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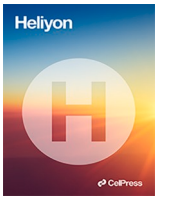

Research article

OIP5-AS1 enhances the malignant characteristics and resistance to chemotherapy of pancreatic cancer cells by targeting miR-30d-5p/MARCH8

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ABSTRACT

MARCH8, an E3 ubiquitin ligase, plays a crucial role in regulating various cellular processes such as protein degradation and signaling pathways and is implicated in the development and spread of pancreatic cancer. Analysis of pancreatic cancer tissues compared to adjacent normal tissues showed a decrease in miRNA-30d-5p levels and an increase in OIP5-AS1 and MARCH8 levels, as confirmed by qRT-PCR and Western blot analysis. The dual-luciferase reporter assay demonstrated a binding relationship between OIP5-AS1 and miRNA-30d-5p, as well as between miRNA-30d-5p and MARCH8 in PACN-1 cells, derived from a human pancreatic carcinoma specimen. Further investigations utilizing various assays revealed that OIP5-AS1 inhibited apoptosis and promoted cell proliferation, invasion, and migration in PACN-1 cells via the miRNA-30d-5p/MARCH8 axis *in vitro*. Tumor experiments in nude mice confirmed that OIP5-AS1 enhanced PACN-1 cell growth *in vivo* through the miRNA-30d-5p/MARCH8 axis. Additionally, OIP5-AS1 was found to activate downstream genes of the JAK-STAT pathway, namely IFNAR2, SOCS3, and JAK1, in PACN-1 cells. Furthermore, OIP5-AS1 increased the IC50 values for doxorubicin, gemcitabine, and cisplatin in PACN-1 cells, as determined by the Cell Counting Kit-8 assay. Overall, OIP5-AS1 was shown to promote aggressive traits and resistance to chemotherapy in PACN-1 cells through the miRNA-30d-5p/MARCH8 axis.

1. Introduction

Pancreatic cancer is highly aggressive, with a generally poor prognosis due to its progressive development [1]. However, the risk factors for pancreatic cancer are sporadic, with no standard identifiable factors [2]. Therefore, early identification of pancreatic cancer

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is crucial. Membrane-associated ring finger (C3HC4) 8 (MARCH8) is the first mammalian membrane-associated ring-CH to be identified [3]. According to a study, pancreatic cancer cells that overexpressed MARCH8 experienced enhanced migration, proliferation, and invasion both *in vivo* and *in vitro* [4]. Researchers found that MARCH8 increased pancreatic cancer development and invasion by degrading PTPN4 and activating STAT3 phosphorylation [4]. Furthermore, through phosphatase and tensin homolog (PTEN) ubiquitination and degradation, MARCH8 was discovered to increase the growth, emigration, and cell cycle progression of hepatocellular carcinoma cells in another study [5]. However, research on MARCH8's biological role in pancreatic cancer is lacking. This pioneering study examined MARCH8's biological function in pancreatic cancer.

By directly binding to messenger RNA (mRNA) and preventing its expression, micro-ribonucleic acids (miRNAs) have been found to have a significant function in controlling pancreatic cancer [6]. Our use of bioinformatics analysis enabled us to determine that MARCH8 may be a miR-30d-5p target. A study found that miR-30d-5p negatively regulated ecto-5′-nucleotidase (CD73) to reduce prostate cancer cell invasion and progression [7] and targeted autophagy-related gene 5 (ATG5) to suppress renal cell carcinoma proliferation and autophagy [8]. However, miR-30d-5p′s effect on pancreatic cancer cells was poorly understood.

Additionally, the bioinformatics study assisted in identifying a long non-coding RNA (LncRNA), Opa interacting protein 5 antisense RNA 1 (OIP5-AS1), as a potential miR-30d-5p target. According to one study, the miR-342–3p/protein kinase B (AKT)/extracellular signal-regulated kinase (ERK) axis facilitated the proliferation of pancreatic cancer cells [9] and promoted the malignant growth of pancreatic ductal adenocarcinoma via controlling the miR-429/forkhead box D1/ERK axis [10]. Thus, OIP5-AS1 may activate the miR-30d-5p/MARCH8 axis to cause pancreatic cancer malignancy.

Chemotherapy is an important part of comprehensive treatment for pancreatic cancer, but chemoresistance is inevitable due to its high heterogeneity [11]. Dealing with multidrug resistance in pancreatic cancer is a major hurdle in clinical treatment, creating effective methods to improve the sensitivity of pancreatic carcinoma cells to chemotherapy is widely recognized as a key approach to advancing future treatments [12]. Therefore, finding ways to facilitate chemosensitivity has become the focus of clinical treatment. This study investigated how OIP5-AS1 affects pancreatic cancer's sensitivity to chemotherapy.

2. Materials and methods

2.1. Cell culture

Zhongqiao Xinzhou Biotechnology Co., Ltd. (Shanghai, China) provided the human PANC-1 cells, which are a commonly used cell line in pancreatic cancer research. These cells are derived from a human pancreatic carcinoma specimen and are widely utilized in studying various aspects of pancreatic cancer, such as cell growth, differentiation, chemoresistance, and signaling pathways [13]. The PANC-1 cells were cultured in Dulbecco's Modified Eagle Medium (Novizan Biotechnology Co., Ltd., Shanghai, China) with 5 % (vol/vol) CO2 in a humidified chamber at 37 °C. The medium also contained 10 % fetal bovine serum, 1 % penicillin, and 1 % streptomycin, all provided by Novizan Biotechnology Co., Ltd.

2.2. Human tissues

The human tissue samples were sourced from the Fudan University Shanghai Cancer Center. The samples consisted of 10 pancreatic cancer tissues and 10 paired nearby non-cancerous tissues. All patients provided their informed consent, and the Research Ethics Committee at Fudan University Shanghai Cancer Center granted approval for the study.

2.3. Bioinformatics analysis

TargetScan (http://www.targetscan.org/vert_72/) was used to predict the binding sites for miR-30d-5p and OIP5-AS1. Additionally, the starBase website (http://starbase.sysu.edu.cn/) predicted binding sites for MARCH8 and miR-30d-5p. The correlation between OIP5-AS1, interferon alpha and beta receptor subunit 2 (IFNAR2), suppressor of cytokine signaling 3 (SOCS3), janus kinase 1 (JAK1) were conducted using the Pearson correlation test based on The Cancer Genome Atlas (TCGA) pancreatic cancer dataset (https://portal.gdc.cancer.gov/).

2.4. Cell transfection

Lipofectamine 2000 (Thermo Fisher Scientific, Cleveland, OH, USA) was used to transfect cells on 6-well plates (Beyotime, Shanghai, China). The cells were then divided into various groups including small interfering RNA (siRNA) negative control (NC), overexpressing (OE) NC, OIP5-AS1-OE, siRNA-OIP5-AS1, mimic NC, miR-30d-5p mimic, inhibitor NC, miR-30d-5p-inhibitor, siMARCH8, and their corresponding control vector groups. In the miR-30d-5p-inhibitor + siMARCH8 group, cells were treated with both miR-30d-5p-inhibitor and siMARCH8. All plasmids and siRNAs used in this study were supplied by Guangdong Ruibo Biotechnology Co., Ltd. (Guangzhou, Guangdong, China) (Supplementary Table 1).

2.5. Wound healing test

Initially, a 6-well plate was inoculated with 2×10^6 PANC-1 cells. Once the cell fusion percentage reached 100 %, sterilized scratching needles were used to create scratches on the cells, which were then washed with phosphate-buffered saline (PBS, Sigma

Aldrich Inc., St. Louis, MO, USA). Wound healing was recorded using a microscope (Olympus IX71, Olympus, Japan) at 0 h and 48 h.

2.6. 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) test

The MTT assay was used to assess cell viability. A total of 3000–5000 cells per 96-well plate were grown overnight. After removing the medium, 100 μL of 500 μg/mL MTT reagent (Sigma Aldrich Inc.) was added to each well. The cells were then incubated at 37 °C for 4 h. Following this, the culture was treated with a 20 % sodium dodecyl sulfate (SDS, 100 μL) solution and incubated for an additional 20 h. Finally, the absorbance at 570 nm was determined using a microplate reader (SpectraMax M2e, Molecular Devices, USA).

2.7. Quantitative real-time polymerase chain reaction (qRT-PCR)

After isolating total RNA from cells using the TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA) was used to produce complementary deoxyribonucleic acid (cDNA). SYBR Premix EX TaqTMII (Takara, Japan) was used for PCR. The relative expression was calculated using the $2-\Delta\Delta Ct$ method. Supplementary Table 2 contains a list of primers used in this investigation.

2.8. Western blot (WB)

To facilitate the extraction of cytoplasmic and nuclear proteins, tissues or cells were lysed following the manufacturer's instructions using a Cytoplasmic and Nuclear Protein Enrichment reagent (AMRESCO, Chicago, IL, USA). The Bradford method (Bio-Rad Laboratories) was utilized to quantify the protein with bovine serum albumin (BSA) (Sigma Aldrich Inc.) serving as the standard. On 12 % SDS-polyacrylamide gels, total cellular proteins and cytoplasmic and nuclear protein fractions (100 μg protein/lane) were resolved. Subsequently, the proteins were transferred to polyvinylidene fluoride (PVDF) membranes manufactured by Sigma Aldrich Inc., where they were immunolabeled with specific primary antibodies: α-tubulin (1:3000, ab7291, Abcam, Cambridge, MA, USA), Bax (1:2000, ab32503, Abcam), Bcl-2 (1:2000, ab182858, Abcam), cyclin-dependent kinase 4 (CDK4) (1:1800, ab108357, Abcam), cyclin D1 (1:2000, ab16663, Abcam), IFNAR2 (1:1500, ab190664, Abcam), SOCS3 (1:2000, ab236519, Abcam), JAK1 (1:2000, ab133666, Abcam), and MARCH8 (1:2500, ab79054, Abcam). These were then incubated at room temperature for an hour in 1 % BSA. Moreover, secondary antibodies (Sigma Aldrich Inc.) labeled with horseradish peroxidase (HRP) were used to incubate PVDF membranes. HRP-immunolabeled proteins were detected using Enhanced Chemiluminescence (Thermo Scientific, Wilmington, DE, USA). Group integrated density values (IDV) were calculated using ImageJ (https://imagej.nih.gov). Data normalization involved dividing the corrected IDV of proteins in each group by the corrected IDV of α-tubulin protein in the corresponding group.

2.9. Dual-luciferase report

Bioinformatics was utilized to predict the locations of promoter transcription factor binding sites. Lipofectamine 2000 (Invitrogen Life Technologies) was used to co-transfect 200 ng of OIP5-AS1-wt, mut, MARCH8-wt (Sangon Biotech, Shanghai, China), or mut into PANC-1 cells. The same method was employed to transfect PANC-1 cells with NC and miR-30d-5p-mimic. Following 48 h of transfection, the relative luciferase activity was normalized to Renilla luciferase activity. Three sets of transfections were performed.

2.10. Animal experiments

Shanghai Bikai Biotechnology Co., Ltd. (Shanghai, China) provided BALB/c-nude mice that were 4–5 weeks old, with an equal distribution of male and female mice. The mice were injected subcutaneously with 5×10^7 PANC-1 cells/0.2 mL using a serum-free medium. Tumor volumes were measured using a caliper, and formula $V = \pi(\text{length} \times \text{width}^2)/6$ was used to calculate the volumes. 16 nude mice were divided into four groups: control, NC, OIP5-AS1-OE, and siOIP5-AS1 when the tumor volume reached around 300 mmˆ3. Additionally, 24 nude mice were randomly assigned to six groups: NC, control, miR-30d-5p mimic, miR-30d-5p inhibitor, siMARCH8, and miR-30d-5p inhibitor + siMARCH8. After 45 days, the mice were euthanized via spinal dislocation, and the tumors were extracted, weighed, and frozen in liquid nitrogen. Group differences were analyzed using a one-way analysis of variance (ANOVA). Approval for all animal experiments was obtained from Fudan University's Animal Ethics Committee.

2.11. Apoptosis assay using flow cytometry

After being collected into a 10 mL centrifuge tube, approximately 2×10^6 PANC-1 cells were centrifuged for 5 min at 500–1000 rpm. The cells then underwent a 10–15 min dark incubation period at room temperature after being resuspended in 100 μL of the labeling solution. A fluorescent (SA-FLOUS) solution from Origin Biosciences Inc., Nanjing, Jiangsu, China, was added to the cells, which were then incubated for 20 min at 4 °C, avoiding light and agitating several times. Propidium iodide (PI) was detected at a wavelength of 560 nm, and fluorescein isothiocyanate (FITC) fluorescence was detected at 515 nm using Facscalibur from BD Biosciences in San Jose, CA, USA.

2.12. TdT-mediated dUTP nick-end labeling (TUNEL) apoptosis assay

The PANC-1 cells, extracted from tumor tissue in the tumor-burdened model, were utilized for TUNEL detection. The tumor tissue was sliced into 5 μm thick sections. After an hour of incubation at 37 °C with a 50 μL TUNEL combination (Guangdong Ruibo Biotechnology Co., Ltd., China) consisting of 50 μL enzyme solution and 450 μL marker solution, the tumor sections were stained with α-actin and 4′,6-diamidino-2-phenylindole (DAPI). The sections were then examined under a fluorescence microscope. The apoptosis rate was calculated by dividing the number of apoptotic cells (red) by the total number (blue) and multiplying by 100 %. All materials were sourced from the same company (Guangdong Ruibo Biotechnology Co., Ltd.).

2.13. Gene set enrichment analysis (GSEA)

The pancreatic cancer expression profile was obtained from TCGA. Samples were divided into high (N = 89) and low (N = 88) groups based on median OIP5-AS1 expression. GSEA was used to analyze the differences in pathways between the two groups. A false discovery rate (FDR) of $\leq$0.001 was applied as the screening criterion.

2.14. In vitro chemoresistance assay

PANC-1 cells were seeded in 96-well plates at a density of 1×10^5 cells/well. Each well was treated with a culture medium containing varying concentrations of doxorubicin (1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 μM, HY-15142A, MedChemExpress, Shanghai, China), gemcitabine (10, 20, 40, 80, 160, 320, 640, 1280, and 2560 nM, HY-17026, MedChemExpress), and cisplatin (10, 20, 40, 60, 80, 100, 120, 140, and 160 μM, HY-17394, MedChemExpress). After 48 h, Cell Counting Kit-8 (CCK-8) solution (10 μL per 100 μL of media in each well; Sigma Aldrich Inc.) was added, and the cells were incubated for an additional 2 h. Absorbance was measured using a Thermo Fisher Scientific microplate reader at 450 nm. IC50 values for cisplatin, doxorubicin, and gemcitabine were then calculated.

2.15. Data analysis

The statistical package for social sciences (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the data. T-test or one-way ANOVA was employed to examine the variations across the groups. All experiments were run at least three times, and the results are shown as mean ± standard deviation (SD). When $P < 0.05$, it was considered statistically significant.

3. Results

3.1. OIP5-AS1 sponged miR-30d-5p to promote MARCH8 expression

The RT-PCR analysis revealed that pancreatic tumor tissues exhibited higher levels of MARCH8 mRNA (Fig. 1A) and protein

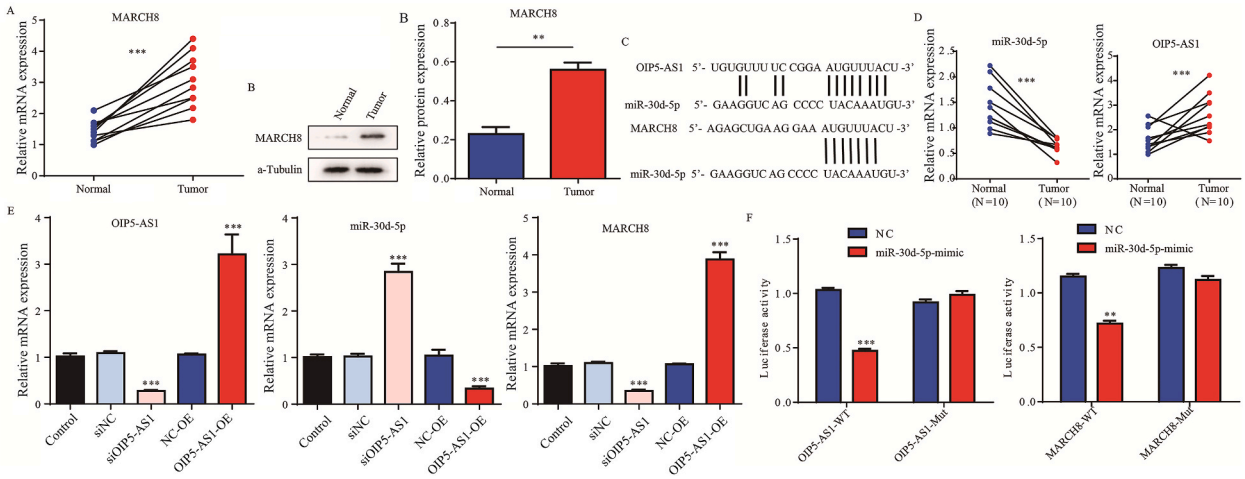

Fig. 1. OIP5-AS1 promoted the expression of MARCH8 by sponging miR-30d-5p *in vitro*. (A) RT-PCR and (B) WB were used to detect the expression of MARCH8 in pancreatic tumor tissues and adjacent normal tissues. (C) The binding sites of OIP5-AS1, miR-30d-5p, and MARCH8 were predicted using the miRWalk (http://mirwalk.umm.uni-heidelberg.de/) database. (D) The figure represents the expression of OIP5-AS1 and miR-30d-5p in pancreatic tumor tissues and adjacent normal tissues. (E) RT-PCR was used to detect the effect of OIP5-AS1-OE and siOIP5-AS1 on the expression of miR-30d-5p and MARCH8. (F) Dual-luciferase report assays were used to detect the combination of OIP5-AS1, miR-30d-5p, and MARCH8. SiNC: siRNA negative control; siOIP5-AS1: siRNA-OIP5-AS1; NC-OE: overexpression negative control; OIP5-AS1-OE: OIP5-AS1 overexpression; WT: wild type; Mut: mutation. Data are presented as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

(Fig. 1B) expression compared to the adjacent normal tissues. Bioinformatics predictions indicated that OIP5-AS1, miR-30d-5p, and MARCH8 share binding sites, suggesting that lncRNA OIP5-AS1 may upregulate MARCH8 expression by acting as a competitive endogenous RNA (ceRNA) for miR-30d-5p (Fig. 1C). Furthermore, RT-PCR analysis demonstrated significantly increased expression of OIP5-AS1 and decreased levels of miR-30d-5p mRNA in pancreatic tumor tissues versus adjacent normal tissues (Fig. 1D), implicating OIP5-AS1 in the suppression of miR-30d-5p expression and subsequent promotion of MARCH8 expression. To validate these findings, siRNA and plasmids were used to manipulate OIP5-AS1 expression in PACN-1 cells (Fig. 1E). The RT-PCR results in Fig. 1E indicated that silencing OIP5-AS1 in PACN-1 cells led to increased miR-30d-5p expression and decreased MARCH8 expression, whereas overexpression of OIP5-AS1 resulted in decreased miR-30d-5p and increased MARCH8 expression. Luciferase reporter assays confirmed direct binding between OIP5-AS1/miR-30d-5p and miR-30d-5p/MARCH8 in the OIP5-AS1 and MARCH8 mutants (Fig. 1F), supporting the notion that OIP5-AS1 acts as a sponge for miR-30d-5p to enhance MARCH8 expression. Overall, these results suggest that OIP5-AS1 may play a role in regulating MARCH8 expression through miR-30d-5p sponging.

3.2. *OIP5-AS1 promoted the carcinogenic characteristics of PACN-1 cells in vitro*

In the MTT assay, silencing OIP5-AS1 (siOIP5-AS1) decreased PACN-1 cell proliferation, while overexpression (OIP5-AS1-OE) enhanced it (Fig. 2A). Transwell assays demonstrated that OIP5-AS1-OE promoted PACN-1 cell invasion. Conversely, siOIP5-AS1 decreased the invading ability of PACN-1 cells (Fig. 2B). Results from scratch experiments showed that siOIP5-AS1 decreased the migrating ability of PACN-1 cells, while OIP5-AS1-OE encouraged PACN-1 cell migration (Fig. 2C). Flow cytometry apoptosis experiments indicated that siOIP5-AS1 promoted PACN-1 cell apoptosis, whereas OIP5-AS1-OE inhibited it (Fig. 2D). Western blot experiments demonstrated that siOIP5-AS1 inhibited the protein expression of Bcl-2, cyclin D1, and CKD4 in PACN-1 cells, and promoted the protein expression of Bax in PACN-1 cells (Fig. 2E). Conversely, OIP5-AS1-OE had the opposite effect on the protein expression profile (Fig. 2E). Overall, these results suggest that OIP5-AS1 facilitates PACN-1 cell invasion and migration while preventing apoptosis.

3.3. *miR-30d-5p inhibited the carcinogenic characteristics of PACN-1 cells in vitro by down-regulating MARCH8*

The MTT assays demonstrated that silencing MARCH8 (siMARCH8) and overexpressing miR-30d-5p (miR-30d-5p mimic) decreased PACN-1 cell proliferation, while silencing miR-30d-5p (miR-30d-5p inhibitor) increased PACN-1 cell proliferation (Fig. 3A). The difference between NC v. s. miR-30d-5p mimic group, NC v. s. miR-30d-5p inhibitor group, NC v. s. siMARCH8 group, miR-30d-5p inhibitor group v. s. miR-30d-5p inhibitor group + siMARCH8 group, siMARCH8 group v. s. miR-30d-5p inhibitor group + siMARCH8 group were statistically analyzed. In comparison to the miR-30d-5p inhibitor group, PACN-1 cell proliferation significantly decreased in the siMARCH8 + miR-30d-5p inhibitor group (Fig. 3A). The miR-30d-5p inhibitor + siMARCH8 group showed an increase in PACN-1 cell proliferation compared to siMARCH8 alone (Fig. 3A). PACN-1 cell invasion was significantly reduced in Transwell assays in the

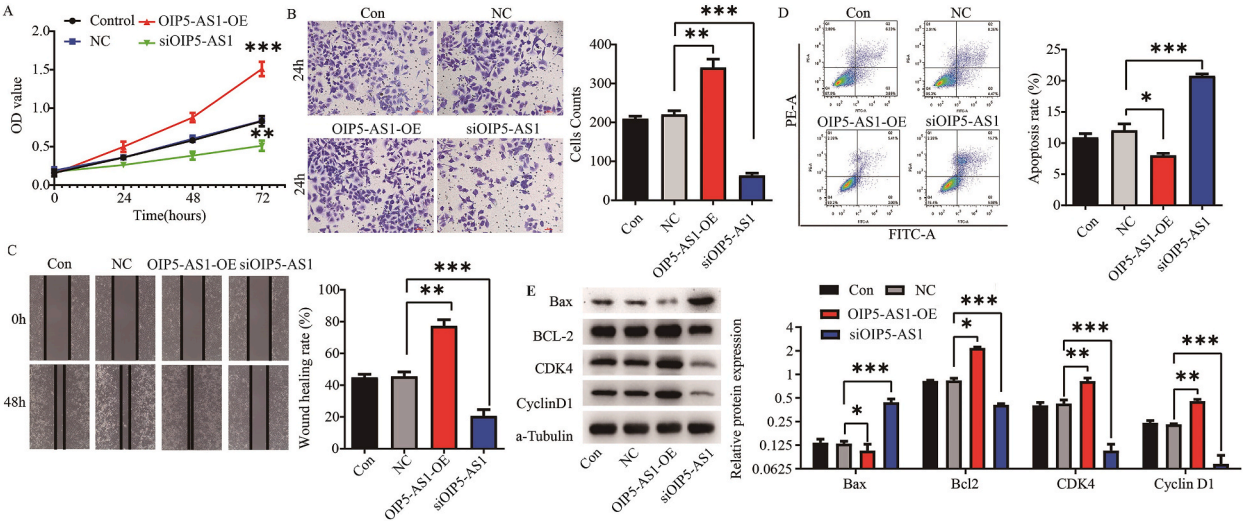

Fig. 2. OIP5-AS1 promoted the malignant characteristics of PACN-1 cells *in vitro*. (A) MTT assay was used to detect the effect of OIP5-AS1-OE and siOIP5-AS1 on the proliferation of PACN-1 cells *in vitro*. (B) Transwell assay was used to detect the effect of OIP5-AS1-OE and siOIP5-AS1 on the invasion of PACN-1 cells *in vitro*. (C) Wound healing assay was used to detect the effect of OIP5-AS1-OE and siOIP5-AS1 on the migration of PACN-1 cells *in vitro*. (D) Flow cytometry apoptosis experiments were conducted to detect the effect of OIP5-AS1-OE and siOIP5-AS1 on the apoptosis of PACN-1 cells *in vitro*. (E) WB experiments were conducted to detect the effect of OIP5-AS1-OE and siOIP5-AS1 on the expression of Bax, Bcl-2, CDK4, and cyclin D1 *in vitro*. Con: control; NC: negative control; siOIP5-AS1: siRNA-OIP5-AS1; OIP5-AS1-OE: OIP5-AS1 overexpression; CDK4: cyclin-dependent kinase 4. Data are presented as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

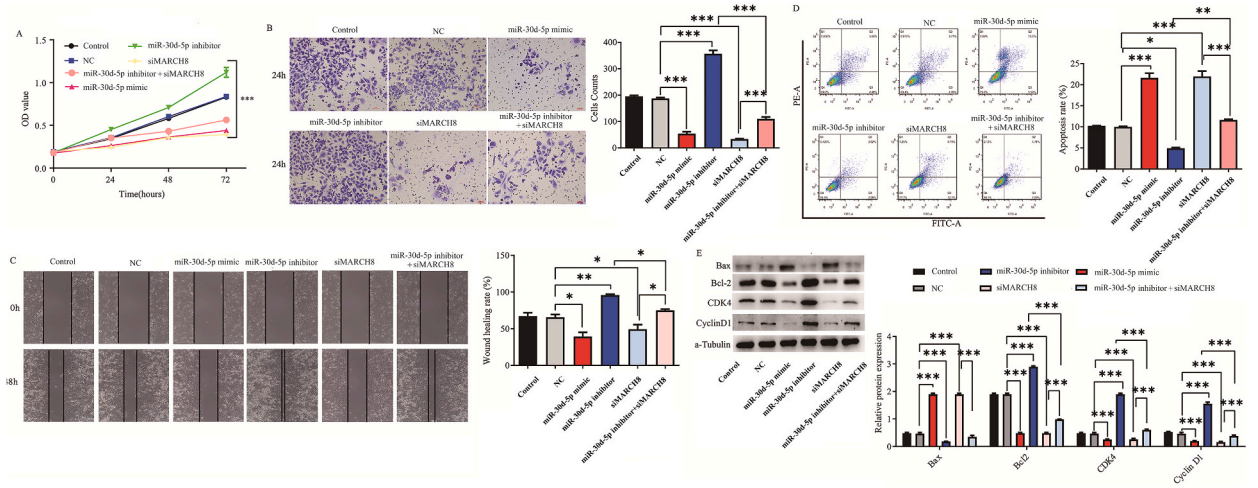

Fig. 3. miR-30d-5p inhibited the carcinogenic properties of PACN-1 cells through MARCH8 *in vitro*. (A) MTT assay was used to detect the effect of miR-30d-5p mimic, miR-30d-5p inhibitor, and siMARCH8 on the proliferation of PACN-1 cells *in vitro*. (B) Transwell assay was used to detect the effect of miR-30d-5p mimic, miR-30d-5p inhibitor, and siMARCH8 on the invasion of PACN-1 cells *in vitro*. (C) Wound healing assay was used to detect the effect of miR-30d-5p mimic, miR-30d-5p inhibitor, and siMARCH8 on the migration of PACN-1 cells *in vitro*. (D) Flow cytometry apoptosis experiments were conducted to detect the effect of miR-30d-5p mimic, miR-30d-5p inhibitor, and siMARCH8 on the apoptosis of PACN-1 cells *in vitro*. (E) WB experiments were conducted to detect the effect of miR-30d-5p mimic, miR-30d-5p inhibitor, and siMARCH8 on the expression of Bax, Bcl-2, CDK4, and cyclin D1 *in vitro*. WB: Western blot; siMARCH8: siRNA- MARCH8. Data are presented as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

miR-30d-5p mimic and siMARCH8 groups compared to NC. The miR-30d-5p inhibitor group also exhibited a reduction in PACN-1 cell invasion compared to NC (Fig. 3B). Additionally, scratch experiments revealed that the wound healing rates of PACN-1 cells were significantly reduced in the group overexpressing miR-30d-5p (miR-30d-5p mimic) or suppressing MARCH8 (siMARCH8) compared to NC. Similarly, the PACN-1 cells in the miR-30d-5p inhibitor group had significantly worse wound healing rates compared to NC (Fig. 3C).

Flow cytometry experiments showed that siMARCH8 and miR-30d-5p mimic significantly accelerated the apoptosis of PACN-1 cells compared to NC. On the other hand, PACN-1 cell apoptosis was significantly suppressed by miR-30d-5p inhibitor compared to NC (Fig. 3D). Furthermore, miR-30d-5p inhibitor + siMARCH8 reversed the effects of miR-30d-5p inhibitor and siMARCH8 on PACN-1 cell apoptosis (Fig. 3D, miR-30d-5p inhibitor group v. s. miR-30d-5p inhibitor group + siMARCH8 group, siMARCH8 group v. s. miR-30d-5p inhibitor group + siMARCH8 group). WB experiments indicated that siMARCH8 and miR-30d-5p mimic increased the ratio of Bax/Bcl-2 in PACN-1 cells, but decreased the protein expression of cyclin D1 and CKD4 compared to NC (Fig. 3E). siMARCH8 counteracted the effects of miR-30d-5p inhibitor on cyclin D1, CKD4, and Bax/Bcl-2 (miR-30d-5p inhibitor group v. s. miR-30d-5p inhibitor group + siMARCH8 group), while miR-30d-5p inhibitor reversed siMARCH8's action on these proteins (Fig. 3E, siMARCH8 group v. s. miR-30d-5p inhibitor group + siMARCH8 group). Overall, by down-regulating MARCH8, miR-30d-5p enhanced apoptosis in PACN-1 cells and prevented their invasion and migration ability.

3.4. OIP5-AS1 promoted the carcinogenic characteristics of PACN-1 cells through the miR-30d-5p/MARCH8 axis in vivo

Firstly, the results of subcutaneous tumor burdens have shown that overexpressing OIP5-AS1 (OIP5-AS1-OE) stimulated the growth of PACN-1 cells, while silencing OIP5-AS1 (siOIP5-AS1) decreased PACN-1 tumor growth (Fig. 4A). Overexpressing miR-30d-5p (mimic) and silencing MARCH8 (siMARCH8) reduced PACN-1 tumor growth, while silencing miR-30d-5p (inhibitor) enhanced it (Fig. 4B). The difference between NC v. s. miR-30d-5p mimic group, NC v. s. miR-30d-5p inhibitor group, NC v. s. siMARCH8 group, miR-30d-5p inhibitor group v. s. miR-30d-5p inhibitor group + siMARCH8 group, siMARCH8 group v. s. miR-30d-5p inhibitor group + siMARCH8 group were statistically analyzed. The group treated with both the miR-30d-5p inhibitor and siMARCH8 exhibited slower PACN-1 cell proliferation compared to the group treated only with the miR-30d-5p inhibitor (Fig. 4B). Moreover, flow cytometry experiments demonstrated that overexpressing OIP5-AS1 and silencing miR-30d-5p inhibited PACN-1 cell apoptosis *in vivo* (Fig. 4C), whereas silencing OIP5-AS1, silencing MARCH8, and overexpressing miR-30d-5p promoted PACN-1 cell apoptosis *in vivo* (Fig. 4C). The difference between NC v. s. OIP5-AS1-OE group, NC v. s. siOIP5-AS1 group, NC v. s. miR-30d-5p mimic group, NC v. s. miR-30d-5p inhibitor group, NC v. s. siMARCH8 group, siMARCH8 group v. s. miR-30d-5p inhibitor group + siMARCH8 group were statistically analyzed. Compared to the siMARCH8 group, the combination treatment of miR-30d-5p inhibitor and siMARCH8 resulted in a reduction in PACN-1 cell apoptosis (Fig. 4C, siMARCH8 group v. s. miR-30d-5p inhibitor group + siMARCH8 group). In conclusion, OIP5-AS1 facilitated the carcinogenic characteristics of PACN-1 cells *in vivo* through the miR-30d-5p/MARCH8 axis.

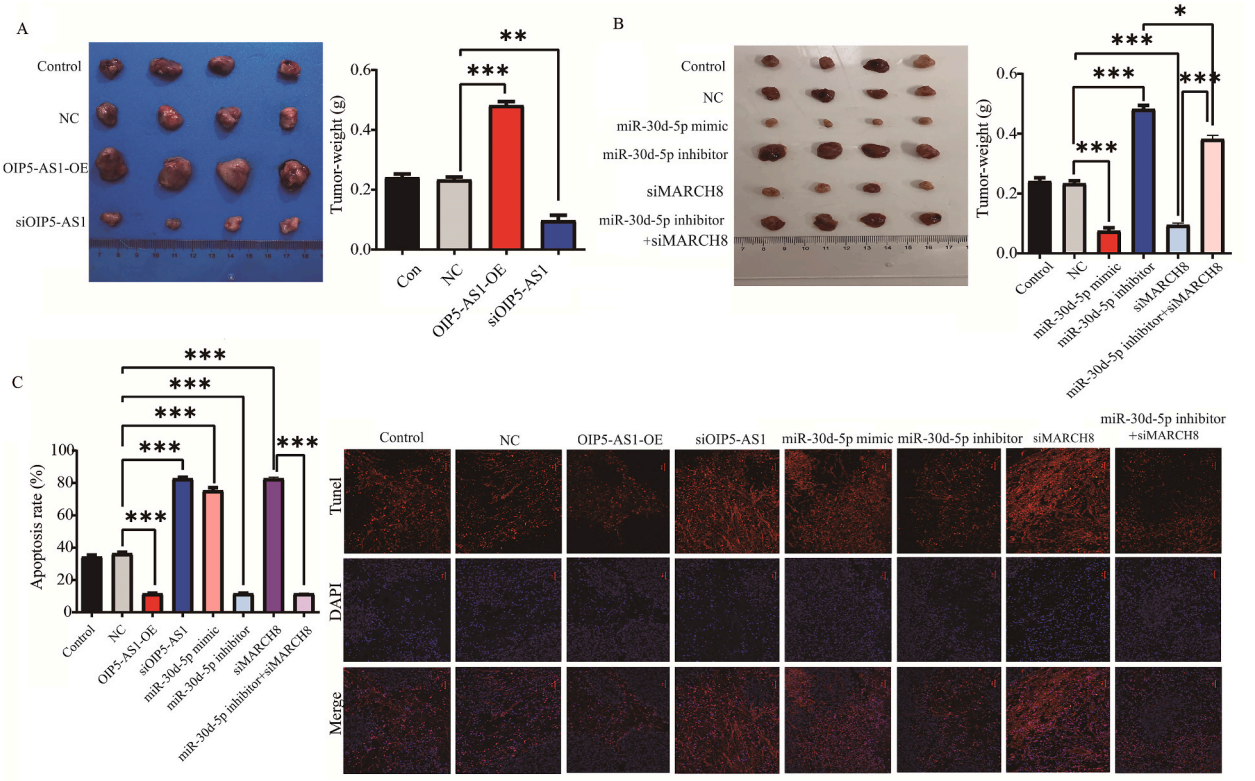

Fig. 4. OIP5-AS1 promoted the malignant characteristics of PACN-1 cells through the miR-30d-5p/MARCH8 axis *in vivo*. (A) Tumor-bearing experiments were conducted to detect the effect of OIP5-AS1-OE and siOIP5-AS1 on the growth of PACN-1 cells *in vivo*. (B) Tumor-bearing experiments were conducted to detect the effect of miR-30d-5p mimic, miR-30d-5p inhibitor, and siMARCH8 on the growth of PACN-1 cells *in vivo*. (C) Flow cytometry was used to detect the effect of miR-30d-5p mimic, miR-30d-5p inhibitor, and siMARCH8 on the apoptosis of PACN-1 cells *in vivo*. Con: control; NC: negative control; siOIP5-AS1: siRNA-OIP5-AS1; OIP5-AS1-OE: OIP5-AS1 overexpression. Data are presented as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

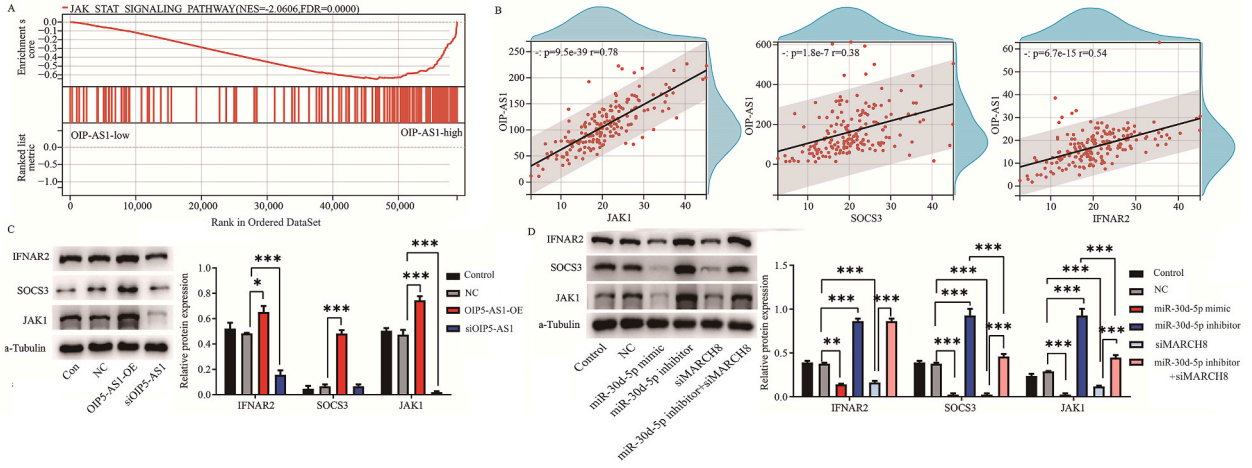

Fig. 5. OIP5-AS1 activated the JAK-STAT signaling pathway through the miR-30d-5p/MARCH8 axis *in vitro*. (A) GSEA assay was used to identify the JAK-STAT signaling pathway between OIP5-AS1-low expression (N = 88) and OIP5-AS1-high expression (N = 89) groups. (B) The correlation between OIP5-AS1 and IFNAR2, SOCS3, and JAK1 was calculated using the Pearson correlation test. (C) WB experiments were conducted to detect the effect of OIP5-AS1-OE and siOIP5-AS1 on the expression of IFNAR2, SOCS3, and JAK1 *in vitro*. (D) WB experiments were conducted to detect the effect of miR-30d-5p mimic, miR-30d-5p inhibitor, and siMARCH8 on the expression of IFNAR2, SOCS3, and JAK1 *in vitro*. IFNAR2: interferon alpha and beta receptor subunit 2; SOCS3: suppressor of cytokine signaling 3; JAK1: Janus kinase 1. Data are presented as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

3.5. OIP5-AS1 activated the JAK-STAT signaling pathway through the miR-30d-5p/MARCH8 axis in vitro

The JAK-STAT signaling pathway displayed a significant difference between OIP5-AS1 low expression (N = 88) and OIP5-AS1 high expression (N = 89) groups, as determined by a GSEA assay (Fig. 5A), and the top three downstream genes of the pathway, *IFNAR2*, *SOCS3*, and *JAK1*, were selected. A correlation analysis revealed that OIP5-AS1, *IFNAR2*, *SOCS3*, and *JAK1* were significantly and positively correlated (Fig. 5B). In WB experiments, siOIP5-AS1 decreased PACN-1 cell *IFNAR2*, *SOCS3*, and *JAK1* expression. Conversely, PACN-1 cells exhibited increased expression of *IFNAR2*, *SOCS3*, and *JAK1* in response to OIP5-AS1 overexpression (OIP5-AS1-OE) (Fig. 5C). Further, WB experiments revealed that miR-30d-5p mimic and siMARCH8 substantially inhibited the expression of *IFNAR2*, *SOCS3*, and *JAK1* in PACN-1 cells. On the other hand, PACN-1 cells exhibited a significant up-regulation of *IFNAR2*, *SOCS3*, and *JAK1* expression when treated with miR-30d-5p inhibitor (Fig. 5D). The difference between NC v. s. miR-30d-5p mimic group, NC v. s. miR-30d-5p inhibitor group, NC v. s. siMARCH8 group, siMARCH8 group v. s. miR-30d-5p inhibitor group + siOIP5-AS18 group, miR-30d-5p inhibitor group v. s. miR-30d-5p inhibitor group + siMARCH8 group, were statistically analyzed. Moreover, siOIP5-AS1 restored the stimulatory effect of miR-30d-5p inhibitor on *SOCS3* and *JAK1* (Fig. 5D, miR-30d-5p inhibitor group v. s. miR-30d-5p inhibitor group + siMARCH8), and miR-30d-5p inhibitor restored the suppression caused by siMARCH8 on *SOCS3* and *JAK1* (Fig. 5D, siMARCH8 group v. s. miR-30d-5p inhibitor group + siMARCH8 group). In conclusion, OIP5-AS1 partly activated the JAK-STAT pathway via the miR-30d-5p/MARCH8 axis.

3.6. OIP5-AS1 inhibited the sensitivity of PACN-1 cells to chemotherapy via the miR-30d-5p/MARCH8 axis in vitro

Cisplatin is a platinum-based chemotherapy drug that functions by forming DNA cross-links, resulting in DNA damage and ultimately cell death [14]. It is commonly employed in the treatment of various cancers, including pancreatic cancer. By subjecting PACN-1 cells to cisplatin, we can assess the cells' responsiveness to DNA-damaging agents and evaluate their sensitivity to platinum-based chemotherapy. Gemcitabine, a nucleoside analog, hinders DNA synthesis, triggering cell cycle arrest and apoptosis [15]. This drug is a standard treatment for pancreatic cancer and is often utilized in combination with other chemotherapeutic agents. Testing the sensitivity of PACN-1 cells to gemcitabine can offer valuable insights into their reaction to nucleoside analogs. Doxorubicin, an anthracycline chemotherapy medication, operates by intercalating DNA and inhibiting topoisomerase II, resulting in DNA damage and apoptosis [16]. Although it is not frequently used as a first-line treatment for pancreatic cancer, evaluating the sensitivity of PACN-1 cells to doxorubicin can yield crucial information about their response to anthracycline-based chemotherapy. The OIP5-AS1-OE group exhibited higher IC50 values for cisplatin (95.43 μM vs. 48.6 μM), gemcitabine (158.5 μM vs. 104.1 μM), and doxorubicin (4.382 μM vs. 2.407 μM) compared to the NC group (Fig. 6). Additionally, the OIP5-AS1-OE + miR-30d-5p mimic group and OIP5-AS1-OE + siMARCH8 group both displayed lower IC50 values for cisplatin (76.34 μM, 41.07 μM vs. 95.43 μM), gemcitabine (73.51 μM, 42.32 μM vs. 158.5 μM), and doxorubicin (2.968 μM, 2.432 μM vs. 4.382 μM) when compared to the OIP5-AS1-OE group. Overall, these findings suggest that OIP5-AS1 inhibits the chemosensitivity of PACN-1 cells to cisplatin, doxorubicin, and gemcitabine through the miR-30d-5p/MARCH8 axis.

4. Discussion

Several studies reported that OIP5-AS1 exerts carcinogenic effects in gastric cancer [17], pancreatic cancer [7], breast cancer [18], ovarian cancer [19], and other tumor types [20,21]. Through the miR-153–3p/ZBTB2 axis, OIP5-AS1 increased gastric cancer cell proliferation. A dual-luciferase reporter assay was used to validate the interaction between miR-153–3p and ZBTB2 or OIP5-AS1 [17]. LncRNA OIP5-AS1 regulates miR-216a-5p/glyoxalase 1 (GLO1), which advances the development of breast cancer [18]. Moreover, through ZNF217 and miR-137 signaling, OIP5-AS1 modulates ovarian cancer progression. It increased ZNF217 expression by sponging miR-137 as a competitive endogenous RNA (ceRNA) [19]. The principle of ceRNA is that there is a mutual regulation relationship among mRNA, lncRNA, and miRNA in cells [22]. When two or more RNAs share the same miRNA binding sites, they compete with each other to bind to miRNA, thereby influencing the target selection and downstream regulatory effects of miRNA [23]. In the present study, we found that lncRNA OIP5-AS1 increased the MARCH8 expression by sponging miR-30d-5p as a ceRNA. When lncRNA OIP5-AS1 binds to miR-30d-5p, they reduce the possibility of miRNA binding to other MARCH8 RNA, thereby affecting the expression

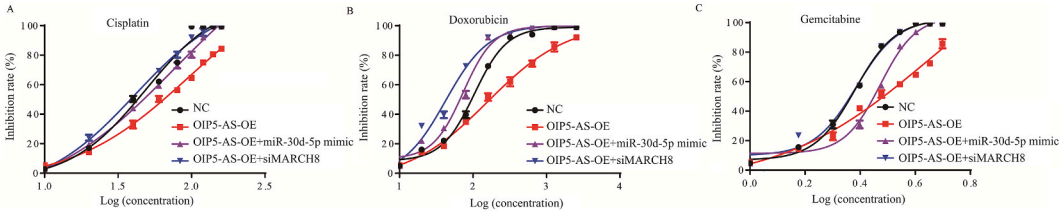

Fig. 6. OIP5-AS1 inhibited the sensitivity of PACN-1 cells to chemotherapy *in vitro*. CCK-8 was used to detect IC50 of PACN-1 cells in each group. (A) the inhibition rate of PACN-1 cells to cisplatin treatment. (B) the inhibition rate of PACN-1 cells to doxorubicin treatment. (C) the inhibition rate of PACN-1 cells to gemcitabine treatment. IFNAR2: interferon alpha and beta receptor subunit 2; SOCS3: suppressor of cytokine signaling 3; JAK1: Janus kinase 1. Data are presented as the mean ± SD.

of MARCH8.

OIP5-AS1 was found to be up-regulated in both pancreatic ductal adenocarcinoma (PDAC) tissues and cell lines [10]. It facilitated epithelial-to-mesenchymal transition (EMT), migration, and cell proliferation by activating the ERK pathway using sponging miR-429, which inhibited forkhead box D1 (FOXD1) expression [10]. The miR-422a/anoctamin-1 axis was targeted by the LncRNA OIP5-AS1, which promoted gastric cancer cell proliferation [24]. Another study showed that LncRNA OIP5-AS1 regulated G1 phase progression and promoted the proliferation of H1299, A549, FaDu, and CAL27 cells [25]. By means of the RT-PCR assay, the current investigation identified a significant up-regulation of OIP5-AS1 in pancreatic cancer tissues relative to adjacent normal tissues. *In vitro* experiments showed that overexpressing OIP5-AS1 increased PACN-1 cell invasion, migration, and proliferation and decreased their apoptosis. PACN-1 cell proliferation was enhanced and apoptosis was impeded, as demonstrated by *in vivo* experiments involving overexpression of OIP5-AS1. Therefore, OIP5-AS1 demonstrated a carcinogenic role in pancreatic cancer.

Dual-luciferase and RT-PCR assays demonstrated that OIP5-AS1 bound directly to miR-30d-5p, thereby inhibiting its expression. According to previous research, miR-30d-5p inhibits tumor formation and progression in non-small cell lung cancer [26], prostate cancer [7], and gallbladder cancer [27]. An RT-PCR assay was performed in this investigation, and the results indicated a significant down-regulation of miR-30d-5p in pancreatic cancer tissues relative to adjacent normal tissues. Overexpression of miR-30d-5p *in vitro* reduced PACN-1 cell growth, invasion, and migration while inducing apoptosis. *In vivo*, miR-30d-5p overexpression decreased PACN-1 cell growth. Therefore, OIP5-AS1 exhibited a carcinogenic effect in pancreatic cancer by inhibiting miR-30d-5p.

Dual-luciferase report and RT-PCR assays revealed that miR-30d-5p inhibited MARCH8 expression by directly binding to MARCH8. As determined using RT-PCR and WB, MARCH8 was significantly up-regulated in pancreatic cancer tissues relative to adjacent normal tissues. MARCH8 has been implicated in the growth of gastric cancer [28], esophageal cancer [3], and glioma [29], according to available reports. Therefore, this study hypothesizes that MARCH8 also exhibits a carcinogenic effect in pancreatic cancer.

Bax is a pro-apoptotic protein that promotes apoptosis by inducing mitochondrial outer membrane permeabilization, leading to the release of cytochrome *c* and activation of downstream caspases [30]. On the other hand, Bcl-2 is an anti-apoptotic protein that inhibits apoptosis by preventing mitochondrial outer membrane permeabilization and cytochrome *c* release [31]. By measuring the expression levels of Bax and Bcl-2, we can assess the balance between pro-apoptotic and anti-apoptotic signals in cells, providing valuable information about the apoptotic status of the cells. The increase in Bax expression and a decrease in Bcl-2 expression is indicative of apoptosis activation, whereas the decrease in Bax expression and an increase in Bcl-2 expression suggests inhibition of apoptosis. Our results showed that silencing OIP5-AS1 inhibited the protein expression of Bcl-2 and promoted the protein expression of Bax in PACN-1 cells, but OIP5-AS1 overexpression had the opposite result (Fig. 2E). We also revealed that silencing MARCH8 and overexpressing miR-30d-5p increased the ratio of Bax/Bcl-2 in PACN-1 cells. Silencing MARCH8 abolished the miR-30d-5p inhibitor's effects on Bax/Bcl-2, while silencing miR-30d-5p abolished siMARCH8's action on Bax/Bcl-2, indicating that the role of miR-30d-5p on apoptosis is dependent on MARCH8.

Cyclin D1 forms a complex with cyclin-dependent kinase 4 (CDK4) to regulate progression through the G1 phase of the cell cycle by phosphorylating and inactivating the retinoblastoma protein (Rb) [32]. By measuring the expression levels of cyclin D1 and CDK4, we can assess the progression of cells through the G1 phase and the commitment to enter the cell cycle. Monitoring the levels of cyclin D1 and CDK4 can provide valuable information about the cell cycle status and potential aberrations in cell cycle control. In the present study, it was found that silencing OIP5-AS1 inhibited the protein expression of cyclin D1 and CKD4 in PACN-1 cells, suggesting its effect on the cell cycle control. In addition, silencing MARCH8 and overexpressing miR-30d-5p decreased the protein expression of cyclin D1 and CKD4 in PACN-1 cells. silencing MARCH8 abolished the miR-30d-5p inhibitor's boosting effects on cyclin D1 and CKD4, while silencing miR-30d-5p abolished siMARCH8's inhibitory action on them, suggesting that by down-regulating MARCH8, miR-30d-5p regulated the cell cycle.

Functional assays demonstrated that MARCH8 knockdown significantly reduced PACN-1 cell migration, invasion, and proliferation while increasing apoptosis *in vitro*. MARCH8 knockdown also decreased PACN-1 cell proliferation *in vitro*. Therefore, this study is the first to report the carcinogenic effect MARCH8 plays in pancreatic cancer. The study uses cell functional gain-and-loss experiments to confirm that miR-30d-5p inhibits MARCH8, which in turn acts as a tumor suppressor. This study concludes that OIP5-AS1 enhances pancreatic cancer malignancy via miR-30d-5p/MARCH8.

Decades of research have shown that JAK-STAT signal transduction controls crucial cellular processes such as proliferation, invasion, survival, inflammation, and immunity [33]. This study found that OIP5-AS1 activated *JAK1*, *IFNAR2*, and *SOCS3* downstream genes via the miR-30d-5p/MARCH8 axis. Studies have reported that *JAK1* and *SOCS3* inhibited pancreatic cancer cell apoptosis [34,35] and enhanced their invasion, proliferation, and migration [36,37]. Consequently, OIP5-AS1 increased pancreatic cancer cell incidence and progression via activating *JAK1* and *SOCS3* via the miR-30d-5p/MARCH8 axis. The impact of *IFNAR2* regulation on pancreatic cancer cell activity remains unclear and requires additional investigation.

Cisplatin, doxorubicin, and gemcitabine are common pancreatic cancer treatments [38]. By examining the response of PACN-1 cells to these three chemotherapeutic agents with distinct mechanisms of action, we can obtain a comprehensive understanding of the cell's sensitivity to different classes of chemotherapy drugs. Some studies have revealed that lncRNAs and miRNAs played an important role in the chemosensitivity of these drugs [39–41]. For example, Qi et al. found that cancer-associated fibroblasts induce gemcitabine resistance in pancreatic cancer cells by secreting exosome-derived ACSL4-targeting miRNAs [42]. The present study utilized the CCK-8 assay and found that overexpressing OIP5-AS1 increased the IC50 of cisplatin, gemcitabine, and doxorubicin in PACN-1 cells, whereas silencing MARCH8 or overexpressing miR-30d-5p reversed the OIP5-AS1-induced IC50 increase in PACN-1 cells. Thus, OIP5-AS1 inhibited the sensitivity of PACN-1 cells to chemotherapy via the miR-30d-5p/MARCH8 axis.

The findings of this study illustrate the carcinogenic role that OIP5-AS1 plays in pancreatic cancer as well as the underlying mechanism via which it activates the JAK-STAT pathway's downstream genes via the miR-30d-5p/MARCH8 axis. Furthermore, OIP5-

AS1 increased PACN-1 cells' resistance to chemotherapy by utilizing the miR-30d-5p/MARCH8 axis. These findings imply that OIP5-AS1 uses the miRNA-30d-5p/MARCH8 axis to enhance the malignant traits and chemoresistance of PACN-1 cells. This finding has significant clinical implications for treating pancreatic cancer by identifying a potential target for mitigating the disease's aggressive behaviors. Future research avenues could focus on further elucidating the molecular mechanisms underlying the interaction between OIP5-AS1, miRNA-30d-5p, and MARCH8 in pancreatic cancer cells. Additionally, future studies could investigate the potential of targeting this pathway as a novel therapeutic strategy for overcoming chemoresistance in pancreatic cancer patients. The study's findings offer vital insights into the mechanisms that contribute to the malignant behaviors of pancreatic cancer cells and propose possible therapeutic opportunities to enhance treatment outcomes for patients with this deadly disease.

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Ethics approval

All procedures in this study involving human participants and animal experiments were performed following the ethical standards of the Institutional Ethics Committee of Fudan University Shanghai Cancer Center (No. SCC2021-013). A written informed consent was obtained from all individual participants included in the study.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Leilei Ying: Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Kening Li:** Writing – original draft, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Chao Chen:** Validation, Software, Methodology, Formal analysis. **Ying Wang:** Software, Resources, Data curation. **Qing Zhao:** Visualization, Validation, Software. **Yaohui Wang:** Visualization, Formal analysis, Data curation. **Lichao Xu:** Visualization, Resources, Conceptualization. **Haozhe Huang:** Visualization, Data curation. **Ge Song:** Visualization, Formal analysis. **Wentao Li:** Resources, Methodology. **Xinhong He:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Xinhong He reports financial support was provided by Natural Science Foundation of Shanghai Science and Technology Commission. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33835.

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