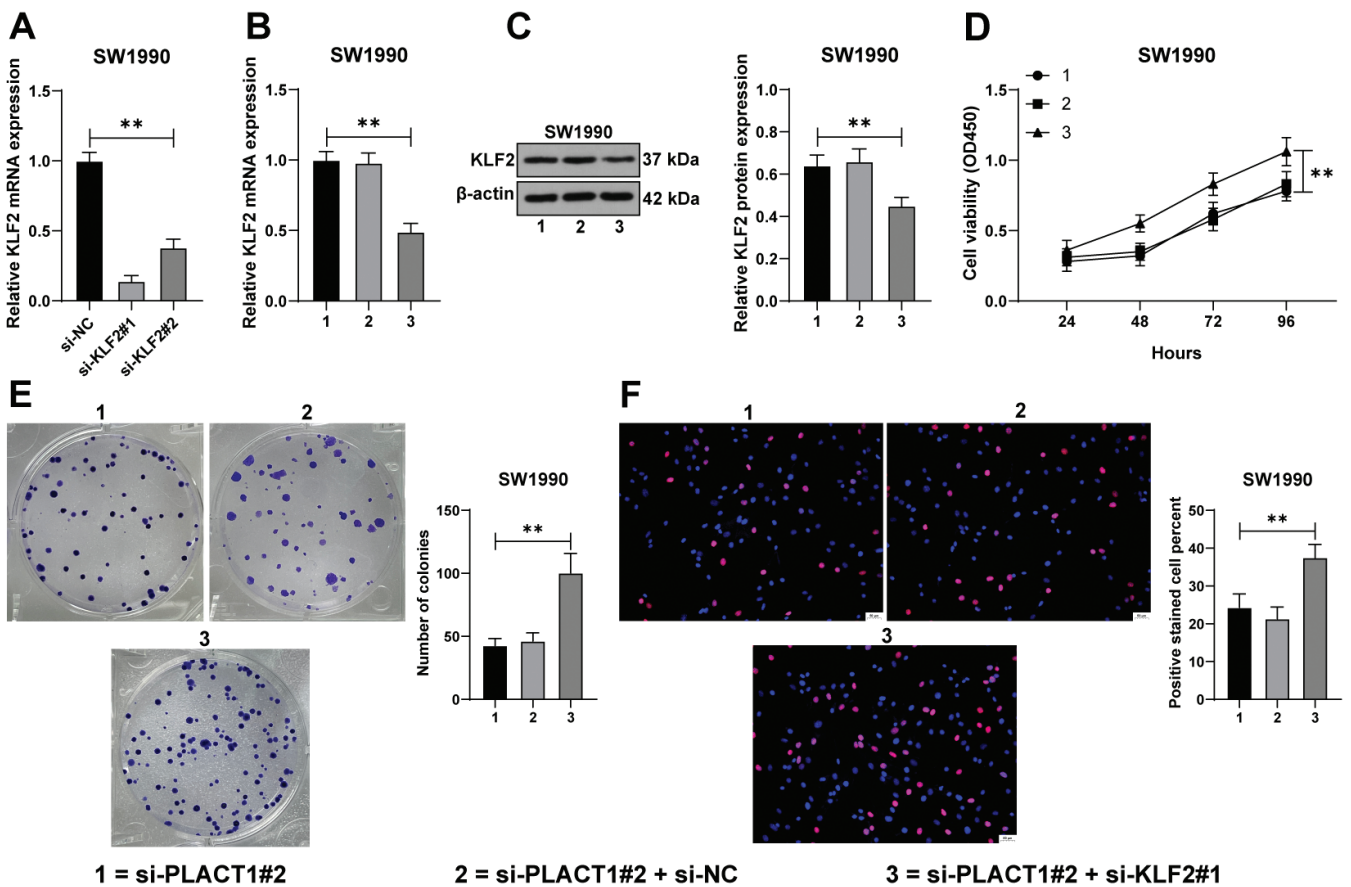
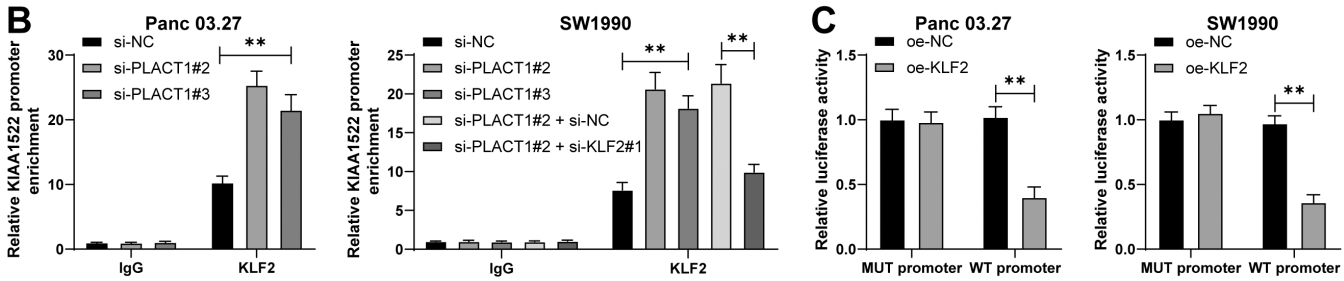
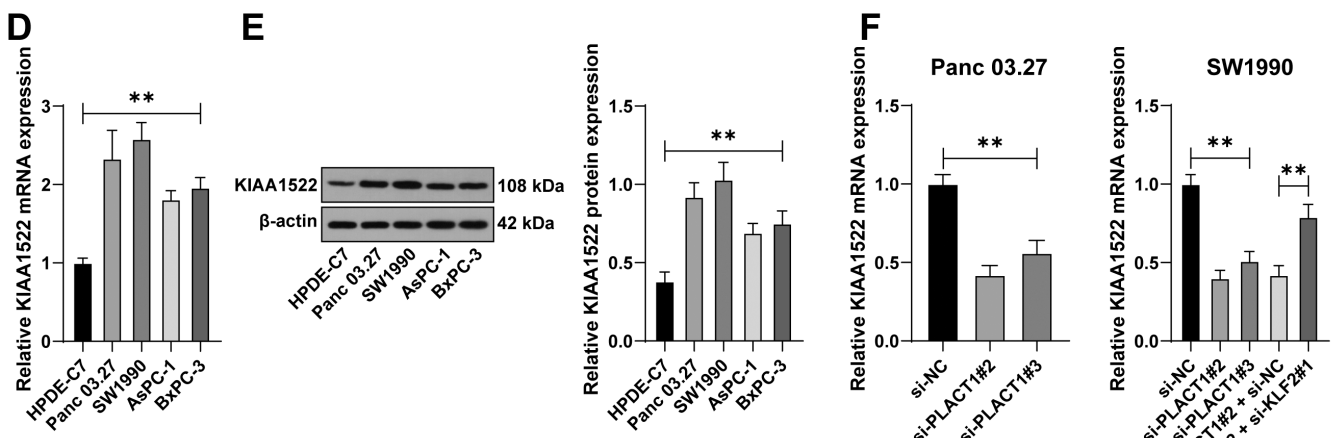


Fig. 3. Reduced KLF2 expression alleviates the inhibitory effect of PLACT1 knockdown on PAAD cell proliferation. KLF2 was knocked down in SW1990 cells, with si-NC transfection as the negative control. **A, B.** KLF2 mRNA expression in the cells was analyzed by RT-qPCR. **C.** KLF2 protein expression (representative bands) was detected by western blot. **D.** Cell proliferation was detected by the CCK-8 assay. **E.** Colony formation of PAAD cells (representative images) was detected by colony formation assay, and the number was counted. **F.** The number of EdU-positive cells in PAAD (representative images) was detected by the EdU staining assay and counted. Three independent replicate detections were performed, and the data are presented as mean ± standard deviation. Comparisons among multiple groups in panels A-C and E-F were analyzed by one-way ANOVA; comparisons among multiple groups in panel D were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons test. $^{**}p<0.01$.

A

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence
MA1515.1	MA1515.1.KLF2	14.105693	0.9848266067790816	NC_000001.11:32739830-32741830	245	255	+	CACCACACCCA





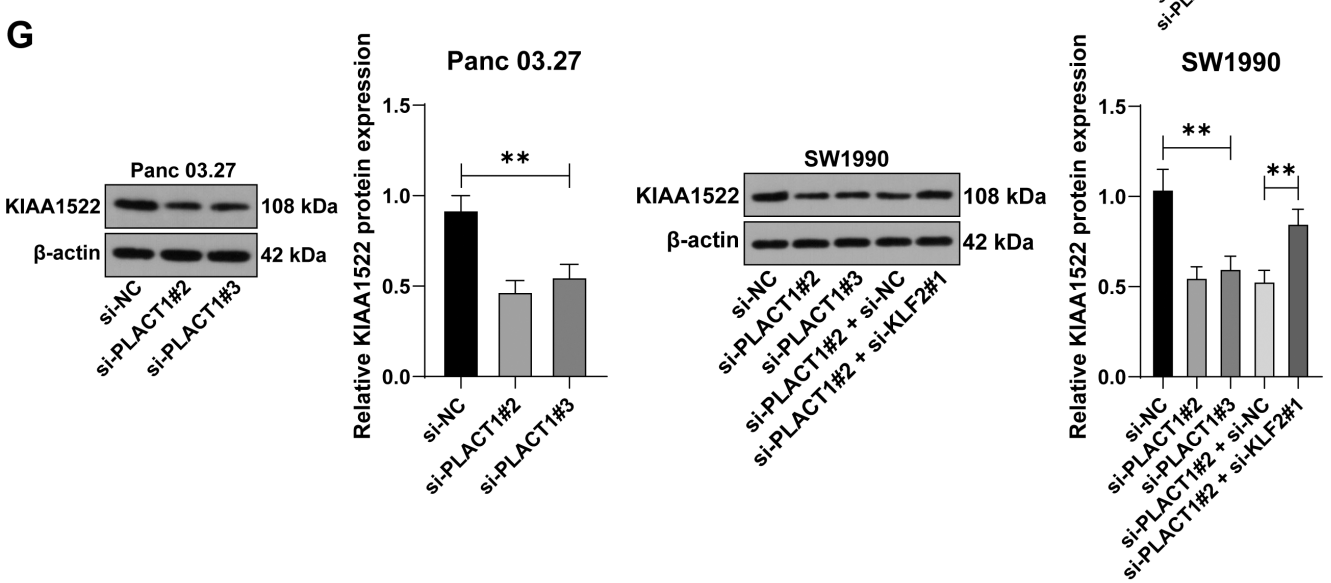


Fig. 4. KLF2 transcriptionally suppresses KIAA1522 expression. **A.** JASPAR database prediction of KLF2 binding sites on the *KIAA1522* promoter. **B, C.** The binding of KLF2 to the KIAA1522 promoter in PAAD cells was analyzed by ChIP and dual-luciferase reporter assays. **D.** KIAA1522 mRNA expression in HPDE-C7 and PAAD cells was detected by RT-qPCR. **E.** KIAA1522 protein expression (representative bands) in HPDE-C7 and PAAD cells was detected by western blot. **F.** KIAA1522 mRNA expression in PAAD cells was detected by RT-qPCR. **G.** KIAA1522 protein expression (representative bands) in PAAD cells was detected by western blot. Three independent replicate detections were performed, and the data are presented as mean ± standard deviation. Comparisons among multiple groups in panels D-G were analyzed by one-way ANOVA; comparisons among multiple groups in panels B-C were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons test. $^{**}p<0.01$.

For instance, lncRNA PMSB8-AS1 promotes proliferation, migration, and invasion of PAAD cells by sponging miRNA and positively correlates with the poorest survival in PAAD patients (Zhang et al., 2020). LncRNA PVT1 restores cellular activity in PAAD and promotes gemcitabine resistance in PAAD cells (Zhou et al., 2020). High expression of lncRNA UCA1 is associated with poor clinical outcomes of PAAD patients and enhances the migratory ability and mitochondrial fusion of PAAD cells (Wang et al., 2023). Here, we found that PLACT1 expression was increased in PAAD and promoted cell proliferation. Silencing PLACT1 significantly reduced cell proliferation and markedly inhibited tumor growth in mice. EHMT2 primarily mediates H3K9me2 and is associated with epithelial-mesenchymal transition, cellular plasticity, and therapy resistance in cancers (Nachiyappan et al., 2022). In liver cancer, EHMT2 mediates H3K9me2 methylation of the *APC* promoter through epigenetic modifications, leading to gene silencing (Guo et al., 2021). Our further exploration indicated that PLACT1 induced H3K9me2 on the *KLF2* promoter region by recruiting EHMT2, thereby suppressing KLF2 expression. Notably, another report stated that PLACT1 suppresses IκBα transcriptional activity through EZH2-induced H3K27me3, leading to continuous activation of the NF-κB signaling pathway, thereby exacerbating PAAD proliferation and invasion (Ren et al., 2020). Taken together, the epigenetic mechanism of PLACT1 may provide new directions for clinical treatment of PAAD.

Epigenetic silencing of KLF2 is a potential pathway for inhibiting tumor growth. A previous report has indicated that lncRNA SH3PXD2A-AS1 recruits EZH2 to the *KLF2* promoter, thereby silencing KLF2 and mediating colorectal cancer cell proliferation (Ma et al., 2018). LncRNA GHET1 mediates H3K27me3

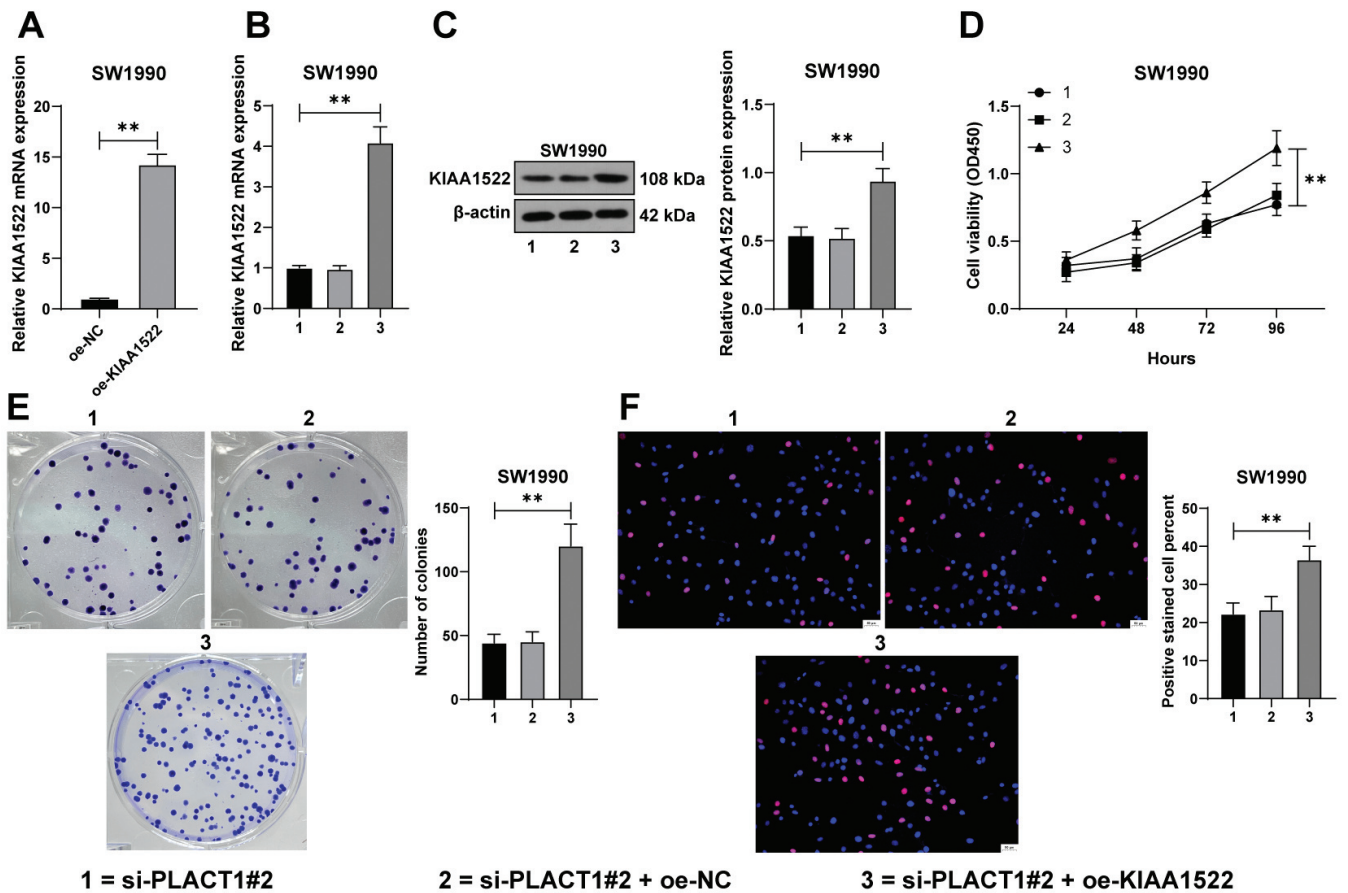


Fig. 5. Overexpression of KIAA1522 alleviates the inhibitory effect of PLACT1 knockdown on PAAD cell proliferation. Overexpression vectors for KIAA1522 (oe-KIAA1522) were transfected into SW1990 cells, with oe-NC transfection as the negative control. **A, B.** KIAA1522 mRNA expression in the cells was detected by RT-qPCR. **C.** KIAA1522 protein expression (representative bands) in the cells was detected by western blot. **D.** Cell proliferation was assessed by the CCK-8 assay. **E.** Colony formation of PAAD cells (representative images) was detected by colony formation assay, and the number was counted. **F.** The number of EdU-positive cells in PAAD (representative images) was detected by the EdU staining assay and counted. Three independent replicate detections were performed, and the data are presented as mean ± standard deviation. Comparisons between two groups in panel A were analyzed by a *t*-test; comparisons among multiple groups in panels B-C and E-F were analyzed by one-way ANOVA; comparisons among multiple groups in panel D were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons test. $^{**}p<0.01$.

modification at the *KLF2* promoter by interacting with EZH2, suppressing KLF2 and promoting hepatocellular carcinoma cell proliferation (Jin et al., 2018). Notably, epigenetically silenced KLF2 promotes PAAD cell proliferation and carcinogenesis (Lian et al., 2018). KLF2 can slow tumor growth in PAAD by inducing senescence marker P21, thereby promoting cellular senescence and inhibiting cancer cell progression (Yuedi et al., 2020). Herein, we verified that after KLF2 silencing, PAAD cell proliferation was markedly increased. In conclusion, inhibition of KLF2 is a potential pathway to prevent PAAD cell proliferation.

KLF2 can regulate downstream genes involved in cancer progression (Li et al., 2021). Further experiments found that KLF2, as a transcription factor, suppressed KIAA1522 expression. Recent studies have identified that KIAA1522 primarily acts as an oncogenic factor involved in cancer progression. In liver cancer, overexpression of KIAA1522 promotes the growth and migration of hepatocellular carcinoma cells (Liu et al., 2023). In colorectal cancer cells, KIAA1522 promotes cancer cell invasion, proliferation, and migration, as well as distant metastasis *in vivo*, through the Notch signaling pathway (Yi et al., 2022). Overexpression of KIAA1522 may accelerate the progression of PAAD by inhibiting cell apoptosis (Lin et al., 2021). Notably, overexpression of KLF9 can suppress KIAA1522 expression, thereby inhibiting PAAD cell proliferation, invasion, and migration (Guo et al., 2024). Our study confirmed that KIAA1522 upregulation promoted PAAD cell proliferative activity, colony numbers, and EdU-positive cells. KIAA1522 may play a role in regulating processes such as invasion, metastasis, and apoptosis in PAAD cells, which needs to be further confirmed in future studies.

Our study still has some limitations. First, our mechanism was only validated at the cellular level. Second, the mechanism is relatively simple. Third, it is unclear whether PLACT1 regulates processes such as migration and apoptosis in PAAD cells. The possibility that PLACT1 participates in PAAD progression through a ceRNA mechanism has not been extensively explored. Finally, the function of PLACT1 in PAAD remains to be integrated with clinical samples. In future studies, we plan to further explore the impact of PLACT1 through the ceRNA mechanism on migration and apoptosis of PAAD cells and include clinical samples, thus providing new theoretical knowledge for PAAD treatment.

In conclusion, PLACT1 suppresses KLF2 expression by recruiting EHMT2 to induce H3K9me2 on the *KLF2* promoter, leading to reduced enrichment of KLF2 on the *KIAA1522* promoter and increased

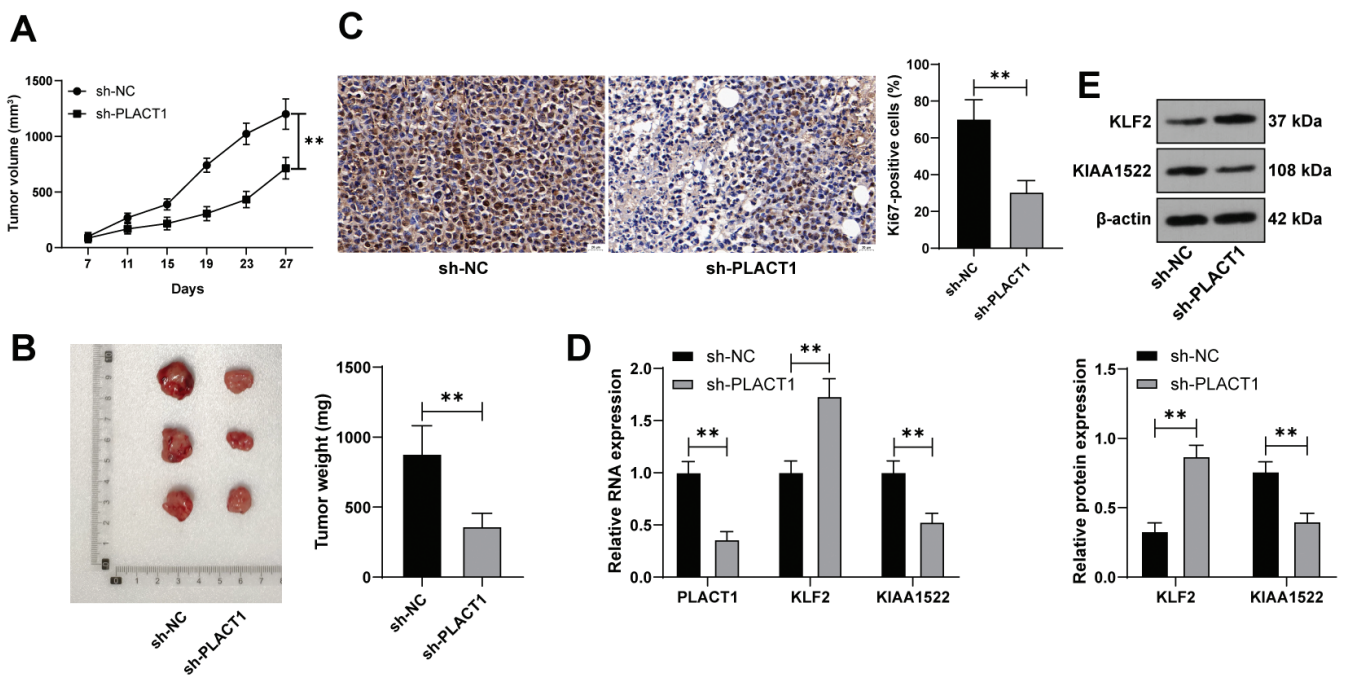


Fig. 6. Downregulation of PLACT1 inhibits tumor growth via the KLF2/KIAA1522 axis. SW1990 cells infected with lentivirus containing PLACT1 shRNA (sh-PLACT1) were used to establish a xenograft model in nude mice, with SW1990 cells infected with sh-NC serving as the negative control. **A.** Tumor volume was measured every four days starting from day 7, and the growth curve was plotted. **B.** Representative photographs were taken, and tumor weights were recorded after euthanizing the nude mice on day 27. **C.** The positivity rate of Ki67 (representative images) in the tumor tissues was detected by IHC and quantified. **D.** The RNA expression of PLACT1, KLF2, and KIAA1522 in the tissues was detected by RT-qPCR. **E.** The protein expression of KLF2 and KIAA1522 (representative bands) in the tissues was detected by western blot. N=6. The data are presented as mean ± standard deviation. Comparisons between two groups in panels B-C were analyzed by a *t*-test; comparisons among multiple groups in panels A, D-E were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons test. $^{**}p<0.01$.

expression of KIAA1522, ultimately promoting PAAD cell proliferation. PLACT1 has the potential to become a novel biomarker and therapeutic target for PAAD.

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Ethics approval and consent to participate. Animal experiments were conducted under the guidance of the Animal Care and Use Ethics Committee of Yangzhou University Medical College (Approval number: 202402111). All procedures involving the use, care, and handling of animals complied with the Guide for the Care and Use of Laboratory Animals of National Institutes of Health (National Research Council (US), 2011).

Availability of data and materials. Datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests. The authors declare that they have no conflict of interest.

Authors' contribution. Fei Wang: Conceptualization, Data curation, Methodology, Visualization, Writing – original draft, Writing – review & editing; Xiaoli Hou: Conceptualization, Data curation, Methodology, Validation; Shutao Wu: Formal analysis, Investigation, Visualization; Wei Sun: Methodology, Supervision; Yixia Wang: Conceptualization, Validation; Yasen Cao: Formal analysis, Validation, Visualization; Hong Cheng: Investigation, Supervision.

References

Bravo-Vázquez L.A., Frías-Reid N., Ramos-Delgado A.G., Osorio-Perez S.M., Zlotnik-Chavez H.R., Pathak S., Banerjee A., Bandyopadhyay A., Duttaroy A.K. and Paul S. (2023). MicroRNAs and long non-coding RNAs in pancreatic cancer: From epigenetics to potential clinical applications. Transl. Oncol. 27, 101579.

Bridges M.C., Daulagala A.C. and Kourtidis A. (2021). LNCcation: lncRNA localization and function. J. Cell Biol. 220, e202009045.

Ghafouri-Fard S., Fathi M., Zhai T., Taheri M. and Dong P. (2021). LncRNAs: Novel biomarkers for pancreatic cancer. Biomolecules 11, 1665.

Guo Y., Zhao Y.R., Liu H., Xin Y., Yu J.Z., Zang Y.J. and Xu Q.G. (2021). EHMT2 promotes the pathogenesis of hepatocellular carcinoma by epigenetically silencing APC expression. Cell Biosci. 11, 152.

Guo Y., Tian S., Li H., Zuo S., Yu C. and Sun C. (2024). Transcription factor KLF9 inhibits the proliferation, invasion, and migration of pancreatic cancer cells by repressing KIAA1522. Asia Pac. J. Clin. Oncol. 20, 423-432.

Hogg S.J., Beavis P.A., Dawson M.A. and Johnstone R.W. (2020). Targeting the epigenetic regulation of antitumour immunity. Nat. Rev. Drug Discov. 19, 776-800.

Jin L., He Y., Tang S. and Huang S. (2018). LncRNA GHET1 predicts poor prognosis in hepatocellular carcinoma and promotes cell proliferation by silencing KLF2. J. Cell. Physiol. 233, 4726-4734.

Klein A.P. (2021). Pancreatic cancer epidemiology: Understanding the role of lifestyle and inherited risk factors. Nat. Rev. Gastroenterol. Hepatol. 18, 493-502.

Li R., Chen J., Gao X. and Jiang G. (2021). Transcription factor KLF2 enhances the sensitivity of breast cancer cells to cisplatin by suppressing kinase WEE1. Cancer Biol. Ther. 22, 465-477.

Lian Y., Yang J., Lian Y., Xiao C., Hu X. and Xu H. (2018). DUXAP8, a pseudogene derived lncRNA, promotes growth of pancreatic carcinoma cells by epigenetically silencing CDKN1A and KLF2. Cancer Commun. 38, 64.

Lin J., Liao S., Liu Z., Li E., Wu X. and Zeng W. (2021). LncRNA FGD5-AS1 accelerates cell proliferation in pancreatic cancer by regulating miR-520a-3p/KIAA1522 axis. Cancer Biol. Ther. 22, 257-266.

Liu C., Ren C., Guo L., Yang C. and Yu Q. (2023). Exosome-mediated circTTLL5 transfer promotes hepatocellular carcinoma malignant progression through miR-136-5p/KIAA1522 axis. Pathol. Res. Pract. 241, 154276.

Livak K.J. and Schmittgen T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402-408.

Ma Z., Peng P., Zhou J., Hui B., Ji H., Wang J. and Wang K. (2018). Long non-coding RNA SH3PXD2A-AS1 promotes cell progression partly through epigenetic silencing P57 and KLF2 in colorectal cancer. Cell Physiol. Biochem. 46, 2197-2214.

McCabe E.M. and Rasmussen T.P. (2021). lncRNA involvement in cancer stem cell function and epithelial-mesenchymal transitions. Semin. Cancer Biol. 75, 38-48.

McGuigan A., Kelly P., Turkington R.C., Jones C., Coleman H.G. and McCain R.S. (2018). Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. World J. Gastroenterol. 24, 4846-4861.

Nachiyappan A., Gupta N. and Taneja R. (2022). EHMT1/EHMT2 in EMT, cancer stemness and drug resistance: Emerging evidence and mechanisms. FEBS J. 289, 1329-1351.

National Research Council (US) In Guide for the Care and Use of Laboratory Animals, 8th ed.; Washington (DC), 2011. https://doi.org/10.17226/12910

Ohguchi H., Hideshima T., Bhasin M.K., Gorgun G.T., Santo L., Cea M., Samur M.K., Mimura N., Suzuki R., Tai Y.T., Carrasco R.D., Raje N., Richardson P.G., Munshi N.C., Harigae H., Sanda T., Sakai J. and Anderson K.C. (2016). The KDM3A-KLF2-IRF4 axis maintains myeloma cell survival. Nat. Commun. 7, 10258.

Rauluseviciute I., Riudavets-Puig R., Blanc-Mathieu R., Castro-Mondragon J.A., Ferenc K., Kumar V., Lemma R.B., Lucas J., Cheneby J., Baranasic D., Khan A., Fornes O., Gundersen S., Johansen M., Hovig E., Lenhard B., Sandelin A., Wasserman W.W., Parcy F. and Mathelier A. (2024). JASPAR 2024: 20th anniversary of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 52, D174-D182.

Ren X., Chen C., Luo Y., Liu M., Li Y., Zheng S., Ye H., Fu Z., Li M., Li Z. and Chen R. (2020). lncRNA-PLACT1 sustains activation of NF-kappaB pathway through a positive feedback loop with IkappaBalpha/E2F1 axis in pancreatic cancer. Mol. Cancer 19, 35.

Rong Z., Xu J., Shi S., Tan Z., Meng Q., Hua J., Liu J., Zhang B., Wang W., Yu X. and Liang C. (2021). Circular RNA in pancreatic cancer: A novel avenue for the roles of diagnosis and treatment. Theranostics 11, 2755-2769.

Shi Y., Xiao Q., Huang S., Pan Q., Jia H., Zhou Q., Wei J., Kang J., Li Z. and Hu Y. (2023). Poor prognostic biomarker KIAA1522 is associated with immune infiltrates in hepatocellular carcinoma. J. Oncol. 2023, 3538928.

Song W., Gu Y., Lu S., Wu H., Cheng Z., Hu J., Qian Y., Zheng Y. and Fan H. (2019). LncRNA TRERNA1 facilitates hepatocellular

carcinoma metastasis by dimethylating H3K9 in the CDH1 promoter region via the recruitment of the EHMT2/SNAI1 complex. Cell Prolif. 52, e12621.

Taghehchian N., Maharati A., Akhlaghipour I., Zangouei A.S. and Moghbeli M. (2023). PRC2 mediated KLF2 down regulation: A therapeutic and diagnostic axis during tumor progression. Cancer Cell Int. 23, 233.

Terashima M., Ishimura A., Wanna-Udom S. and Suzuki T. (2018). MEG8 long noncoding RNA contributes to epigenetic progression of the epithelial-mesenchymal transition of lung and pancreatic cancer cells. J. Biol. Chem. 293, 18016-18030.

Wang H., Ding Y., He Y., Yu Z., Zhou Y., Gong A. and Xu M. (2023). LncRNA UCA1 promotes pancreatic cancer cell migration by regulating mitochondrial dynamics via the MAPK pathway. Arch. Biochem. Biophys. 748, 109783.

Xie Z.H., Yu J., Shang L., Zhu Y.Q., Hao J.J., Cai Y., Xu X., Zhang Y. and Wang M.R. (2017). KIAA1522 overexpression promotes tumorigenicity and metastasis of esophageal cancer cells through potentiating the ERK activity. OncoTargets Ther. 10, 3743-3754.

Xu Y., Sun C., Han B., Xi Y., Zhang M., Yang J. and Chen Z. (2020). High KIAA1522 expression predicts a poor prognosis in patients with hepatocellular carcinoma. Oncol. Lett. 20, 509-516.

Yi X., Hu C., Zhang C., Shao K., Sun H., Jiang Y., Sun N. and Zhi X. (2022). KIAA1522 is a new biomarker of promoting the tumorigenesis and distant metastasis of colorectal carcinoma. Cell Signal. 90, 110202.

Yuedi D., Houbao L., Pinxiang L., Hui W., Min T. and Dexiang Z. (2020). KLF2 induces the senescence of pancreatic cancer cells by cooperating with FOXO4 to upregulate p21. Exp. Cell Res. 388, 111784.

Zhang D., Dai Y., Cai Y., Suo T., Liu H., Wang Y., Cheng Z. and Liu H. (2016). KLF2 is downregulated in pancreatic ductal adenocarcinoma and inhibits the growth and migration of cancer cells. Tumour Biol. 37, 3425-3431.

Zhang H., Zhu C., He Z., Chen S., Li L. and Sun C. (2020). LncRNA PSMB8-AS1 contributes to pancreatic cancer progression via modulating miR-382-3p/STAT1/PD-L1 axis. J. Exp. Clin. Cancer Res. 39, 179.

Zhou C., Yi C., Yi Y., Qin W., Yan Y., Dong X., Zhang X., Huang Y., Zhang R., Wei J., Ali D.W., Michalak M., Chen X.Z. and Tang J. (2020). LncRNA PVT1 promotes gemcitabine resistance of pancreatic cancer via activating Wnt/beta-catenin and autophagy pathway through modulating the miR-619-5p/Pygo2 and miR-619-5p/ATG14 axes. Mol. Cancer 19, 118.

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