

ECT2 promotes proliferation and stemness of pancreatic cancer cells via the Wnt/ β -catenin signaling pathway

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ABSTRACT: Epithelial cell transformation 2 (ECT2) plays a critical role in human oncogenesis, but its biological role in pancreatic adenocarcinoma (PAAD) remains unclear. Bioinformatics analysis was performed to evaluate the expression of ECT2 in PAAD and its prognostic value. Functional assays and Western blot were performed to explore the effects of ECT2 on the proliferation and stemness of pancreatic cancer cells. TCGA and GTEx databases revealed upregulated ECT2 expression in PAAD tissues ($n = 178$), which was associated with advanced T3/T4 stage, deceased status, and disease-specific survival (DSS) events ($p < 0.05$). Furthermore, high ECT2 expression correlated with poor overall survival (OS), relapse-free survival (RFS), and progression-free interval (PFI) ($p < 0.05$). ECT2 knockout inhibited proliferation, colony formation, and sphere formation ($p < 0.05$), and decreased the expression of Cyclin D1, CDC25C, CD24, OCT4, and SOX2. Conversely, overexpression of ECT2 enhanced proliferation, colony formation, and sphere formation ($p < 0.05$), and increased the expression of Cyclin D1, CDC25C, CD24, OCT4, and SOX2. Gene Set Enrichment Analysis (GSEA) revealed that high ECT2 expression was linked to pancreatic cancer, the cell cycle, and classical Wnt signaling. ECT2 expression was positively correlated with β -catenin levels ($R = 0.481$, $p < 0.001$). ECT2 knockout reduced β -catenin expression, but increased p- β -catenin, GSK-3 β , and Axin1 expression. In contrast, ECT2 overexpression increased β -catenin expression while reducing p- β -catenin, GSK-3 β , and Axin1 expression. This study revealed that ECT2 promotes the proliferation and stemness of pancreatic cancer cells via the Wnt/ β -catenin signaling pathway. ECT2 may serve as a prognostic biomarker and therapeutic target for pancreatic cancer.

KEYWORDS: epithelial cell transformation2, Wnt/ β -catenin, pancreatic cancer, proliferation, stemness

INTRODUCTION

Pancreatic cancer remains as one of the most aggressive and deadly malignancies, with both its incidence and economic burden steadily rising. By 2040, the overall incidence of pancreatic cancer is projected to rise by approximately 30% [1]. Due to challenges in early diagnosis and the lack of effective therapeutic treatments, the 5-year survival rate for pancreatic cancer patients remains below 9% [2]. Therefore, exploring the pathogenesis of pancreatic cancer is important to early prediction and diagnosis.

Epithelial cell transforming sequence 2 (ECT2) functions as a guanine nucleotide exchange factor (GEF) for Rho family GTPases, playing a crucial role in regulating the cell cycle and cytokinesis. ECT2 promotes mitosis by catalyzing the exchange of guanine nucleotides on small GTPases, including RhoA, Rac1, and Cdc42. The phosphorylation of ECT2 during the G2/M phase is essential for its exchange activity [3, 4]. Abnormal expression of ECT2 can lead to uncontrolled cell division, and increasing evidence suggests that

its dysregulation is associated with tumor proliferation and growth. In oral squamous cell carcinoma (OSCC)-derived cells, ECT2 knockdown upregulates p21^{Cip1} and p27^{Kip1}, while downregulating Cyclin D1, cyclin E, and CDK4. The resulting elevation of the Cip/Kip family members suppresses cyclin D1-CDK complex activity, thereby inducing G1-phase cell cycle arrest and inhibiting cell proliferation [5]. ECT2 facilitates proliferation by stabilizing the deubiquitinase PSMD14, which inhibits E2F1 degradation through the ubiquitin-proteasome pathway. This reduction in E2F1 ubiquitination further promotes PTTG1 expression, an oncogenic transcription factor, thereby driving glioma cell proliferation [6]. Additionally, the role of ECT2 in promoting tumor progression has been well established in breast, colorectal, and liver cancers [7–9].

Tumor cells, particularly cancer stem cells (CSCs), are characterized by self-renewal, immune evasion, chemo-radioresistance, and unlimited proliferation. The Wnt/ β -catenin signaling pathway plays a central role in maintaining CSC phenotypes and tumor proliferative capacity [10]. Upon binding of Wnt

ligands to receptor complexes, Disheveled (DVL) is activated, leading to the recruitment of the cytoplasmic destruction complex (composed of AXIN, GSK3 β , CK1, and APC). This process results in the stabilization and nuclear translocation of β -catenin, which subsequently associates with T-cell factor (TCF)/lymphoid enhancer-binding factor (LEF) and co-activators (such as Pygopus and Bcl-9) to drive the expression of downstream target genes, including c-Myc, cyclin D1, and CDKN1A, thereby regulating cell cycle progression and stemness maintenance [10, 11].

Given the critical role of ECT2 in cell cycle regulation, we hypothesized that it modulates both proliferation and stemness in pancreatic cancer cells. However, the mechanisms underlying these processes remain largely unexplored. Therefore, this study aims to clarify the biological functions of ECT2. Specifically, we investigated how ECT2 drives cell proliferation and stemness via the Wnt/ β -catenin pathway, and evaluated its potential as a therapeutic target.

MATERIALS AND METHODS

Bioinformatics analysis

Data from the TCGA and GTEx databases were integrated to analyze the expression, clinical characteristics, and prognostic of ECT2 in pancreatic cancer patients. The diagnostic efficacy of ECT2 for pancreatic cancer was assessed by receiver operating characteristic (ROC) curve analysis. Based on the univariate regression results and clinical relevance, relevant prognostic factors were selected to construct a nomogram for predicting the 1-year overall survival (OS) probability. Furthermore, Gene Set Enrichment Analysis (GSEA) was conducted to identify potential signaling pathways associated with ECT2.

Cell culture

The hTERT-HPNE cell line and Pancreatic cancer cell lines (MIA PaCa-2, PaTu 8988, and PANC-1) were purchased from Wuhan Pricella Biotechnology Co., Ltd (Wuhan, China). All cell lines described in this study were verified via STR analysis using the PowerPlex 21 system. Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; Procell, China) and maintained in a humidified incubator at 37°C with 5% CO₂.

Lentivirus packaging

The ECT2-overexpressing and knockout lentiviral vectors were constructed using pHBLV-CMV-MCS-3FLAG-EF1-ZsGreen-T2A-PURO and pHBLV-U6-gRNA-EF1-Cas9-PURO, respectively. The corresponding amplification primers, target single-guide RNA (sgRNA) sequences, and verification primers are detailed in Table S1.

The ECT2 knockout and overexpression plasmids were successfully constructed and verified by sequenc-

ing. The purified shuttle vector was co-transfected into 293T cells with packaging plasmids (pSPAX2 and pMD2G) using the Lipofiter™ transfection reagent (Hanbio Biotechnology, Shanghai, China). Viral supernatants were harvested at 48 and 72 h post-transfection and stored at -80°C. Subsequently, MIA PaCa-2, PaTu 8988, and PANC-1 cells were transduced with 1×10^7 viral particles for 48 h. Following selection with puromycin (1:10,000 dilution), stable cell pools expressing EGFP, KO-ECT2, 3×Flag vector, or 3×Flag-ECT2 were successfully established.

RNA extraction and quantitative polymerase chain reaction (qPCR)

Total RNA was extracted using TRIzol (Thermo Fisher Scientific, USA). cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The qPCR was conducted using the PowerUp SYBR™ Green Master Mix (Thermo Fisher Scientific). The amplification protocol was, as follows: initial denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 30 s. The relative mRNA expression levels were calculated using the $2^{-\Delta\Delta CT}$ method, with GAPDH as the endogenous control. The primers are listed in Table S1.

Western blot

Cells were washed with phosphate-buffered saline (PBS) and lysed with RIPA lysis buffer on ice for 30 min. Protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit. Approximately 20 μ g of protein per sample was separated by 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were then blocked and incubated with the following primary antibodies: rabbit anti- β -Tubulin (1:1000, 2128S; Cell Signaling Technology (CST), USA), mouse anti-ECT2 (1:1000, abcam-236502, Cambridge, UK), anti-CD24 (1:500, bs-0528R, Bioss (bs), Beijing, China), anti-SOX2 (1:500, bs-0523R), anti-OCT4 (1:1000, bs-0830R), anti-Cyclin D1 (1:1000, bs-0623R), anti-CDC25C (1:500, bs-9597R), anti-Phospho- β -Catenin (1:1000, 9561T; CST), anti-Axin1 (1:1000, 2087T; CST), anti-GSK3 β (1:1000, ET1607-71; HUABIO, Hangzhou, China), anti- β -Catenin (1:1000, 8480T; CST), and anti-FLAG (1:1000, 14793T; CST).

Cell counting kit-8 (CCK-8)

Cell proliferation was assessed using Cell Counting Kit-8 (CCK8) (Biosharp, BS350B). Cells were seeded in 96-well plates at a density of 3,000–5,000 cells/well. On days 1, 2, and 3, 90 μ l of serum-free medium and 10 μ l of CCK-8 solution were added to each well. The plates were incubated for 2 h at 37°C in the dark. Absorbance was measured at 450 nm using a

SuPerMax3100 multifunctional microplate reader. The experiments were performed in triplicate and repeated three independent times.

Colony formation assay

EGFP, KO-ECT2, 3×Flag-vector, or 3×Flag-ECT2-stably transfected cells were seeded into 6-well plates at a density of approximately 1,000 cells per well. The cells were cultured for 10–14 days. Subsequently, colonies were fixed with 4% paraformaldehyde for 20 min, washed with PBS, and stained with 0.05% crystal violet. Colonies were observed and photographed.

Sphere formation assay

EGFP, KO-ECT2, 3×Flag-vector, or 3×Flag-ECT2-stably transfected cells were seeded into ultra-low attachment 6-well plates (Corning, 3471, USA) at a density of 400–1,000 cells per well. Cells were cultured in complete medium supplemented with 10% fetal bovine serum for 7–14 days at 37 °C. Spheroid formation was monitored using a microscope. Spheroids with a diameter >50 µm were counted for quantitative analysis using ImageJ software. The spheroid formation rate was calculated as: (number of spheroids/ number of seeded cells) × 100%.

Drug intervention

Stable ECT2-overexpressing PANC-1 and MIA PaCa-2 cells were seeded in 6-well plates (1×10^5 cells/well). After 8 h of incubation (30%–50% confluence), cells were treated with various concentrations of the Wnt inhibitor IWR-1 (MedChemExpress, Shanghai, China; 10, 20, and 30 µM). Cells were harvested for functional assays at 48 h post-treatment.

Statistical analysis

The experiments were performed in triplicate and repeated three independent times. Data are expressed as mean ± standard deviation (SD) from at least three independent experiments. Statistical comparisons between two groups were performed using Student's *t*-test, and comparisons among multiple groups were performed using one-way analysis of variance (ANOVA). $p < 0.05$ was considered statistically significant.

RESULTS

Correlation between the ECT2 expression and clinical features in pancreatic cancer patients

The expression of ECT2 in pancreatic cancer tissues ($n = 178$) and adjacent normal tissues ($n = 171$) was analyzed using the TCGA and GTEx database. Compared with normal pancreatic tissues, ECT2 expression was significantly upregulated in pancreatic cancer tissues (Fig. 1A). Furthermore, the association between the ECT2 expression and other clinical characteristics in pancreatic cancer patients was further explored. The

results revealed that the ECT2 expression was higher in patients with advanced T stage (T3/T4) (Fig. 1G), deceased patients (Fig. 1J), and patients with a disease-specific survival (DSS) event (Fig. 1K) ($p < 0.05$). However, there was no significant association between ECT2 expression and patient age (Fig. 1B), gender (Fig. 1C), smoking history (Fig. 1D), alcohol history (Fig. 1E), histologic grade (Fig. 1F), pathologic stage (Fig. 1H), M-stage (Fig. 1I), or surgical treatment outcomes (Fig. 1L) (all $p > 0.05$).

High ECT2 expression is associated with poor prognosis in pancreatic cancer patients

Kaplan-Meier survival analysis indicated that high ECT2 expression was significantly associated with poor overall survival (OS) (hazard ratio [HR]: 2.09, $p < 0.001$) (Fig. 2A), DSS (HR: 2.34, $p < 0.001$) (Fig. 2B), and progression-free interval (PFI; HR: 1.74, $p = 0.006$) (Fig. 2C). Additionally, the diagnostic performance of ECT2 in pancreatic cancer was evaluated using the ROC curve and nomogram analyses based on the TCGA database. The results showed that the area under the ROC curve (AUC) for ECT2 was 0.897 (95% CI: 0.846–0.947), indicating that ECT2 has high diagnostic value as a biomarker for pancreatic cancer (Fig. 2D). Furthermore, the nomogram survival prediction model revealed that ECT2 has superior predictive value compared to traditional clinical variables (e.g., age and M-stage) (Fig. 2E).

ECT2 promotes proliferation in pancreatic cancer cells

Compared with the normal pancreatic ductal epithelial cell line (hTERT-HPNE), ECT2 expression was markedly upregulated in all pancreatic cancer cell lines ($p < 0.05$). Furthermore, ECT2 mRNA and protein levels were highest in PaTu 8988 cells, followed by MIA PaCa-2 cells, and lowest in PANC-1 cells (Fig. 3A,B). ECT2 knockdown in PaTu 8988 and MIA PaCa-2 cells significantly downregulated its mRNA (Fig. 3C) and protein (Fig. 4C) expression. Conversely, ECT2 overexpression in PANC-1 and MIA PaCa-2 cells upregulated both mRNA (Fig. 3D) and protein (Fig. 4D) levels.

CCK-8 assay revealed that the ECT2 knockout significantly reduced the proliferation ability of PaTu 8988 and MIA PaCa-2 cells ($p < 0.05$) (Fig. 3E,F), whereas ECT2 overexpression significantly increased the growth of PANC-1 and MIA PaCa-2 cells ($p < 0.05$) (Fig. 3G,H). The colony formation assay showed that ECT2 knockout reduced the number of colonies formed by PaTu 8988 and MIA PaCa-2 cells ($p < 0.05$) (Fig. 4A), while ECT2 overexpression increased colony formation in PANC-1 and MIA PaCa-2 cells ($p < 0.05$) (Fig. 4B). Furthermore, Western blot results revealed that the protein levels of cyclin D1 and CDC25c decreased after the ECT2 knockout (Fig. 4C), and increased after ECT2 overexpression (Fig. 4D).

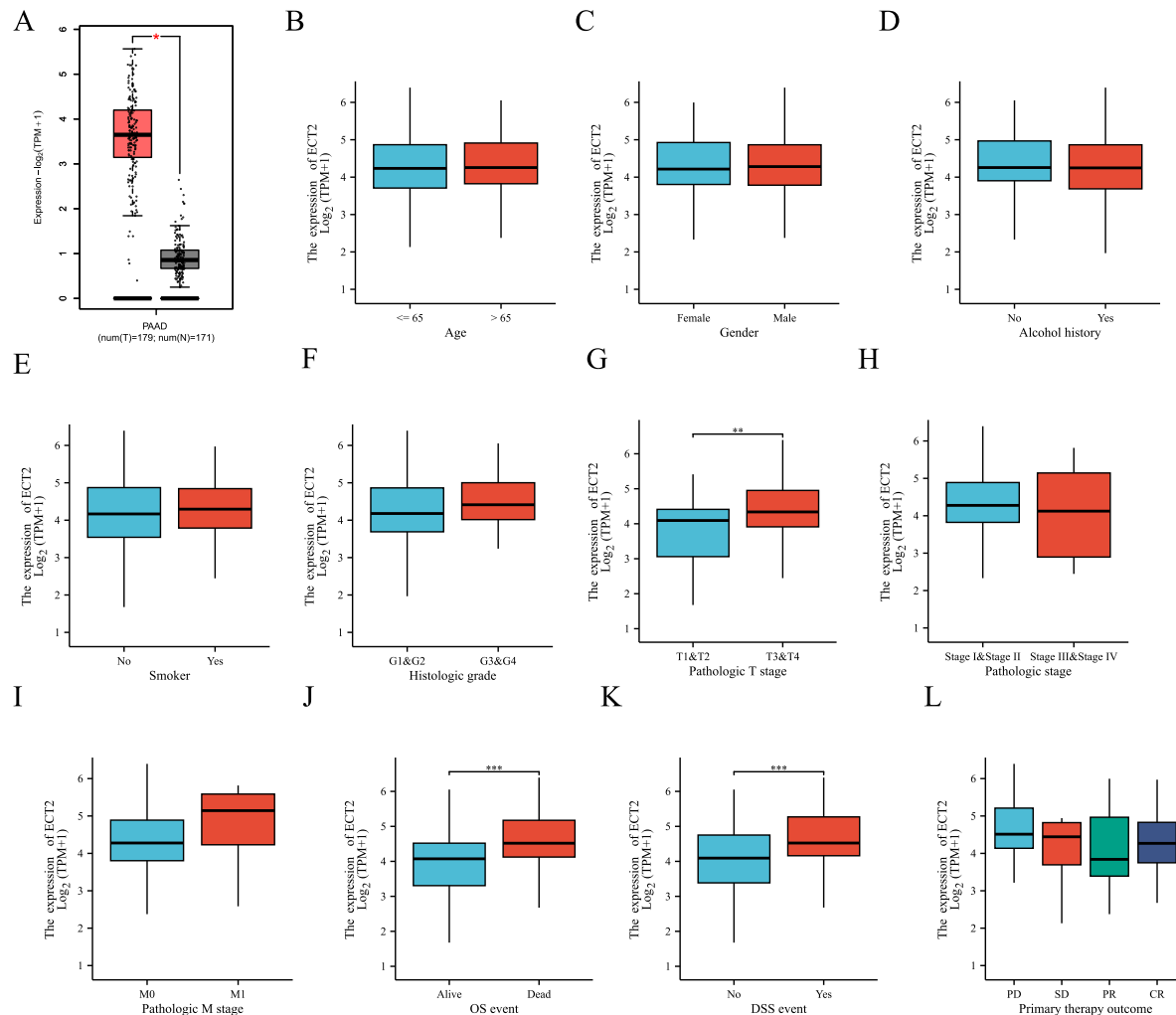


Fig. 1 Correlation between the ECT2 expression and clinical features in pancreatic cancer patients. The Cancer Genome Atlas (TCGA) and GTEx database revealed that the ECT2 expression was significantly upregulated in pancreatic cancer tissues (A), as well as in patients in the T3/T4 stage (G), patients with deceased status (J), and patients with a disease-specific survival (DSS) event (K) ($p < 0.05$). No significant correlation was identified between the ECT2 expression, and patient age (B), gender (C), smoking history (D), alcohol history (E), histologic grade (F), pathologic stage (H), M-stage (I), or surgical treatment outcomes (L) ($p > 0.05$).

ECT2 maintains stemness in pancreatic cancer cells

Knockdown of ECT2 in PaTu 8988 and MIA PaCa-2 cells resulted in fewer and smaller spheroids (Fig. 5A). Western blot analysis revealed decreased expression of the stemness-related proteins CD24, OCT4, and SOX2 (Fig. 5C). In contrast, overexpression of ECT2 increased both the number and size of spheroids (Fig. 5B) and elevated the expression levels of CD24, OCT4, and SOX2 (Fig. 5D).

ECT2 promotes proliferation and stemness through the Wnt/ β -catenin pathway in pancreatic cancer cells

GSEA revealed that the expression of gene sets related to pancreatic cancer (Fig. 6A), the cell cycle

(Fig. 6B), the G1-S phase (Fig. 6C), and the classical Wnt signaling pathway (Fig. 6D) correlated with high ECT2 expression. The heatmap showed that β -catenin (CTNNB1) exhibited a similar expression pattern to ECT2 between tumor and normal tissues (Fig. 6E). Further correlation analysis demonstrated that ECT2 expression was positively correlated with β -catenin (CTNNB1) levels ($R = 0.481$, $p < 0.001$) (Fig. 6F). Accordingly, we hypothesize that ECT2 promotes the proliferation and stemness of pancreatic cancer cells through activation of the Wnt signaling pathway. Therefore, key molecules associated with the Wnt/ β -catenin signaling pathway, including β -catenin, p- β -catenin, Axin1, and GSK3- β , were examined. Knock-out of ECT2 resulted in increased expression of GSK3- β , Axin1, and p- β -catenin, along with decreased expression of β -catenin (Fig. 6G). Conversely, over-

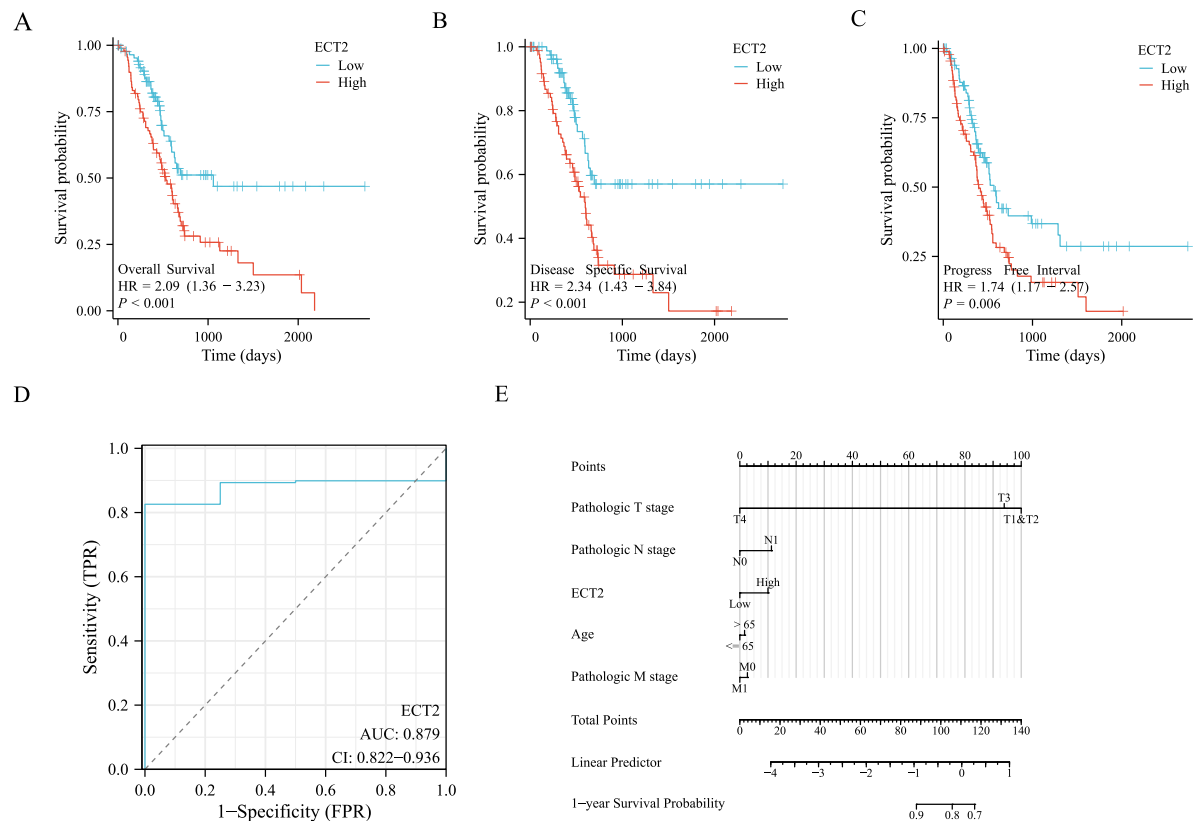


Fig. 2 Prognostic and diagnostic value of ECT2 in pancreatic cancer. (A, B, and C) The Kaplan-Meier survival analysis shows that high ECT2 expression is significantly associated with poor overall survival (OS, $p < 0.001$), disease-specific survival (DSS, $p < 0.001$), and progression-free interval (PFI, $p = 0.006$). (D) The ROC curve analysis shows that ECT2 has high diagnostic value for pancreatic cancer, with an AUC of 0.897. (E) The nomogram survival prediction model indicates that ECT2 has superior predictive value, when compared to traditional clinical variables.

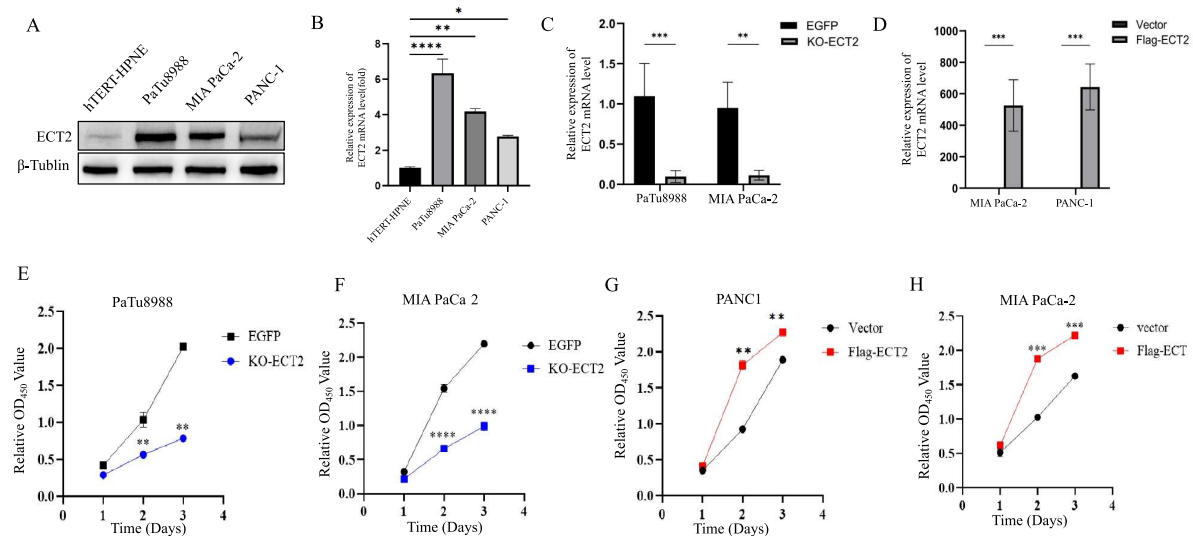


Fig. 3 ECT2 promotes proliferation in pancreatic cancer cells. Western blot (A) and qPCR (B) detected the ECT2 protein and mRNA levels in hTERT-HPNE, PaTu 8988, MIA PaCa-2, and PANC-1 cells. The ECT2 mRNA levels were assessed by qPCR after ECT2 knockout (C) or overexpression (D). The CCK-8 cell counting assay shows that the ECT2 knockdown significantly reduced the growth of PaTu 8988 (E) and MIA PaCa-2 (F) cells ($p < 0.05$). The ECT2 overexpression significantly enhanced the growth of PANC-1 (G) and MIA PaCa-2 (H) cells ($p < 0.05$).

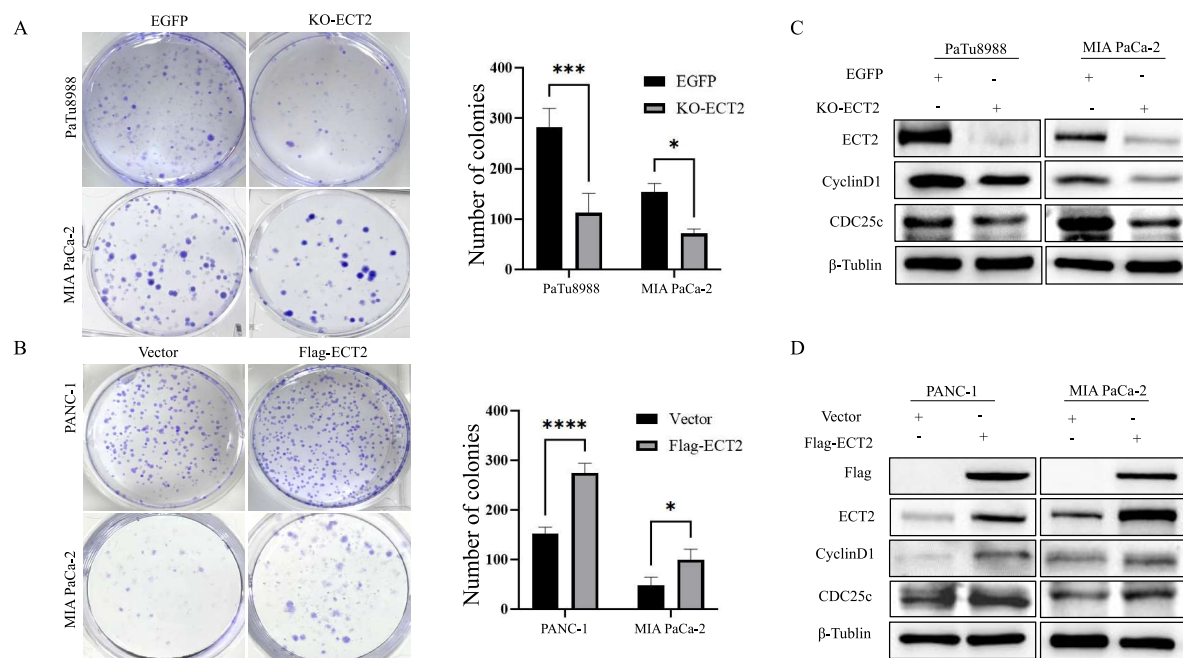


Fig. 4 Effect of ECT2 on colony formation in pancreatic cancer cells. The colony formation assay shows that the ECT2 knockout reduced the colony formation in PaTu 8988 and MIA PaCa-2 cells (A, $p < 0.05$). The ECT2 overexpression increased the colony formation in PANC-1 and MIA PaCa-2 cells (B, $p < 0.05$). Western Blot detected the cyclin D1 and CDC25c protein levels after ECT2 knockout or overexpression (C and D).

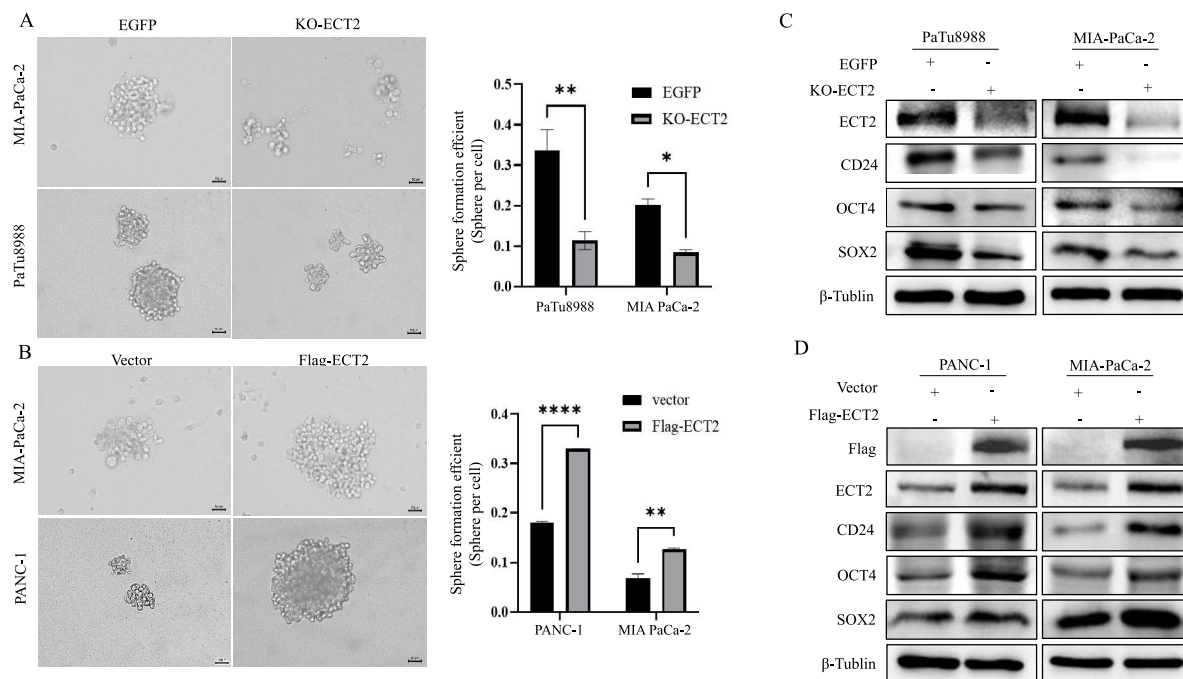


Fig. 5 ECT2 regulates stemness in pancreatic cancer cells. (A and C) The ECT2 knockout decreased the number and size of tumor spheres, and downregulated stemness-related proteins, including CD24, OCT4 and SOX2. (B and D) The ECT2 overexpression increased the number and size of tumor spheres, and upregulated stemness-related proteins, including CD24, OCT4 and SOX2.

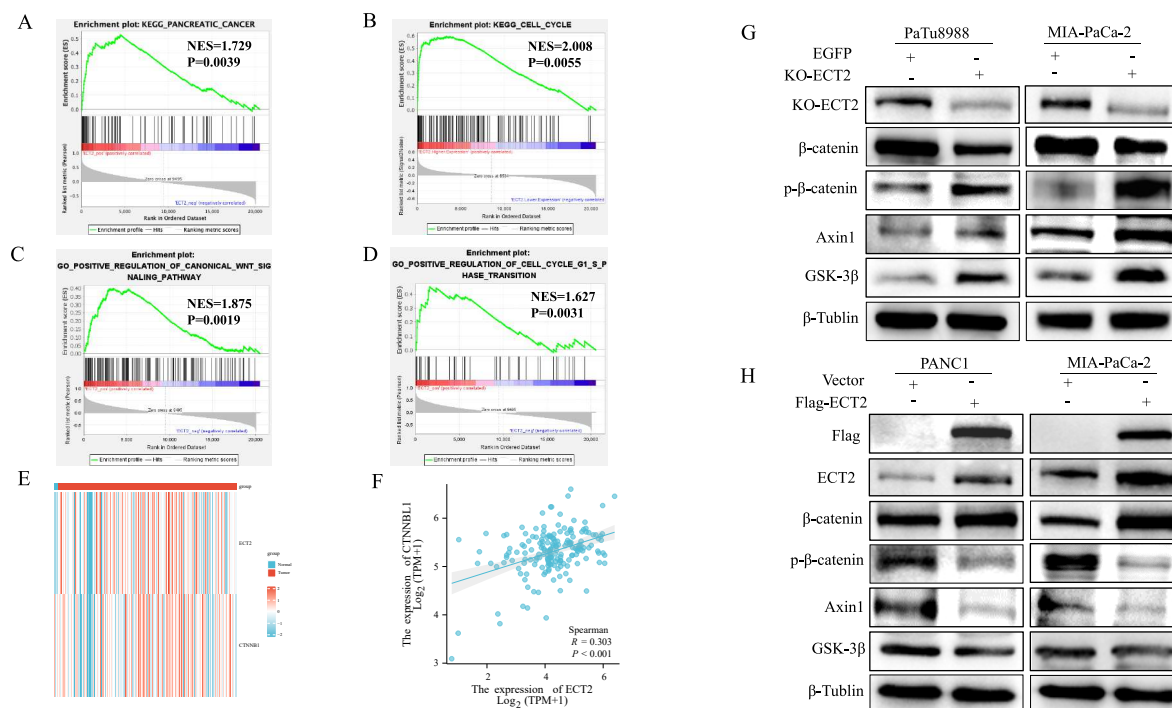


Fig. 6 ECT2 promotes proliferation and stemness via the Wnt/β-catenin pathway in pancreatic cancer cells. The GSEA revealed that high ECT2 expression is associated with gene sets related to pancreatic cancer (A), the cell cycle (B), the G1-S phase (C), and the Wnt/β-catenin signaling pathway (D). The heatmap shows the expression levels of ECT2 and β-catenin in pancreatic cancer samples (E). Correlation analysis between the ECT2 and β-catenin expression (F). Western blot detected the GSK-3β, Axin1, β-catenin, and p-β-catenin protein levels after ECT2 knockout or overexpression (G and H).

expression of ECT2 led to decreased levels of Axin1 and p-β-catenin and increased expression of β-catenin (Fig. 6H).

IWR-1 reverses ECT2 overexpression-induced proliferation and stemness

To further verify whether ECT2 mediates its biological functions through the Wnt signaling pathway, ECT2-overexpressing PANC-1 and MIA PaCa-2 cells were treated with the Wnt inhibitor IWR-1 to observe its effects on cell functions. Colony formation (Fig. 7A), sphere formation assay (Fig. 7B), and CCK-8 assays (Fig. 7C,D) demonstrated that IWR-1 treatment significantly reversed the enhanced cell proliferation and stemness mediated by ECT2 overexpression ($p < 0.05$).

DISCUSSION

In this study, the bioinformatics analysis revealed that ECT2 is highly expressed in pancreatic cancer patients. The overexpression of ECT2 in pancreatic cancer may be primarily driven by genomic amplification. This hypothesis is supported by evidence that its localized chromosomal region, the 3q26 amplicon (which also harbors PRKCI and SOX2), exhibits widespread copy number gains in various tumors, such as lung and

head and neck cancers [12, 13]. Notably, high ECT2 levels correlated with advanced TNM staging and were significantly associated with shortened OS, DSS, and PFS. These findings indicate that ECT2 plays a critical role in the development of pancreatic cancer.

Aberrant cell proliferation is an early, critical event driving pancreatic cancer initiation and subsequent metastasis [14]. Consequently, we investigated the functional role of ECT2 in regulating pancreatic cancer cell proliferation and stemness, aiming to explore the underlying molecular mechanisms driving these oncogenic processes.

In vitro functional assays demonstrated that ECT2 positively regulates pancreatic cancer cell proliferation and the expression of Cyclin D1 and CDC25, both of which are key regulators of cell cycle progression. Cyclin D1 activates cyclin-dependent kinase 4 (CDK4), which promotes the phosphorylation of the retinoblastoma protein (Rb). Phosphorylated Rb dissociates from the E2F transcription factor, thereby relieving its suppression of E2F, and promoting the transition from the G1 phase to the S phase of the cell cycle. Amplification of the CCND1 gene or overexpression of Cyclin D1 disrupts CDK activity, alters the cell cycle, bypasses critical cell cycle checkpoints, and induces uncontrolled cell proliferation, ultimately contributing to tumor growth [15]. The CDC25 phosphatase family,

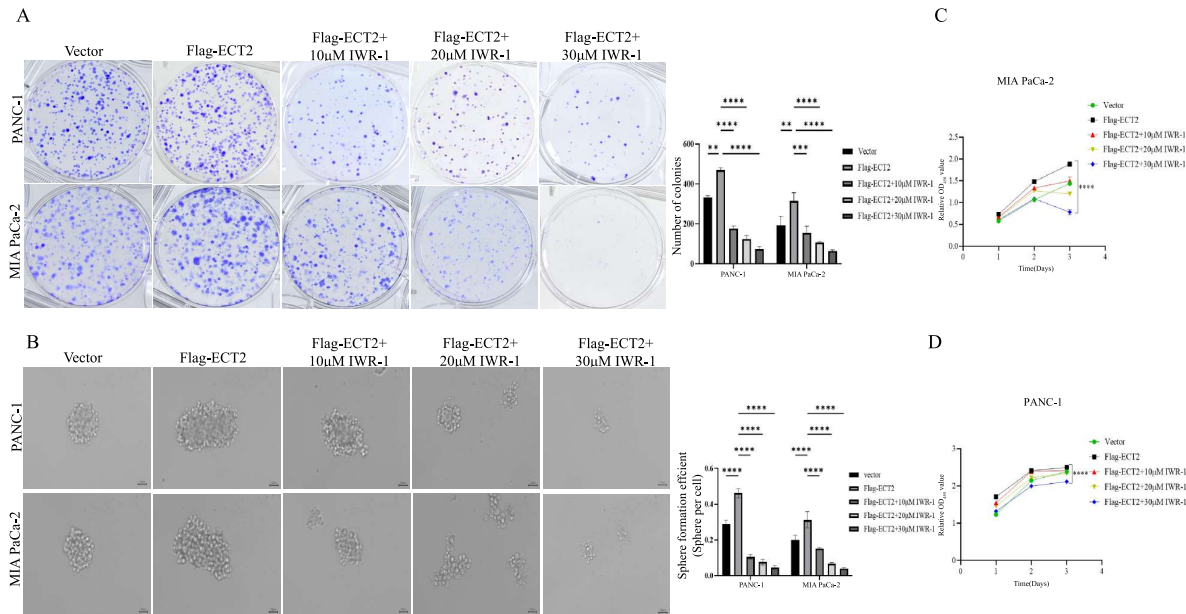


Fig. 7 IWR-1 inhibits pancreatic cancer cell proliferation and stemness. Colony formation assay shows the reversal of ECT2 overexpression-mediated cell proliferation by the Wnt inhibitor (A). Sphere formation assay shows the reversal of ECT2 overexpression-mediated self-renewal capacity by the Wnt inhibitor (B). CCK-8 shows the reversal of ECT2 overexpression-mediated cell proliferation by the Wnt inhibitor (C and D).

another core regulator of the cell cycle, removes the inhibitory phosphate group from tyrosine 15 (Tyr15) on CDKs, thereby promoting progression through cell cycle checkpoints. All three isoforms of CDC25 are considered potential oncogenes, with their overexpression or sustained activation leading to premature entry into the S or M phase of the cell cycle [16]. Enrichment analysis revealed that ECT2 is associated with cell cycle-related gene sets, particularly the G1-S phase, indicating that ECT2 may regulate cell cycle progression. Collectively, those findings suggest that ECT2 overexpression enhances the expression of Cyclin D1 and CDC25, which may accelerate cell division and promote the proliferative capacity of pancreatic cancer cells.

Beyond its role in cell cycle regulation, our findings establish that ECT2 plays a vital role in maintaining the stemness characteristics of pancreatic cancer cells. Specifically, loss- and gain-of-function assays demonstrate that ECT2 expression levels positively influence the spheroid-forming capacity of these cells, accompanied by the regulation of key stemness-related markers, including CD24, OCT4, and SOX2. Approximately 3–28% of pancreatic cancer cells express CD24 [17, 18]. CD24⁺ pancreatic cancer cells exhibit tumorigenic potential that is at least 10 times greater than CD24[−] cells [19]. Similarly, OCT4 and SOX2 serve as key drivers of CSCs stemness [20]. Previous studies have revealed that OCT4 inhibits pancreatic cancer cell proliferation by suppressing the AKT pathway in

a time-dependent manner [21]. SOX2 is upregulated through direct binding of the nuclear receptor NR5A2 to its promoter/enhancer region, thereby enhancing CSC stemness. Targeting SOX2 expression with the NR5A2 inhibitor Cpd3 can effectively reduce CSC stemness, disrupt its maintenance mechanisms, significantly improve chemotherapy sensitivity, and delay recurrence [22]. Notably, our data suggest that the regulatory effect of ECT2 on cyclins (cyclin D1 and CDC25) may synergistically enhance its influence on stemness proteins.

Enrichment analysis was performed, revealing a prominent correlation between ECT2 and the canonical Wnt/ β -catenin signaling pathway. Aberrant Wnt activation is well-established to drive CSC renewal, cell proliferation, and therapeutic responses [23]. Crucially, these literature findings are highly consistent with our current; for instance, Wnt/ β -catenin activation has been shown to enhance the stemness properties of CD24⁺ CSCs and directly transactivate downstream cell cycle drivers, including c-Myc and Cyclin D1 [24, 25]. Furthermore, blocking this cascade—either by disrupting receptor complexes or hindering the nuclear translocation of β -catenin—effectively suppresses CSC spheroid formation, downregulates stemness markers (e.g., CD44, CD133), and restores drug sensitivity [26, 27]. Consequently, ECT2 likely intersects with the Wnt/ β -catenin axis to drive cell proliferation and stemness maintenance in pancreatic cancer.

Our further investigation revealed that ECT2 activates the Wnt/ β -catenin pathway by regulating its core destruction complex. Experimentally, ECT2 overexpression in pancreatic cancer cells decreased the expression of GSK-3 β and Axin1, both of which are core components of the destruction complex responsible for phosphorylating and degrading β -catenin to maintain its intracellular levels at a low concentration [28, 29]. This pathway plays a well-established role in pancreatic cancer development. For example, the long non-coding RNA PVT1 has been shown to activate TCF/LEF-dependent downstream targets—such as c-Myc, Cyclin D1, and Axin2—ultimately contributing to gemcitabine resistance [30]. Additionally, Wnt pathway activation can induce the β -catenin/TCF7 complex to directly bind to the promoter region of glutamine synthetase (GS), enhancing its expression and promoting the tumorigenic potential of pancreatic stellate cells [31]. Within this context, our data suggest that ECT2 overexpression alters the levels of GSK-3 β , Axin1, and β -catenin, which is closely associated with activation of the Wnt signaling pathway. Importantly, Wnt inhibition successfully reversed the malignant phenotypes induced by ECT2 upregulation. These results demonstrate that ECT2 promotes pancreatic cancer progression by activating the Wnt/ β -catenin axis to drive cell growth and stemness.

Several limitations of this study should be acknowledged. First, our exploration of the underlying signaling pathway mechanisms remains insufficient. Due to technical constraints, we did not perform nuclear/cytoplasmic fractionation assays or immunofluorescence confocal microscopy to directly track the nuclear accumulation of active β -catenin. Furthermore, the precise role of ECT2 as a GEF to regulate Rho GTPases and β -catenin requires further elucidation. Second, *in vivo* animal experiments were not conducted to validate these findings.

In conclusion, our study demonstrates that ECT2 is highly expressed in pancreatic cancer and is strongly correlated with poor patient survival. High ECT2 expression promotes pancreatic cancer cell proliferation, colony formation, and sphere formation, accompanied by the upregulation of proliferation-related proteins (Cyclin D1 and CDC25C) and stemness markers (CD24, OCT4, and SOX2). ECT2 increases the expression of β -catenin while reducing the levels of GSK-3 β , Axin1, and p- β -catenin, thereby driving pancreatic cancer progression through the activation of the Wnt signaling pathway. These results suggest that ECT2 serves as a promising prognostic biomarker and therapeutic target for pancreatic cancer.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at <https://dx.doi.org/10.2306/scienceasia1513-1874.2026.082>.

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Appendix A. Supplementary data

Table S1 DNA and RNA nucleotide sequences.

Name	DNA and RNA nucleotide sequences
ECT2-all-F	ACTAGAGGATCTATTTCCGGTGAATTCGCCACCATGGCTGAAAATAGT
ECT2-all-R	TCACTTAAGCTTGGTACCGAGGATCCTATCAAATGAGTTGTAGATCT
ECT2-gRNA1	CACACTGGGAGGACTAGCT
ECT2-gRNA2	TCCAAGGAAAACCTACTTAT
ECT2-gRNA3	GATTGAAACAAGAGTGATAT
ECT2-gRNA4	TATTAACATCCACTACTGGG
ECT2-gRNA5	TAAAGTACTGAGATTCCA
ECT2-gRNA6	ATCACTCTTGTTTCAATCTG
ECT2-CAS9-gRNA1-T	CACCGCACTACTGGGAGGACTAGCT
ECT2-CAS9-gRNA1-B	AAACAGCTAGTCCTCCCAGTAGTG
ECT2-CAS9-gRNA2-T	CACCGTCCAAGGAAAACCTTACTTAT
ECT2-CAS9-gRNA2-B	AAACATAAGTAAGTTTTCTTGGAC
ECT2-CAS9-gRNA3-T	CACCGATTGAAACAAGAGTGATAT
ECT2-CAS9-gRNA3-B	AAACATATCACTCTTGTTTCAATC
ECT2-CAS9-gRNA4-T	CACCGTATTAACATCCACTACTGGG
ECT2-CAS9-gRNA4-B	AAACCCAGTAGTGGATGTTAATAC
ECT2-CAS9-gRNA5-T	CACCGTAAAGTACTGAGATTCCA
ECT2-CAS9-gRNA5-B	AAACTGGAAATCTCAGTAACTTTAC
ECT2-CAS9-gRNA6-T	CACCGATCACTCTTGTTTCAATCTG
ECT2-CAS9-gRNA6-B	AAACCAGATTGAAACAAGAGTGATC
ECT2-F	GGAGGACTAGCTTGGCAGAC
ECT2-R	TCCGATTTTCCAGGACAACCTT
GAPDH-F	CGGGTTATGCTGGTTTAGG
GAPDH-R	TGTACCGACTCTTGCCCTT