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The acidic microenvironment promotes pancreatic cancer progression via the lncRNA-LOC100507424/E2F1/FOXM1 axis

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Abstract

Pancreatic cancer is highly aggressive and sensitive to acidic microenvironments, which promote cancer cell survival and invasion. Long non-coding RNAs (lncRNAs) play crucial roles in cancer biology, helping cells adapt to microenvironmental changes, but their functions in the acidic microenvironment of pancreatic cancer are understudied. This study investigated the role of lncRNA LOC100507424 in pancreatic cancer, previously linked to glioma stem cells. Clinical specimens and cell line models cultured under acidic conditions showed that LOC100507424 was upregulated in pancreatic cancer tissues and further increased in acidic environments. Functional assays demonstrated that knockdown of LOC100507424 inhibited cell proliferation, invasion and metastasis. Mechanistically, LOC100507424 transcriptionally regulated FOXM1 expression through its interaction with E2F1. In vivo studies confirmed that LOC100507424 promoted tumor growth in nude mice. These findings highlight the significance of lncRNAs in the acidic microenvironment of pancreatic cancer and suggest potential therapeutic targets.

Keywords Pancreatic cancer, Acidic microenvironment, LncRNA, LOC100507424, Cancer progression

Introduction

Pancreatic cancer is characterized by its high malignancy and often presents at advanced stages, with most patients diagnosed after distant metastases have occurred, rendering surgical intervention impossible. The prognosis for pancreatic cancer is extremely poor, with a 5-year overall survival rate of less than 10% [1]. Patients frequently exhibit primary or secondary resistance to common chemotherapeutic agents, such as 5-fluorouracil and gemcitabine, which severely limits the efficacy of chemotherapy and exacerbates the adverse effects of treatment, thereby increasing the economic burden on patients. Furthermore, pancreatic cancer shows limited sensitivity to radiotherapy and immunotherapy, underscoring the urgent need for novel therapeutic strategies [2].

A thorough investigation into the mechanisms underlying pancreatic cancer development, together with the

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identification of potential therapeutic targets, is essential for enhancing treatment outcomes. The tumor microenvironment (TME), comprising immune cells, fibroblasts, vascular endothelial cells, and the extracellular matrix, often exhibits hypoxic and acidic conditions that significantly contribute to tumor progression and drug resistance [3]. Compared to other malignancies, pancreatic cancer is characterized by extensive fibrosis and pronounced hypovascularity, leading to cellular hypoxia and elevated lactic acid production, which accumulates due to inadequate clearance through the bloodstream, resulting in a distinctly acidic microenvironment [4]. This acidic microenvironment has been shown to promote the progression of pancreatic cancer and contribute to the development of resistance to both chemotherapy and radiotherapy [5].

A significant portion of the human genome consists of non-coding RNAs (ncRNAs), which include primarily short non-coding RNAs (such as microRNAs) and lncRNAs (greater than 200 nucleotides). These ncRNAs regulate gene expression at transcriptional, post-transcriptional, and epigenetic levels [6]. Emerging evidence indicates that ncRNAs play critical roles in various biological processes, including tumor cell proliferation, invasion, metastasis, and chemosensitivity [7, 8]. Recent studies have demonstrated that lncRNAs can undergo adaptive responses to changes in the microenvironment, regulating the expression of target proteins and participating in cellular stress responses. In the context of pancreatic cancer, lncRNAs have been implicated in disease progression through various cell signaling pathways [9, 10]. Specifically, LOC100507424, an 882-nucleotide-lncRNA located at chromosome 12, also known as the antisense lncRNA of FOXM1, has been shown to inhibit Glioma stem cells (GSCs) progression when knocked down [11]. However, LOC100507424 has not yet been reported in pancreatic cancer.

LncRNAs can exert regulatory effects on nearby genes through cis-acting mechanisms [12], and FOXM1 is positioned adjacent to LOC100507424. However, the role of FOXM1 within an acidic microenvironment has not been extensively explored. Cheng et al. found that FOXM1 increases glucose uptake, lactate production, and oxygen consumption in myeloma [13], while Follia et al. reported that FOXM1 functions as a newly discovered glycolytic gene in pancreatic cancer, with lactate production contributing to an acidic microenvironment [14]. This suggests a crucial link between FOXM1 and acidic conditions.

Understanding the oncogenic mechanisms of LOC100507424 in altered microenvironments is essential for developing novel therapeutic strategies and identifying potential therapeutic targets. Thus, we constructed an in vitro acidic microenvironment model of pancreatic cancer cells and a nude mouse pancreatic cancer model, regulated the expression of LOC100507424/FOXM1, and observed the alterations of pancreatic cancer cells' proliferation, migration and invasion, in order to clarify the regulation and molecular mechanism of the acidic microenvironment LOC100507424 on the biological behavior of pancreatic cancer.

Table 1 siRNA/shRNA sequences

si-LOC100507424	**CGCACAAAAAUAUCACAUATT(Sense) UAUGUGAUAUUUUUGUGCGTT(Antisense)**
si-FOXM1	GCCCAACAGGAGUCUAAUCAATT(Sense) UUGAUUAGACUCCUGUUGGGCTT(Antisense)
si-E2F1	GACCACCUGAUGAAUAUCUTT(Sense) AGAUAUUCAUCAGGUGGUCTT(Antisense)
lnc-sh#1	GGATAATGATCTAAGGAAA
lnc-sh#2	GGTCTTCCCTTCTATCAAA
lnc-sh#3	GGAACAGAAGTCAATTGAA

Materials and methods

Clinical specimens and cell culture

The clinical trial was sanctioned by the Ethics Committee of Zhejiang Provincial People's Hospital (2024 No. 261). This work is guided by the Declaration of Helsinki. Participants gave their written consent after being fully informed. The research utilized human pancreatic cancer cell lines PANC-1 and ASPC-1, obtained from the Center for Model Organism Culture Preservation (USA) and the Cancer Research Institute (UK), respectively. For culturing, PANC-1 cells were placed in high-glucose DMEM medium (Gibco), while ASPC-1 cells were kept in RPMI-1640 medium (Gibco). Both media were enriched with 10% fetal bovine serum (Gibco), along with 100 units/mL penicillin and 100 μg/mL streptomycin (Biosharp). Cultures were maintained under conditions of 37 °C and 5% CO_2. To assess the effects of an acidic external environment, the pH levels of the media were modified using PIPES and HEPES buffers (Sigma).

Cell transfection

RiboBio synthesized and purified the plasmids for LOC100507424, FOXM1, E2F1, and the control samples (Table 1). To achieve stable inhibition of LOC100507424, specific shRNA sequences were developed and inserted into lentiviral vectors (Table 1). The study utilized an expression construct for LOC100507424 (pCDH-CMV-LOC100507424) alongside an empty vector (pCDH-CMV). Transfection procedures took place in 6-well plates, followed by culturing the transfected cells in an acidic environment (pH 6.4) for 48 h.

RNA extraction and RT-qPCR

RNA was isolated from cell lines or animal tissues utilizing the Rapid RNA Extraction Kit (ESscience). For

reverse transcription, the riboSCRIPT Reverse Transcription Kit (RiboBio) was employed according to the supplier's protocol. The resulting amplified fragments were examined via a 2% agarose gel. Quantitative PCR was performed using the CFX Manager Real-Time PCR System (Bio-Rad). To quantify mRNA and lncRNA, the riboSCRIPT RT-qPCR Starter Kit was applied. GAPDH was utilized as the internal control for normalization. The primer sequences are provided in Table 2.

Western blot

Cells were lysed with RIPA Buffer (Beyotime) containing a protease inhibitor cocktail (EDTA-free, 100X stock in DMSO, from MCE). The protein extracts were denatured by heating. Proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto PVDF membranes (Millipore). Membranes were blocked with a rapid blocking solution (1X, Epizyme) to reduce non-specific binding. The membranes were then incubated overnight at 4 °C with primary antibodies against E2F1 (Proteintech), used as a loading control. After several washes with Tris-buffered saline with Tween 20 (TBST), the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies—goat anti-rabbit or goat anti-mouse (Beyotime)—for 1 h at room temperature. Blots were visualized and analyzed using film development.

Plate cloning

Cells underwent transfection with siRNA aimed at LOC100507424. Following a 48-hour period, 1000 cells were placed in 6-well plates and allowed to grow for 7 to 14 days. Afterwards, the cells were stabilized with methanol at ambient temperature for half an hour, followed by staining with a 0.1% crystal violet solution for 30 min.

CCK8 assay

Cells were seeded in 96-well plates and transfected with 50 nM siRNA targeting LOC100507424 using JET PRIME transfection reagent (Polyplus). After 24 h of culture, the CCK-8 assay was performed. Cells were incubated in medium containing the transfection mixture, and OD values were measured at 450 nm using a Synergy HTX microplate reader (BioTek).

Table 2 qPCR primer sequences

LOC100507424	**TATGGAGAGGGCCATGCAGA(Sense)** **CTGCAGTGCACGGTTTCTTC(Antisense)**
FOXM1	AAGCCAGGCTGGAAGAACTC(Sense) TGTGATTCCAAGTGCTCGGG(Antisense)
E2F1	AAGAGCAAACAAGGCCCGAT(Sense) ACAACAGCGGTTCTTGCTCC(Antisense)
CD206	GCTGGAGAACTTCTCCACCA(Sense) GCTGTTGTAGAGGTGGCACA(Antisense)
Arginase-1	GGCACATCAGAAGGTGGTG(Sense) GATGTCCAGGCAAAGGTC(Antisense)
GAPDH	GGAGCGAGATCCCTCCAAAAT(Sense) GGCTGTTGTCATACTTCTCATGG(Antisense)

Cell scratching assay

Cells were seeded in 24-well plates and allowed to grow until 90% confluence. A micropipette tip was used to create scratches in the cell monolayers, which were then cultured in serum-free medium. Micrographs were captured after 24 h.

Transwell assay

In the transwell assay, Matrigel (Corning) was combined with a medium devoid of serum and placed into the transwell inserts (Corning). The bottom chamber was filled with a medium that included 10% fetal bovine serum to serve as a chemical attractant. An amount of 30,000 cells, resuspended in serum-free medium, were introduced to the upper chamber and allowed to incubate for a period of 48 h. Subsequently, the cells were fixed with 4% paraformaldehyde, stained using 0.1% crystal violet, and then examined and photographed under a microscope.

Dual luciferase assay

293T cells were initially cultured in 12-well plates and then transfected with the pGL4 plasmid that includes the 10-h-FOXM1-luciferase promoter (NCBIO, Guangzhou, China), alongside the pRL-TK plasmid for Renilla luciferase expression. Co-transfection was performed with the experimental plasmid pCDH-CMV-LOC100507424 and the control plasmid pCDH-CMV. After 48 h, the cells were collected, and luciferase activity was assessed using the Dual Luciferase Reporter Assay System (MCE). The luciferase readings were adjusted to account for transfection efficiency.

ChIRP assay

Designed and synthesized were five specific reverse-complement ChIRP probes targeting LOC100507424, intended to enrich for chromatin-associated RNA-protein complexes specifically involving LOC100507424. Serving as a negative control was a LacZ probe (Axl-Bio), ensuring the specificity of the results and controlling for non-specific binding. ChIRP-DNA experiments were conducted in PANC-1 cells to enrich for DNA endogenously bound to LOC100507424, focusing on a 2000 bp promoter region of the target gene FOXM1. Primers were designed at 300 bp intervals across the FOXM1 promoter, and seven pairs of qPCR primers were validated for this purpose. The sequences of the seven qPCR primer pairs are listed in Table 3. The binding region of LOC100507424 to the FOXM1 promoter was identified using a set of LOC100507424 ChIRP probes (labeled with 5' biotin):

Table 3 7 Pairs of qPCR primers for FOXM1

Primer1: 149 bp
CAGGAGGTTGAGGTTGTAGTG (Sense)
ACTACAAAGCAATGCTCAGAATG (Antisense)
Primer2: 178 bp
GCCCTTGGTCAGGGAATAG (Sense)
GGAGAGAGAGAGAGGAAGAGAA (Antisense)
Primer3: 172 bp
AAACTTCTCGGTATGGCTAAGG (Sense)
TCCTCTAAGCAGAGGGAATCA (Antisense)
Primer4: 153 bp
TTCCTGTCCTACCTCTCAAGAT (Sense)
ATGATCACACCACTGCACTC (Antisense)
Primer5: 152 bp
CCTCCTAAAGTGCTGGGATTAC (Sense)
CTAGGCCCTGAAGATACAATGG (Antisense)
Primer6: 127 bp
TCTATTATATCCGAAGGCTTGGC (Sense)
CCTTTGAGGGCTGCGTATTA (Antisense)
Primer7: 144 bp
CCCACGGCCACTTCTTC (Sense)
ACGGAACGTCGCCAATC (Antisense)

- Probe 1: `tggttcct ctgacctgagctgctgctgtaaggca`.
- Probe 2: `atagtgtgtgacctgtgtgtgtgtgtaat`.
- Probe 3: `Agtggatgatgatctctctgctgtgtgccagagagagagt`.
- Probe 4: `attat cctctctctctactaattaattaatgt`.
- Probe 5: `tcctctctctctctctctctgcaggacca`.

RNA Pull-Down assay

Biotin-labeled lncRNA-LOC100507424 was mixed with magnetic beads and cell extracts, with a LacZ probe (Axl-Bio) serving as the negative control. Protein complexes were collected, heat-denatured by boiling, and subsequently subjected to silver staining and Western blotting.

RIP assay

In the RIP experiment, cells were lysed using polysome lysis buffer supplemented with Protease and RNase inhibitors, followed by immunoprecipitation and washing with polysome washing buffers 1 and 2, including a DNase treatment step to remove DNA contamination. RNA was extracted using phenol-chloroform-isoamyl alcohol extraction, precipitated with ethanol, and dissolved in RNase-free water. The study focused on LOC100507424 and GAPDH as controls, using specific primers for PCR and qPCR to confirm the binding of these RNAs with the E2F1 protein. Primer sequences used were: for LOC100507424, forward primer (5'-CT GCAGTGAAGAACCCAAGA-3') and reverse primer (5'-CAGGGTGGTCCGTGTAAATAG-3'), producing a product length of 225 bp; for GAPDH, forward primer (5'-GTCAACGGATTTGGTCGTATTG-3') and reverse primer (5'-TGGAAGATGGTGATGGGATTT-3'), with a product length of 212 bp.

Chromatin Immunoprecipitation (ChIP) assay

Chromatin Immunoprecipitation (ChIP) assays were performed to investigate the binding of E2F1 to the FOXM1 promoter in siRNA control (sicon) and siRNA-mediated LOC100507424 knockdown (siLOC100507424) cells. Cells were cross-linked with 1% formaldehyde, quenched with glycine, and lysed. Chromatin was sonicated to obtain DNA fragments ranging from 100 to 500 bp. The lysates were then immunoprecipitated using an anti-E2F1 antibody or control IgG. After washing and elution, the cross-links were reversed, and DNA was purified. The enriched DNA was quantified by qPCR using primers specific to the FOXM1 promoter region (primer 4 from Table 3). Fold enrichment was calculated relative to input DNA, and results were normalized to the sicon group to assess the impact of LOC100507424 silencing on E2F1 binding. This procedure revealed a significant reduction in E2F1 binding to the FOXM1 promoter at primer 4 in siLOC100507424 cells compared to the sicon controls.

Subcutaneous tumor formation in nude mice

Animal experiments were authorized and monitored by the Animal Research Committee at Zhengzhou University's School of Medicine (NO. 22 cc-lac 20240427[02]), ensuring all animal care and handling followed the guidelines of the Institutional Animal Care and Use Committee. To develop the pancreatic tumor model, we used 6- to 8-week-old BALB/c nude mice provided by Jiangsu Jik Rui Pharmaceutical Biotechnology Co., Ltd. In the experiment, the PANC-1 pancreatic cancer cell line was used for injecting into nude mice. To ensure consistent tumor cell implantation across groups, healthy cells were selected, processed with trypsin digestion, and washed multiple times with PBS before being resuspended to $1 \times 10\text{^}6$ cells/ml. Each mouse received a 0.3 ml subcutaneous injection of the cell mixture in the back, following disinfection with 75% alcohol. The injection site was gently pressed to prevent leakage. From the injection day, tumor sizes (length L, width W) were checked every three days and recorded over five days. Tumor volumes (V) were calculated with the formula $V = (L \times W^2) / 2$, and a growth curve was generated from weekly observations. At the study's end, the nude mice were euthanized by intravenous injection of a large dose (150 mg/kg) of pentobarbital sodium into the tail veins, followed by cervical dislocation to ensure death, and the tumors were quickly excised for weighing and immunohistochemical processing.

Statistics

Data analysis was performed using GraphPad Prism 8.0 software. Findings are displayed as the average ± standard deviation. When comparing two groups, we utilized a two-sample Student's t-test. For pairwise comparisons,

we first conducted one-way ANOVA, followed by Dunnett's post hoc test to determine which specific groups differed significantly from the control group. Dunnett's test adjusts the *P*-values to reduce the likelihood of false positives. Statistical significance was acknowledged for differences where the *P* value was less than 0.05. The creation of charts also utilized GraphPad Prism 8.0 software.

Results

LOC100507424 is highly expressed in human pancreatic cancer tissues with acidic microenvironment pancreatic cancer cell lines

To understand the regulatory profile of lncRNAs in pancreatic cancer, we conducted microarray analysis on patient tissues and paracancerous tissues (data uploaded to GEO dataset, accession: GSE193109). The differential clustering heatmap showed that LOC100507424 was highly expressed in pancreatic cancer tissues compared to paracancerous tissues (Fig. 1A). KEGG and GO enrichment analyses indicated that high expression of LOC100507424 in pancreatic cancer tissues was closely associated with tumor immunity (Fig. 1B). This suggests the importance of the pancreatic cancer microenvironment. The localization of LOC100507424 was primarily nuclear, as shown by nucleoplasmic separation experiments (Fig. 1C).

An acidic microenvironment was constructed for PANC-1 and ASPC-1 cells. RT-qPCR results showed that the acidic microenvironment promoted LOC100507424 expression compared to the normal environment (Fig. 1D). Further functional assays are discussed in the following section.

Acidic microenvironment promotes pancreatic cancer development by upregulating LOC100507424

To validate the function of LOC100507424, its expression was interfered with in the acidic microenvironment (Fig. 2A). CCK-8 and plate cloning experiments revealed that the acidic microenvironment enhanced ASPC-1 and PANC-1 cells viability and proliferation compared to the normal environment. Knocking down LOC100507424 in the acidic microenvironment reversed these effects (Fig. 2B-C).

Cell scratch and invasion assays revealed that the acidic microenvironment enhanced the migration and invasion capabilities of ASPC-1 and PANC-1 cells relative to a normal setting. Knocking down LOC100507424 under acidic conditions mitigated these enhanced effects (Fig. 2D-E).

Transcriptional regulation of FOXM1 by LOC100507424

High expression of FOXM1 in pancreatic cancer tissues (Fig. 3A-B) was identified through GEPIA (see Discussion section for further details on the clinical significance of FOXM1). The lncRNA microarray gene correlation analysis (GSE193109) indicated that FOXM1

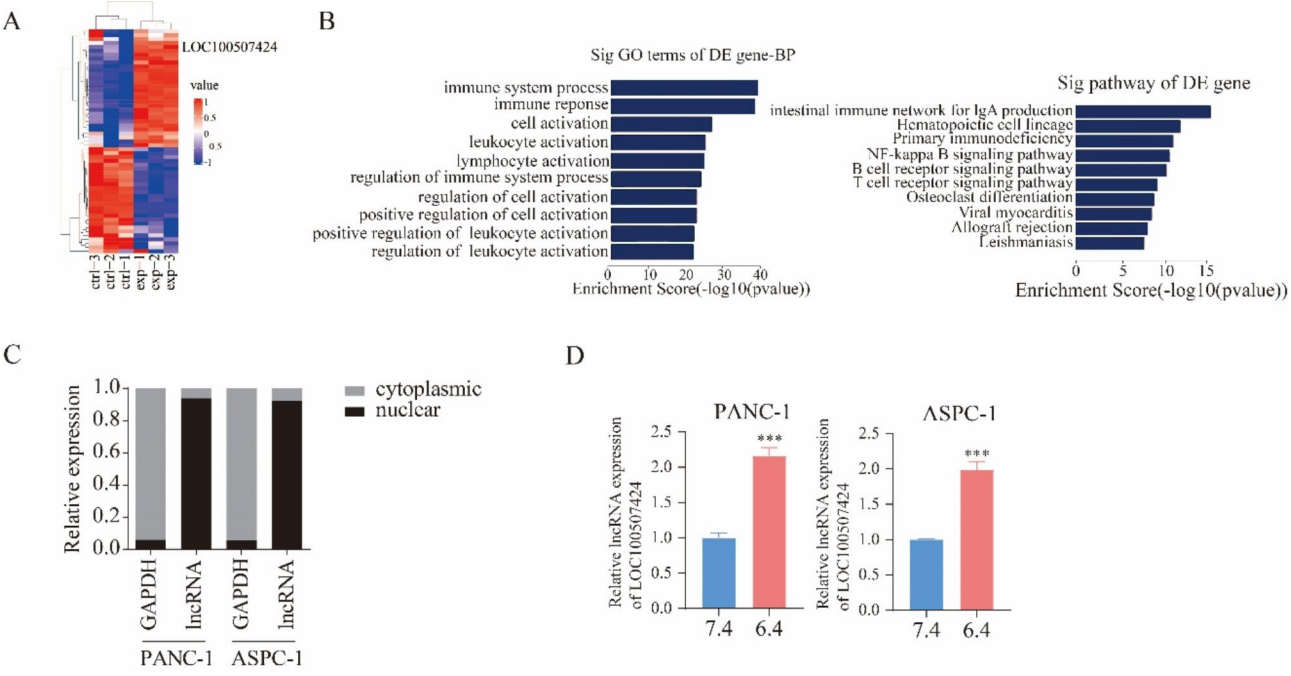

Fig. 1 LOC100507424 is highly expressed in human pancreatic cancer tissues with acidic microenvironment pancreatic cancer cell lines. **A**: Differential clustering heatmap showing high expression of LOC100507424 in pancreatic cancer tissues from 3 patients, including 3 pancreatic cancer samples and 3 adjacent non-tumor tissues. **B**: Graphical representation of GO and KEGG enrichment analysis. **C**: Nucleoplasmic separation assay localizing LOC100507424. **D**: Upregulation of LOC100507424 expression in acidic microenvironment

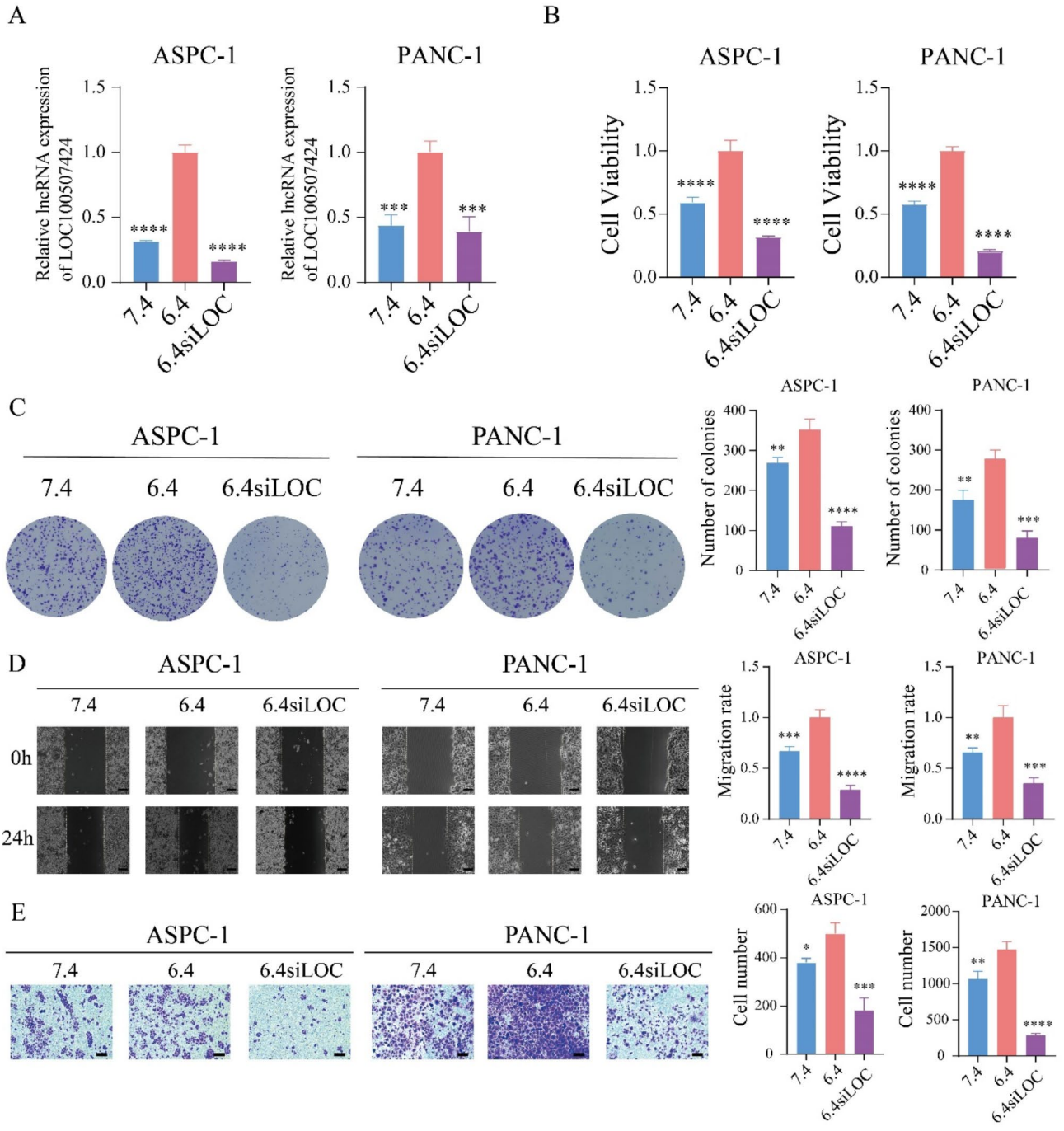

Fig. 2 Acidic microenvironment promotes pancreatic cancer development by upregulating LOC100507424. **A**: Validation of transfection efficiency. **B-C**: CCK-8 and colony formation assays for assessing cell viability and proliferation. **D**: Scratch assay for evaluating cell migration ability. **E**: Transwell assay for measuring cell invasive capacity. Scale of 200 μm

may be a target of LOC100507424 (Fig. S1). Dual-luciferase reporter assays demonstrated that LOC100507424 increased FOXM1 promoter activity (Fig. 3C). To further investigate this interaction, we truncated the FOXM1 promoter region (2000 bp) into seven segments (primers 1–7). ChIRP experiments revealed that LOC100507424 endogenously binds to the FOXM1 promoter in primers 3, 4, and 5, with the strongest binding observed in primer 4 (Fig. 3D-F). RT-qPCR showed that the acidic microenvironment promotes FOXM1 expression. Additionally, knocking down LOC100507424 under acidic conditions inhibited FOXM1 expression (Fig. 3G).

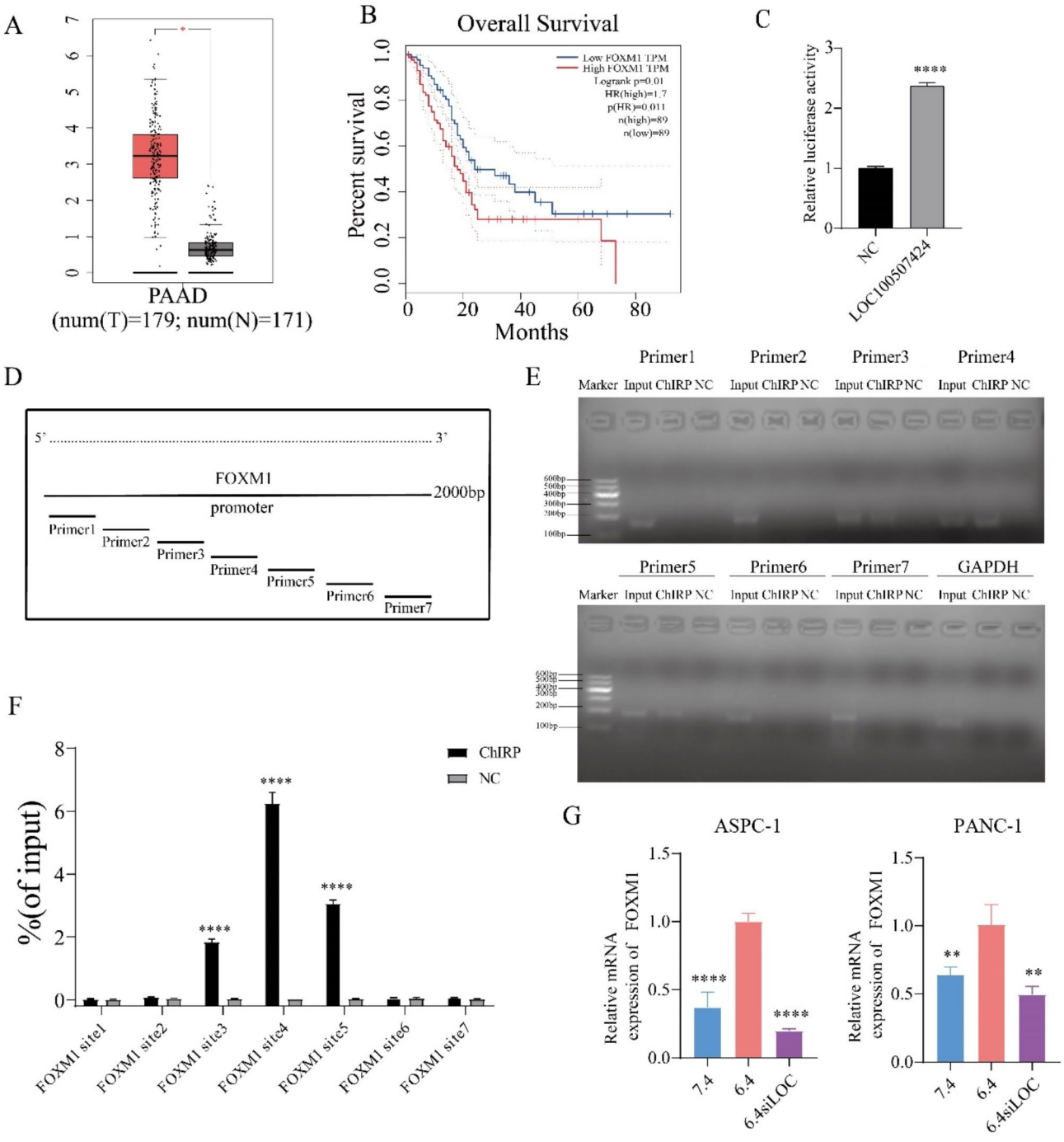

Fig. 3 Transcriptional regulation of FOXM1 by LOC100507424. **A-B**: GEPIA analysis showing high FOXM1 expression in pancreatic cancer tissues and its correlation with poor prognosis. **C**: Dual luciferase activity assay showing the effect of LOC100507424 on FOXM1 promoter activity. **D**: Schematic diagram of primers 1–7 used for promoter region analysis. **E**: Electrophoresis gel image of ChIRP assay products. **F**: ChIRP assay identifying specific binding sites of LOC100507424 on the FOXM1 promoter. **G**: RT-qPCR detecting FOXM1 expression

FOXM1: role in acidic microenvironment-promotes pancreatic cancer development

To explore the role of FOXM1 under acidic conditions, we performed experiments targeting FOXM1 (Fig. 4A). Results from CCK-8 and clonogenic assays indicated that inhibiting FOXM1 under these conditions significantly reduced the viability and proliferation of ASPC-1 and PANC-1 cells (Fig. 4B-C). Furthermore, wound healing and invasion assays demonstrated that this inhibition

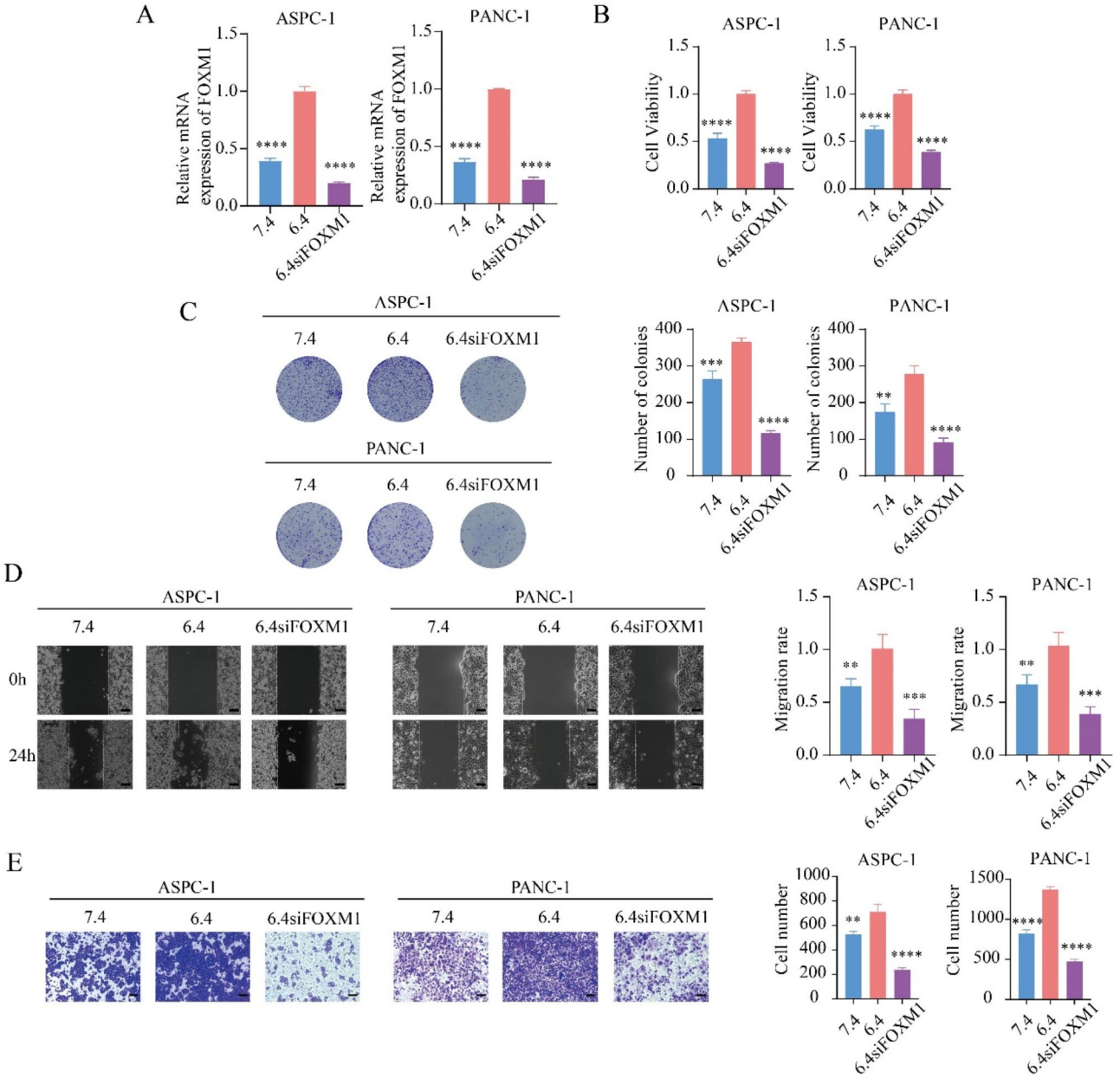

Fig. 4 FOXM1: role in acidic microenvironment-promotes pancreatic cancer development. **A**: Verification of transfection efficiency. **B-C**: Assessment of cell viability and proliferation using CCK-8 and colony formation assays. **D**: Evaluation of cell migration ability using the scratch assay. **E**: Evaluation of cell invasion ability using the transwell assay. Scale of 200 μm

also substantially diminished the migratory and invasive capacities of these cells (Fig. 4D-E).

LOC100507424 interacts with E2F1

LOC100507424 was predicted to bind to E2F1 using catRAPID (Fig. 5A). The RNA pull-down assay was conducted in ASPC-1 cells using the biotinylated LOC100507424 probe and the negative lacz probe. A distinct band was detected by western blot, confirming the binding of LOC100507424 to E2F1 (Fig. 5B-C). RIP assay further verified this interaction. LOC100507424 was notably enriched in the E2F1 immunoprecipitation group relative to IgG controls (Fig. 5D-E).

GEPIA analysis demonstrated the positive correlation between E2F1 and FOXM1 expression (Fig. 5F). Subsequently, we constructed LOC100507424 overexpression plasmid in acidic microenvironment for ASPC-1 and PANC-1 cells, and found that the upregulation of LOC100507424 had no significant effect on E2F1 by RT-qPCR, whereas knockdown of E2F1 significantly downregulated the expression of E2F1 (Fig. 5G-H). Therefore, our ChIP experiments revealed that silencing

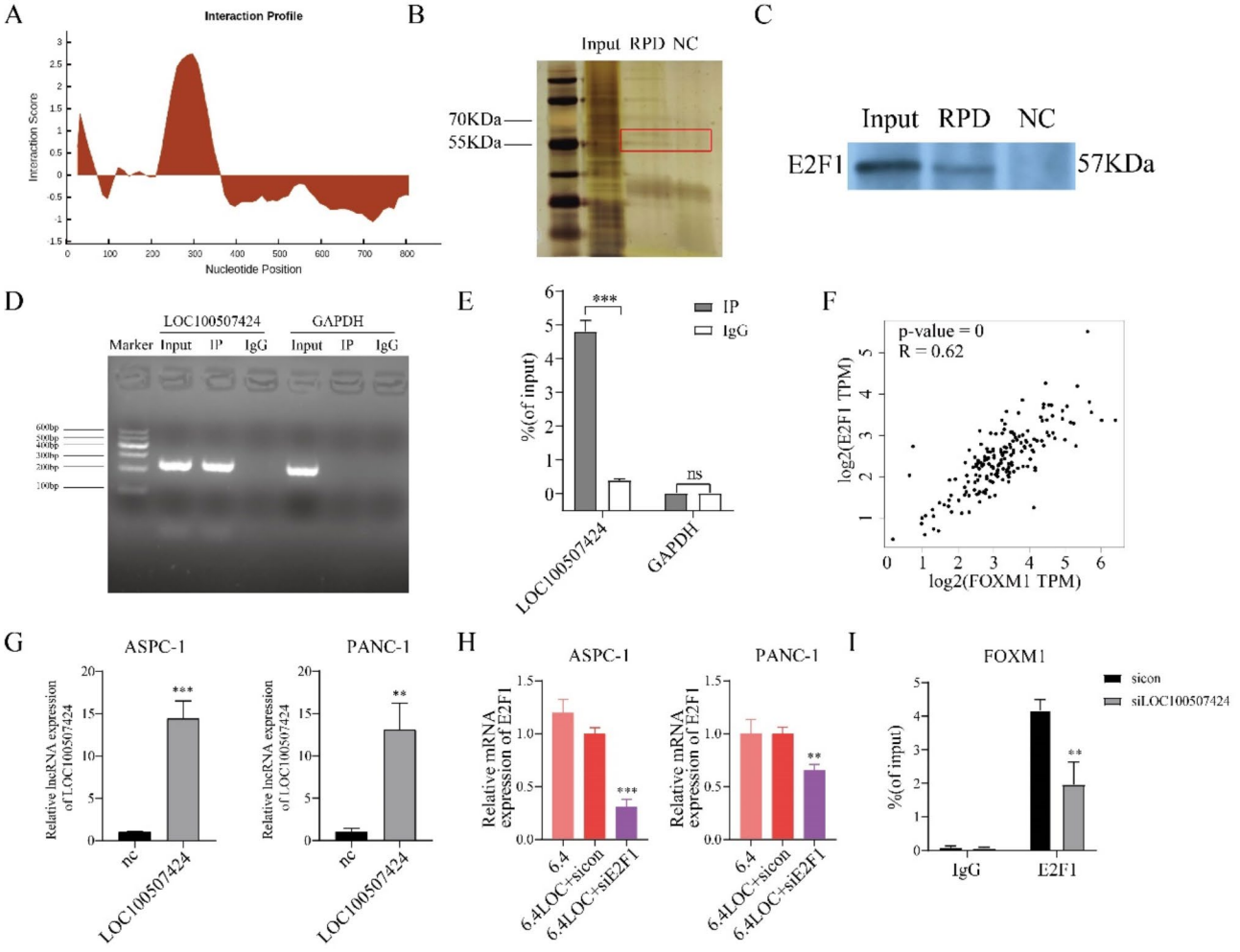

Fig. 5 LOC100507424 interacts with E2F1. **A**: catRAPID prediction of LOC100507424 binding to E2F1. **B**: Silver staining image from RNA pull-down assay. **C**: Western blot analysis of RNA pull-down products. **D**: Electropherogram from RIP assay. **E**: Enrichment analysis of RIP products. **F**: GEPIA analysis showing the correlation between E2F1 and FOXM1. **G-H**: Validation of transfection efficiency. **I**: ChIP analysis of E2F1 binding to the FOXM1 promoter in sicon vs. siLOC100507424 cells

LOC100507424 led to a decreased ability of E2F1 to bind to the FOXM1 promoter compared to the sicon group (Fig. 5I).

Promotion of pancreatic cancer development by the LOC100507424-FOXM1 axis in acidic microenvironments is dependent on E2F1

Rescue experiments were carried out. CCK8 and plate cloning assays showed that when E2F1 expression was knocked down in the context of LOC100507424 overexpression, the viability and proliferation of ASPC-1 and PANC-1 cells were significantly reduced (Fig. 6A-B). Cell scratch and invasion assays indicated that inhibiting E2F1 under conditions of LOC100507424 overexpression led to decreased migration and invasion capabilities in both cell lines (Fig. 6C-D).

RT-qPCR analysis revealed that, upon E2F1 knockdown combined with LOC100507424 overexpression, the expression levels of FOXM1 were markedly decreased (Fig. 6E). It suggests that the regulation of FOXM1 and the promotion of pancreatic cancer progression by LOC100507424 are mediated through E2F1.

In vivo, LOC100507424 promotes pancreatic cancer development

To validate the in vivo function of LOC100507424, we conducted stable transient silencing in PANC-1 cells (Fig. 7A). Using the subcutaneous tumor formation assay in nude mice, we observed that silencing LOC100507424 inhibited tumor growth (Fig. 7B-D). RT-qPCR results indicated that silencing LOC100507424 reduced FOXM1, CD206 and Arginase-1 expression (Fig. 7E-F). IHC analysis revealed that silencing LOC100507424 led to decreased Ki67 expression and increased E-cadherin expression compared to the NC group (Fig. 7G-H). These

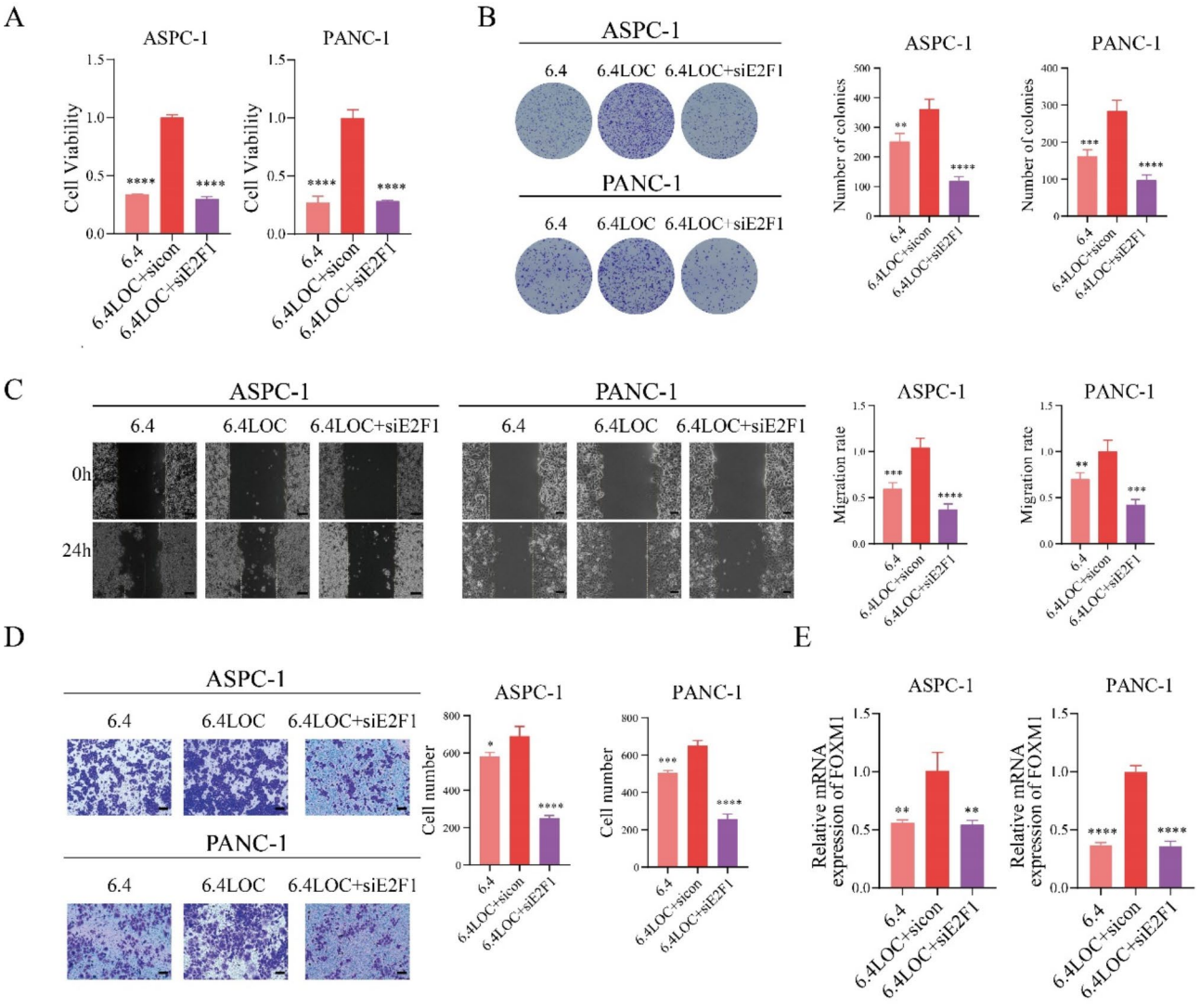

Fig. 6 Promotion of pancreatic cancer development by the LOC100507424-FOXM1 axis in acidic microenvironments is dependent on E2F1. **A-B**: Assessment of cell viability and proliferation using CCK-8 and colony formation assays. **C**: Evaluation of cell migration using the scratch assay. **D**: Evaluation of cell invasion using the transwell assay. **E**: RT-qPCR analysis of FOXM1 expression. Scale of 200 μm

findings confirm that LOC100507424 promotes pancreatic cancer proliferation and metastasis in vivo.

Discussion

TME comprises a network of cytokines, immune cells, extracellular matrix components, and peripheral blood vessels [15]. Metabolic disturbances, hypoxia, and increased acidity can cause the TME to evolve into a tumor-acidic microenvironment [16–21]. In this acidic setting, cancer cells display enhanced proliferation, migration, and invasion [22]. Research shows that tumor cells' adaptation to this modified microenvironment under stress is critical for initiating and advancing tumorigenesis. This adaptation also drives the development of a more aggressive malignant phenotype [23–27]. LncRNAs play essential roles in the TME's response mechanisms and cancer cell signaling pathways [28]. Studies have demonstrated that in an acidic microenvironment, lactic acid upregulates lncRNA HISLA expression in tumor-associated macrophages, resulting in the secretion of HISLA-enriched extracellular vesicles. These vesicles induce changes in breast cancer cell metabolism and promote drug resistance [29]. Moreover, cancer cells exposed to an acidic microenvironment may undergo endoplasmic reticulum (ER) stress as a means to adapt. This ER stress activates the unfolded protein response (UPR), where downstream transcription factors can regulate lncRNA expression [30]. For instance, lncRNA GOLGA2P10 has been shown to inhibit CHOP-induced apoptosis, thus supporting cellular survival and tumor progression [31].

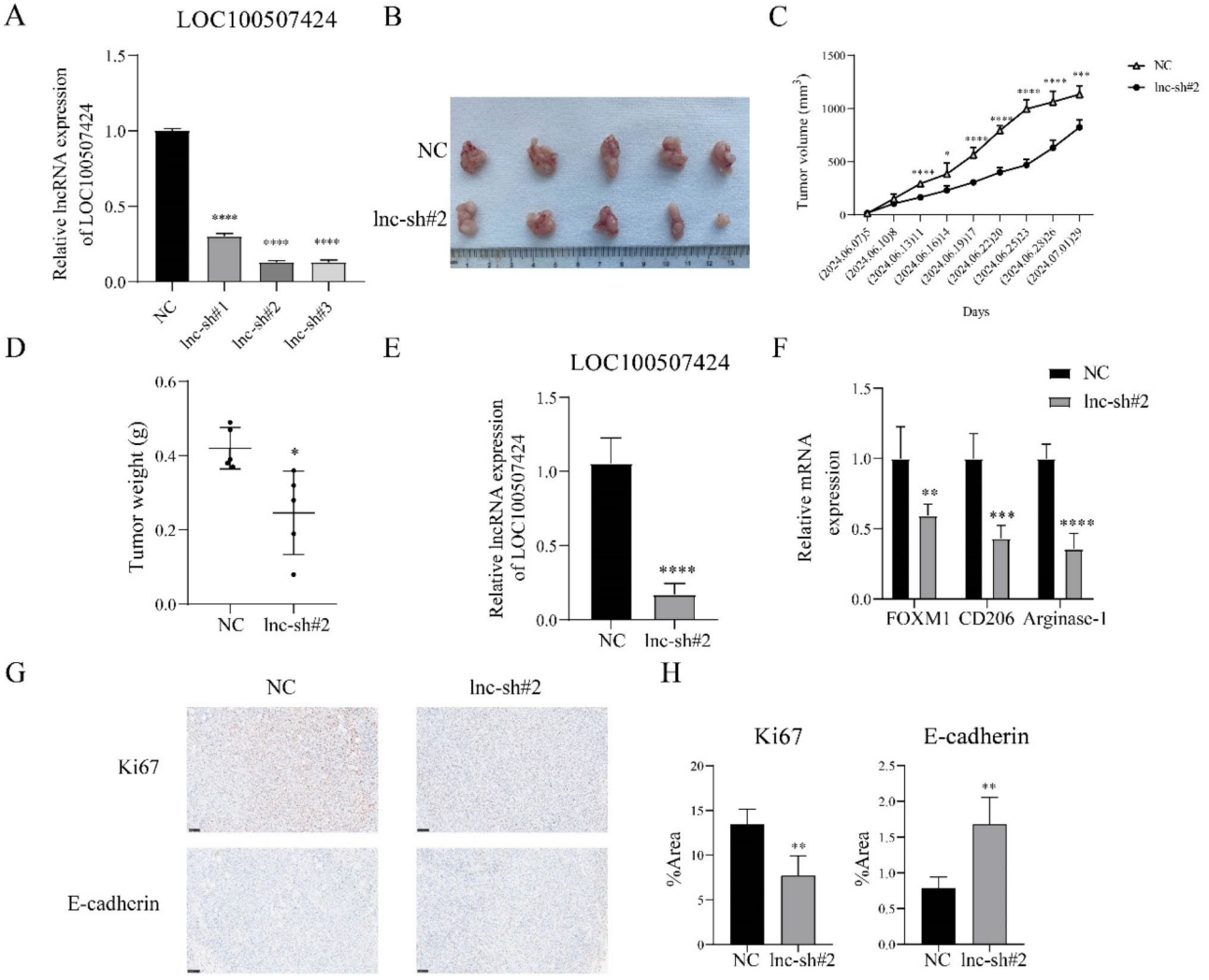

Fig. 7 In Vivo, LOC100507424 Promotes Pancreatic Cancer Development. **A**: Verification of silencing efficiency in stably transfected PANC-1. **B**: Illustration of tumor mass. **C**: Changes in tumor volume. **D**: Tumor mass weight. **E**: In vivo validation of LOC100507424 silencing efficiency. **F**: FOXM1, CD206 and Arginase-1 expression detected by RT-qPCR in vivo. **G**: IHC staining illustration. **H**: Ki67 and E-cadherin expression levels ($n = 5$). Scale of 100 μm

Our research primarily focuses on the function and mechanisms of LOC100507424 in pancreatic cancer within acidic microenvironment. Further studies suggests that LOC100507424 is upregulated in acidic conditions. It serves as a key marker of stress response in pancreatic cancer cells. It promotes cell proliferation, invasion and metastasis. In vivo experiments demonstrated that reducing LOC100507424 levels inhibits tumor growth, supporting our in vitro findings. LncRNAs can interact with DNA, RNA, or proteins, affecting cellular functions. They can act as oncogenes or tumor suppressors in different cancers, showing promise as diagnostic markers [32]. In conclusion, our findings indicate that LOC100507424 may serve as a biomarker for pancreatic cancer. Its role highlights its importance in diagnosis and therapy.

FOXM1 features a conserved winged helix DNA-binding domain of more than 100 amino acids. Our findings indicate that high expression of FOXM1 in pancreatic cancer tissues is significantly associated with poor prognosis. It is essential in cell proliferation, invasion, metastasis, chemoresistance, and angiogenesis, especially in tumor biology. This makes FOXM1 a promising target for cancer therapy [33]. Tan X et al. demonstrated that FOXM1 boosts the proliferation, invasion, and EMT of breast cancer cells. It also increases their resistance to chemotherapy and radiotherapy [34]. Wu et al. reported that FOXM1 overexpression promotes aerobic glycolysis and malignant behavior in multiple osteosarcoma cells [35]. Battistini, C et al. shows that microenvironment-induced FOXM1 maintains ovarian cancer stemness. Inactivating FOXM1 lowers the survival rate of cancer stem cells [36]. The study revealed that FAT10 induces epithelial-mesenchymal transition (EMT) and chemoresistance in pancreatic cancer by stabilizing FOXM1

expression [37]. Studies reveal that FOXM1 interacts with Smad3, keeping the Smad3/Smad4 complex active in the nucleus. FOXM1 regulates the TGF-β/Smad3 signaling pathway, which is involved in tumor cell metastasis and stromal fibrosis [38, 39]. Studies have shown that activation of this pathway can upregulate the expression of pro-carcinogenic genes such as MMPs and Snail to promote cancer progression [40]. Our study found that FOXM1 advances the progression of pancreatic cancer cells in an acidic microenvironment. This process is governed by LOC100507424. Zhang S et al. noted that silencing LOC100507424 decreases FOXM1 expression in GSCs, and this aligns with our findings [11]. We discovered that LOC100507424 binds to the promoter of FOXM1, transcriptionally controlling its expression, driving the malignant progression of pancreatic cancer. These results highlight the importance of the LOC100507424-FOXM1 axis in the development and progression of pancreatic cancer.

E2F1 is a crucial transcription factor that promotes cell proliferation by binding to the promoter regions of various genes. Its role in gene transcription and cell growth varies based on cellular conditions [41]. Research shows that E2F1 can control the transcription of FOXM1 [42, 43]. Previous studies have reported that the lncRNA PVT1 promotes gastric cancer progression by enhancing the transcription of FOXM1. This enhancement may be due to lncRNA-mediated chromatin remodeling or through the recruitment of co-activators to the FOXM1 promoter region [44]. Additionally, lncRNA HIT can form complexes with specific transcription factors such as E2F1, thereby enhancing or diminishing their binding affinity to target gene promoters and regulating the expression levels of these genes [45].

Building on these findings, we investigated the interaction between LOC100507424 and E2F1. Using RNA-pull down and RNA immunoprecipitation assays, we confirmed both exogenous and endogenous binding between LOC100507424 and E2F1. RT-qPCR experiments revealed that overexpression of LOC100507424 did not significantly affect the expression levels of E2F1. However, we hypothesized that the two molecules might form a complex and exert transcriptional regulatory effects on the target gene FOXM1. To test this hypothesis, we conducted rescue experiments. The results demonstrated that silencing E2F1 in the context of LOC100507424 overexpression led to a suppression of FOXM1 expression, consistent with the aforementioned studies. These findings highlight the intricate relationship between lncRNAs and transcription factors in controlling critical oncogenic pathways. These indicate that the LOC100507424/E2F1/FOXM1 axis could be a new target for pancreatic cancer therapy.

In summary, our study highlights the role of the LOC100507424/E2F1/FOXM1 axis in advancing pancreatic cancer growth under acidic conditions. We adjusted the pH of the culture medium to mimic an acidic microenvironment. This condition can also arise from hypoxia or metabolic shifts. It is crucial to acknowledge that our methods do not completely mirror the in vivo acidic microenvironment found in pancreatic tumors. Our results offer fresh insights into the mechanisms driving pancreatic cancer progression. These also underscore the potential of LOC100507424 as the therapeutic target. Future studies should investigate the clinical relevance of LOC100507424 expression and evaluate its suitability as the therapeutic target for pancreatic cancer treatment.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Author contributions

Deyang Mu and Ying Shi: manuscript writing, investigation, methodology, and validation; Deyang Mu and Runxuan Sun: data collection, data curation and analysis; Bing Han and Kai Zhong: data curation and analysis and draft editing; Yilu Ye and Jungang Zhang: study conceptualization, supervision, reviewing, revising, and editing. All authors contributed to the discussion of results and commented on the final manuscript. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Data availability

Not all raw data from this study is publicly available due to its ongoing use in subsequent research projects. However, it can be requested from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declare no competing interests.

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