

Ciprofol suppresses proliferation, invasion and migration of human pancreatic cancer cells

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Abstract: Pancreatic cancer (PC) is heterogeneous cancer having a high death rate and poor prognosis. The perioperative variables, such as anesthetics, may affect the cancer progression. Ciprofol is an intravenous anesthetic widely used recently. We aimed to explore the influence of ciprofol on PC and investigate its possible pathway. The proliferation, migration and invasion roles and apoptosis of ciprofol in human PC cells were examined using methylthiazolyldiphenyl-tetrazolium bromide, transwell, and flow cytometry analysis. Then the putative targeted genes were examined using RNA-sequencing (RNA-seq) analysis. When differentially expressed genes (DEGs) were found, a protein-protein interaction network and pathway analyses were made. Moreover, MMP1 gene expression was confirmed in PC cells using quantitative real-time PCR. PANC-1 cells of PC were significantly suppressed with ciprofol in a dose-dependent and time-dependent way, and 20µg/mL ciprofol significantly suppressed tumor cell aggressiveness. Additionally, the RNA-seq analysis demonstrated that ciprofol controls the expression of 929 DEGs. 5 of 20 hub genes with increased connection were selected. Survival analysis demonstrated that MMP1 may be involved in the carcinogenesis and establishment of PC, reflecting the possible roles associated with ciprofol. Moreover, one target miRNA (hsa-miR-330-5p) of MMP1 was identified.

Keywords: Ciprofol, pancreatic cancer, MMP1, micro-RNA, anesthesia.

INTRODUCTION

Pancreatic cancer (PC) is among the highest fatal cancer kinds. PC Patients frequently experience a high incidence of tumor relapse, progression, and metastasis due to the heterogeneity and multiple disease difficulties. PC has a five-year OS rate of less than 5% (Siegel *et al.*, 2022). Although the continuous improvement of medical technology, diagnosing and treating PC in time is still difficult (Singhi *et al.*, 2019; Feng *et al.*, 2019). The most important method for treating PC is surgery. The quality of life and survival rate of patients suffer significantly as a result of its propensity for recurrence following surgery (Mizrahi *et al.*, 2020). Perioperative variables, such as surgical handling, anesthetics and analgesics, may change cancer development (Perry *et al.*, 2019). Intravenous anesthetics have a certain protective effect on PC by acting on tumor cells (Li *et al.*, 2020; Sun *et al.*, 2022; Oshima *et al.*, 2022; Wall and Buggy 2021; Dubowitz *et al.*, 2021).

Propofol is the most used intravenous anesthetic for surgery. Propofol could inhibit PC progression via different pathways (Gao *et al.*, 2019; Xu *et al.*, 2020). Ciprofol (HSK3486) is a new intravenous anesthetic similar to propofol. Propofol introduces a chiral structure, enhances the steric effect, increases the anesthetic effect,

and makes the side effects relatively mild (Liu *et al.*, 2020; Chen *et al.*, 2022). Ciprofol is widely used in anesthesia. Nevertheless, the impact of ciprofol on cancer was undetected yet.

Herein, we investigated the function and the potential molecular pathway of ciprofol on PC. First, different ciprofol concentrations were administered to the human pancreatic cancer (PANC-1) cells at various times. Second, two groups of selected cells experienced RNA-sequencing (RNA-seq). Then, retrieving RNA-seq, combined with GO and KEGG for comprehensive analysis. Finally, one target gene and the interacting micro-RNAs (miRNAs) were discovered. We found that ciprofol significantly suppressed human PC cell aggressiveness. Additionally, ciprofol influenced potential genes and pathways expression to suppress carcinogenesis, especially MMP1.

MATERIALS AND METHODS

Cell culture

PANC-1 was bought from the Chinese Academy of Sciences (Shanghai, China) and the human pancreatic cell line HPDE6-C7 was bought from BeNa Culture Collection (BNCC). The cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; VivaCell). All cells were kept at typical settings (37°C, 95%

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humidity, and 5% CO₂). Commercially available ciprofol (Haisco Pharmaceutical Group, China) was used for this study.

Proliferation assay

The impact of ciprofol on the proliferation of PANC-1 cells was detected utilizing an MTT assay (Dingguo Biotechnology, DH343-2, China). A total of 2.5×10^3 cells were planted into a 96-well plate (Corning, Nexcelom, USA) with ciprofol at different concentrations (0, 5, 10, 20 μg/mL). MTT (5mg/mL) was supplied and incubated for 4h, then 100 μL DMSO was applied after removing the supernatant. The OD values were detected utilizing a microplate reader (Tecan infinite, Switzerland) at 490 nm.

Wound healing assay

In a 6-well plate, a precise number of PANC-1 cells were equally distributed before being incubated for 24 hours. The cells in a plate were coated and a gap was made between them by using the top point of a crystal pipette to draw a single line. Following PBS was washed, images were captured; therefore, drug-containing and standard media were supplied. At 0 and 24h, the gap growth was monitored and captured in each group. The lengths of control and Ciprofol (20 μg/mL) treated groups were measured separately. The cell mobility (%) = the scratch length of (24h- 0h)/the scratch length of 0h. The count of two groups from three separate tests was employed for statistical analysis.

Migration and invasion assay

A transwell system filter (8.0m pore size; BD Biosciences, USA) was used for the migration test. In a 24-well culture plate (Corning, USA), 200 μL of ciprofol-treated or untreated 1×10^5 PACN-1 cells were put in the top chamber of transwell inserts for 24h and 700 μL of 30% FBS media was introduced to the lower chamber. The invasion assay utilized chambers with inserts uniformly covered with Matrigel (BD Biosciences).

The transwell chambers were preserved for 15 min with 4% paraformaldehyde and stained with 0.1% crystal violet for 30 min. After wards, the upper surface of inserts was cleaned with absorbent paper. The images of stained cells were then randomly taken using the microscope after being rinsed with PBS. By measuring the mean count of labeled cells in five regions, the capabilities for cell migration and invasion were evaluated. For statistical analysis, two groups from three independent experiments were employed.

Flow cytometry analysis

The collected cells were resuspended and centrifuged at 1300 rpm for 5min before being treated with 10L Annexin V-APC (catalog #: 88-8007, eBioscience, USA) and 5L

propidium iodide (PI) for 15 min in the dark. Flow cytometry (C6 PLUS, BD Biosciences, USA) was used to assess the apoptosis of cultured cells.

RNA-seq and pathway analysis

After 24h of medication with 0 and 20 μg/mL ciprofol, total RNA isolation from PANC-1 cells was carried out using TRIzol reagent (Invitgen). RNA-seq samples containing three duplications were verified using RNA-seq. Following quality confirmation, the RNA sample was packed on dry ice and delivered to Genechem (Shanghai, China) for RNA sequencing.

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG)

GO is a key bioinformatics approach to annotating and examining the biological activities of multiple genes. KEGG is a database for comprehending biological activities. According to the GO and KEGG databases, different pathway enrichment analysis was carried out, and the activities of genes having major changes were studied. Biological studies were conducted utilizing the DAVID (<http://david.ncifcrf.gov>; v6.8) online database to investigate the function of differentially expressed genes (DEGs).

Protein-protein interaction (PPI) network development and module analysis

The Search Tool for the Retrieval of Interacting Genes (STRING; <http://string-db.org>; v10.0; <http://string-db.org>) database, which contains known and projected PPIs, was used to build the PPI network. Cytoscape (v3.7.2) was used to display the PPI network and identify statistically significant PPI pairs (those with a total score >0.40). We used the Molecular Complex Detection (MCODE; v1.6.1) plug-in in Cytoscape to determine which PPI network modules were the most prominent. The inclusion criteria were MCODE degree cutoff = 2, node score cutoff = 0.2, max depth = 100, and k-score = 2.

Hub genes selection and analysis

The hub genes module was employed to identify the hub genes, and Coexpedia (<https://coexpedia.org>) web service was employed to evaluate the co-expression network. The hub genes were clustered hierarchically employing the UCSC Cancer Genomics Browser (<http://genome-cancer.ucsc.edu>). The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) data were used to evaluate RNA sequencing expression information of tumor and non-tumor samples using Gene Expression Profiling Interactive Analysis (GEPIA), an interactive online service. This analysis looked at differential expression of hub genes in PC patients. The correlation between hub genes and OS was examined using the Kaplan-Meier (KM) method.

RNA extraction and quantitative real-time PCR (qRT-PCR)

TRIzol (TaKaRa) was used to extract the total RNA from PANC-1 cells and the Prime Script RT Reagent Kit (TaKaRa) was used to create cDNA from 5 µg of the total RNA. A qRT-PCR using a SYBR Green PCR Kit from Takara was carried out using an RT-PCR amplifier system (Bio-Rad, USA). Using the $2^{-\Delta\Delta C_t}$ technique, mRNA expression was quantified relatively to GAPDH following being normalized to the endogenous reference GAPDH.

The sequence of human MMP1 PCR primers was 5'-CCATGAACCCGAGTTCGATTAC-3' (forward) and 5'-GGCCCTCCTCATCTTTCAGATT-3' (reverse). The sequences of the GAPDH primers were: 5'-AAGGTGAAGTTCGGAGTCAAC-3' (forward) and 5'-GGGGTCATTGATGGCAACAATA-3' (reverse).

MiRNAs prediction of key genes

The online analysis tools miRDB (<https://mirdb.org/index.html>), miR Walk (<http://mirwalk.umm.uni-heidelberg.de>) and Target Scan (https://www.targetscan.org/vert_72/) were employed to estimate candidate target miRNAs of MMP1. The results were plotted using the online Venn tool (analysis Bioinformatics & Evolutionary Genomics; <http://bioinformatics.psb.ugent.be/webtools/Venn/>). Cancer MIR Nome (http://bioinfo.jialab-ucr.org/Cancer_MIR_Nome) online database was employed to anticipate the miRNA expression in PC.

STATISTICAL ANALYSIS

All statistical analyses were done employing SPSS 22.0 program (Chicago, USA) and Prism software (v8.0, USA). The results were stated as mean \pm SEM (standard error of the mean). An unpaired t-test was utilized to study the means of two groups. One-way ANOVA and the Bonferroni test for multiple comparisons were performed to examine the variations between two or more groups. $p < 0.05$ was considered statistically significant.

RESULTS

Ciprofol suppresses PC cell proliferation

Cell proliferation is essential to tumorigenesis. Accordingly, we first employed MTT to identify the impact of ciprofol on the proliferation of PC cells. MTT demonstrated the efficiency of ciprofol on the proliferation of PANC-1 cells at various concentrations (fig. 1). It demonstrated that the PANC-1 cells were significantly suppressed following therapy with ciprofol in a dose- and time-dependent way. Moreover, 20 µg/mL dose had the most significant inhibitory effect on PC cells. Based on the MTT results, combined with the fact that the operation time of PC is generally within 24h in clinical practice, we chose 20 µg/mL and 24h for the following study.

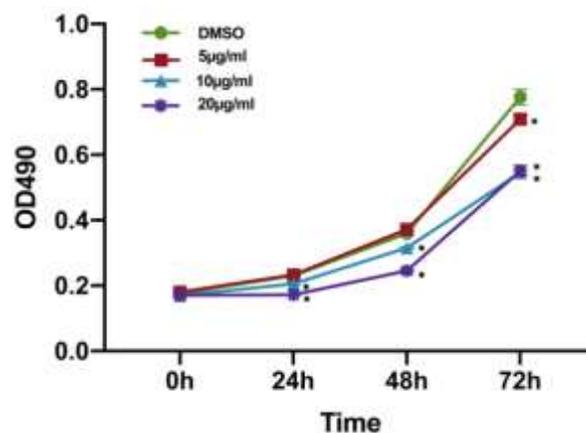


Fig. 1: Ciprofol inhibits cell proliferation in PANC-1 cells of PC. PANC-1 cells are medicated with 0 (DMSO); 5; 10; 20 µg/mL Ciprofol for 0-72h; respectively; cell viability is examined utilizing MTT; comparison of MTT OD490 between experimental and control groups (DMSO); the findings are reported as mean \pm SD (at least three separate experiments). $*p < 0.05$ versus the control group.

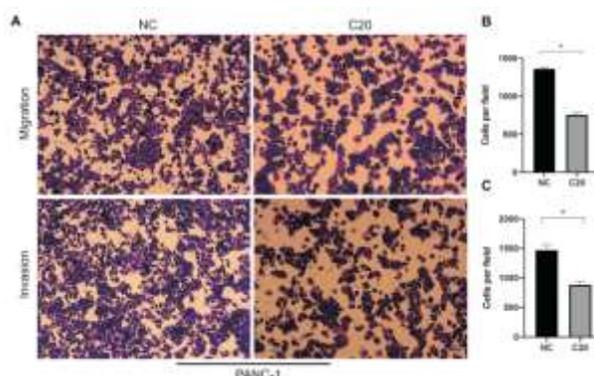


Fig. 2: Ciprofol inhibits cell migration and invasiveness in PANC-1 cells of PC. The cell migration and invasiveness capability are evaluated using trans well assays; Bar = 100 µm; the results are showed as mean \pm SD (at least three separate tests). $*p < 0.01$ versus the control group (NC). C20, cells were treated with 20 µg/mL ciprofol for 24h.

Ciprofol suppresses PC cell migration and invasiveness

The cell migration was studied utilizing trans well assay, and invasiveness was assessed by Matrigel assay respectively. We counted cell numbers and showed their changes compared to the control group. Tran swell and Matrigel assay showed that ciprofol (20 µg/mL, 24h) significantly inhibited PANC-1 cell invasiveness and migration.

To examine the impacts of ciprofol on PANC-1 cells mobility, we evaluated the mobility distance using a wound healing assay (fig. 3). The cell mobility rate revealed that ciprofol significantly suppressed the mobility and migration of PC cells.

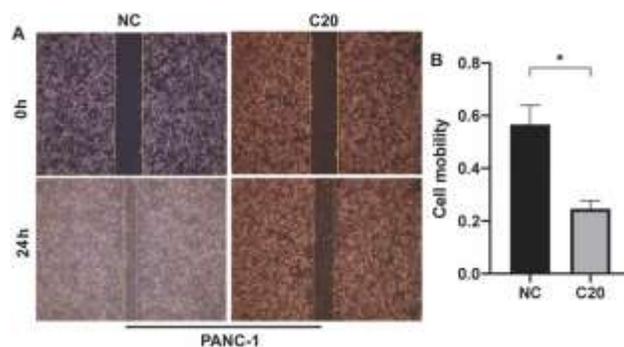


Fig. 3: Ciprofol inhibits PANC-1 cells' mobility. The cell mobility was evaluated using wound healing assays analyzed by the migration rate of cells. Data are reported as mean \pm SD (at least three tests). * $p < 0.01$ versus the control group (NC). C20, cells were treated with 20 μ g/mL ciprofol for 24h.

Ciprofol uninduced PC cell apoptosis

To examine if ciprofol suppresses the proliferation of PC cells via controlling the apoptosis mechanism, an AnnexinV-APC/PI approach was carried out. The PANC-1 cells were medicated with 20 μ g/mL Ciprofol for 24 h. The apoptosis level was evaluated using flow cytometry. The result demonstrated that ciprofol did not increase the apoptosis rate compared to the control group (Supplementary fig. 1).

RNA sequencing analysis demonstrated that ciprofol controls expression of several genes and pathways in PC cells to suppress carcinogenesis

To examine the mechanism of ciprofol in reducing PC development, we sent PANC-1 cells medicated with 0 μ g/mL (DMSO, NC group) and 20 μ g/mL ciprofol for 24 h (C20 group) for RNA sequencing. The analysis demonstrated that ciprofol controlled the expression of 929 DEGs and were enriched for different roles and pathways. fig. 4 shows the obtained DEGs heat map and volcano map. Heatmaps show related genes include matrix metalloproteinase 1 (MMP1), C-C motif chemokine ligand 2 (CCL2), jun proto-oncogene (JUN), intercellular adhesion molecule 1 (ICAM1), tumor necrosis factor receptor-associated factor (TRAF1) and other genes.

Then, DAVID was employed to perform the GO and KEGG pathway analyses. DEGs were enriched in an ECM structure, response to tumor necrosis factor, negative modulation of protein kinase activity, and negative modulation of mitogen-activated protein kinases (MAPK) activity for biological process (BP). Most cellular component (CC) was enriched in cell-substrate junction, focal adhesion, collagen-rich ECM and ruffle.

DEGs displayed enrichment in protein tyrosine phosphatase activity, protein tyrosine/serine/threonine phosphatase activity, mitogen-activated protein kinase binding, MAPK phosphatase activity, MAPK

tyrosine/serine/threonine phosphatase activity and protein tyrosine/serine phosphatase activity.

According to KEGG pathway analysis, DEGs from RNA-seq of PC cells treated with ciprofol were linked to cancer-related pathways, MAPK signaling pathway, TNF signaling pathway, nuclear factor NF-kappa B signaling pathway, transcriptional misregulation in cancer, ECM receptor interaction, nucleotide-binding and oligomerization domain (NOD)-like receptor signaling pathway and focal adhesion.

PPI network development and module analysis

Using STRING, the possibility of correlations between the pathways was examined. A PPI network of DEGs with 266 nodes and 496 interactions was created to discover gene interactions (fig. 5). Using Cytoscape, the most important module was verified. In total, 20 genes with degrees less than 5 were recognized as hub genes, and Coexpedia was used to evaluate the network and co-expression.

Hub gene selection and analysis

Evaluating the hub gene expression in PC and normal samples employing GEPIA demonstrated that 3 of 5 hub genes were upregulated in pancreatic ductal adenocarcinoma (PAAD) than in noncancerous samples. Therefore, we utilized GEPIA to assess the correlation between the five hub genes and PAAD survival. The result showed that the upregulation of MMP1 and TRAF1 was significantly linked to bad PAAD outcomes. However, there was no relationship between the expression of other three hub genes and the outcome of PAAD. These data indicate the possible function of these hub genes, especially MMP1 and TRAF1.

Combined with GEPIA results and the p -value of DEGs (the volcano map), the hub gene MMP1 may be mainly associated with PC prognosis. Then, we verified the MMP1 expression in PC cells. qRT-PCR revealed that MMP1 showed a high expression level in PANC-1 cells in comparison to healthy human pancreatic epithelial cells (HPDE6-C7). The expression levels of MMP1 in the control and ciprofol groups (20 μ g/mL, 24h) (Supplementary fig. 2).

MicroRNAs (miRNAs) prediction of MMP1

Using the miRDB database, 38 target miRNAs of MMP1 were identified, 1422 target miRNAs were identified using the miRWalk database, and 189 target miRNAs of MMP1 were identified using the Target Scan database. A total of 20 overlapping targets were identified using the Venn tool (fig. 7): hsa-miR-330-5p, hsa-miR-5010-3p, hsa-miR-326, hsa-miR-3176, hsa-miR-3922-3p, hsa-miR-4743-3p, hsa-miR-875-3p, hsa-miR-5190, hsa-miR-6857-3p, hsa-miR-558, hsa-miR-6888-3p, hsa-miR-4439, hsa-miR-4458, hsa-miR-518c-5p, hsa-miR-936, hsa-miR-7162-3p, hsa-miR-655-5p, hsa-miR-5094, hsa-miR-514a-5p, hsa-miR-520g-5p.

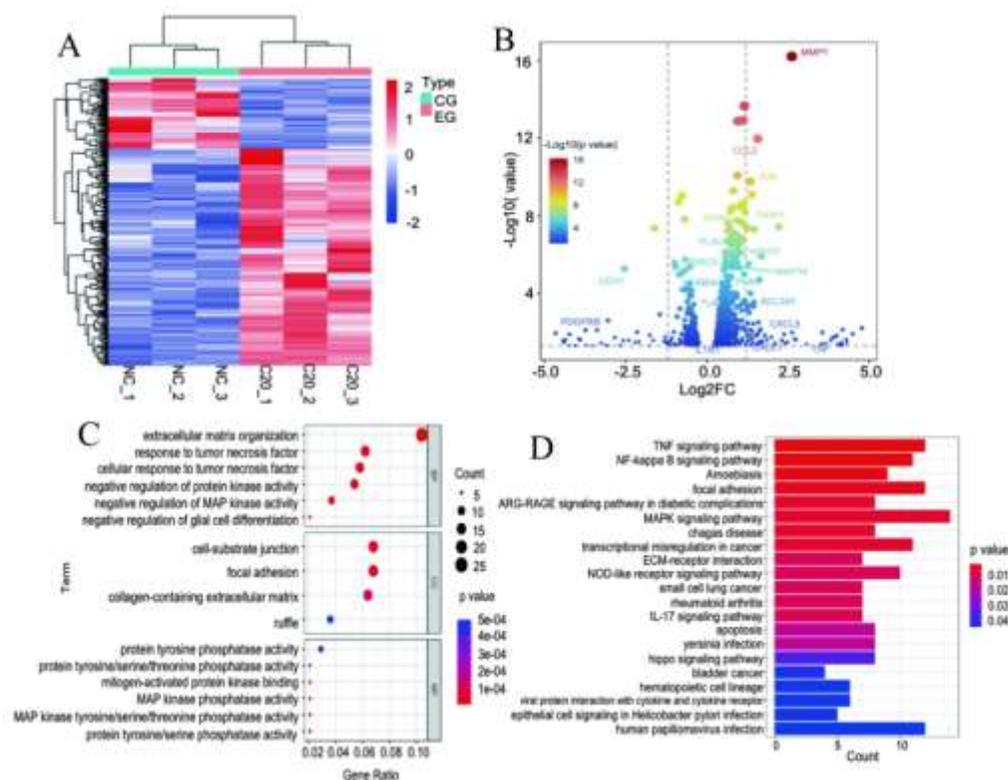


Fig. 4: Ciprofol suppresses several genes and pathways linked to human pancreatic carcinogenesis. Gene expressions of the PANC-1 cells medicated with (20 μ g/mL, 24h) or without ciprofol are detected using RNA-Sequencing; A Heat map of DEGs via the hierarchical clustering; blue color depicts down regulated genes; and red depicts upregulated genes; B DEGs are depicted in the volcano map; C–D GO enrichment process and KEGG pathways enrichment profiles of the PANC-1 cells from two groups. Statistically enriched were considered by false discovery rate (FDR) <0.01. DEGs, Differentially expressed genes; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; NC, control group; C20, ciprofol group.

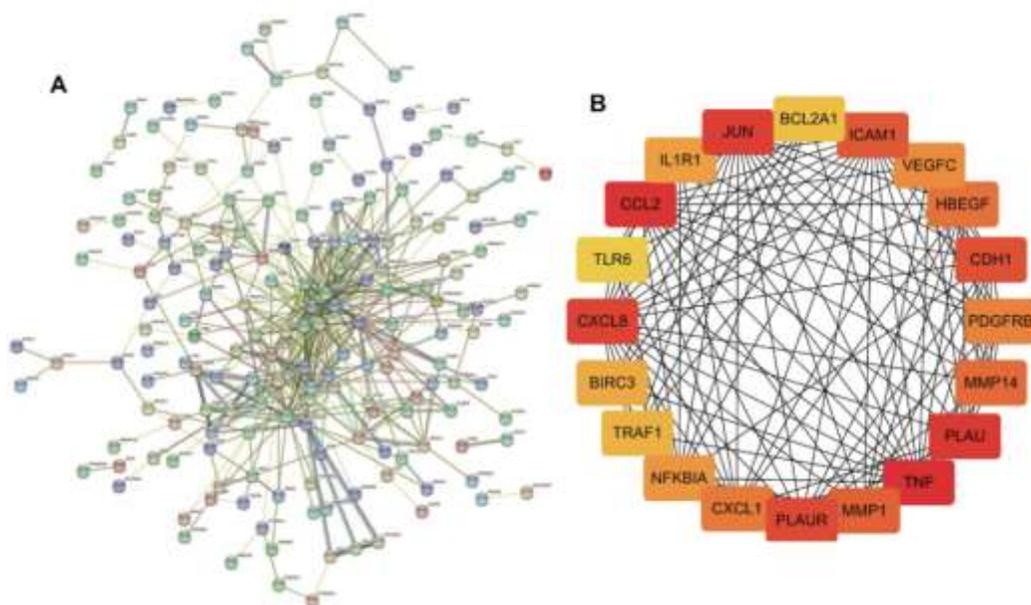


Fig. 5: The PPI network of DEGs is developed employing the STRING database. A The PPI network of DEGs includes 266 nodes and 496 edges; B hub genes module and co-expression network; PPI network; including 20 nodes and 216 edges; encompassing all hub genes; yielded the highest significant module. PPI, protein-protein interaction; DEGs, differentially expressed genes; STRING, search tool for the retrieval of interacting genes.

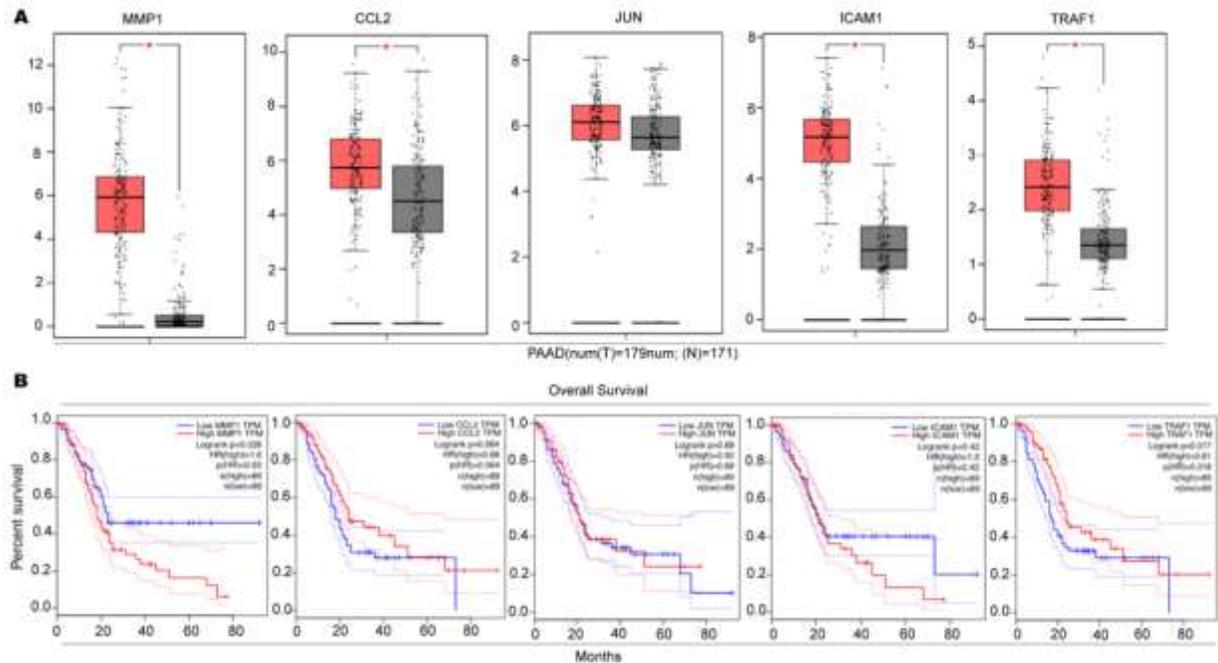


Fig. 6: Differences in hub gene expression in PC and normal tissues and survival analysis. A Five hub genes expression in PAAD and healthy samples through the GEPIA database; two of five hub genes are upregulated in PAAD patients; red depicts cancer tissue; and black depicts healthy tissue; B KM survival curve analysis; the OS of five hub genes expression in PAAD using GEPIA; the red line shows genes with increased expression linked to the bad OS; and the blue line shows genes with decreased expression linked to good survival. GEPIA, gene expression profiling interactive analysis. PAAD, pancreatic ductal adenocarcinoma; MMP1, matrix metalloproteinase 1; CCL2, C-C motif chemokine ligand 2; JUN, jun proto-oncogene; ICAM1, intercellular adhesion molecule 1; TRAF1, TNF receptor associated factor (* $p < 0.01$).

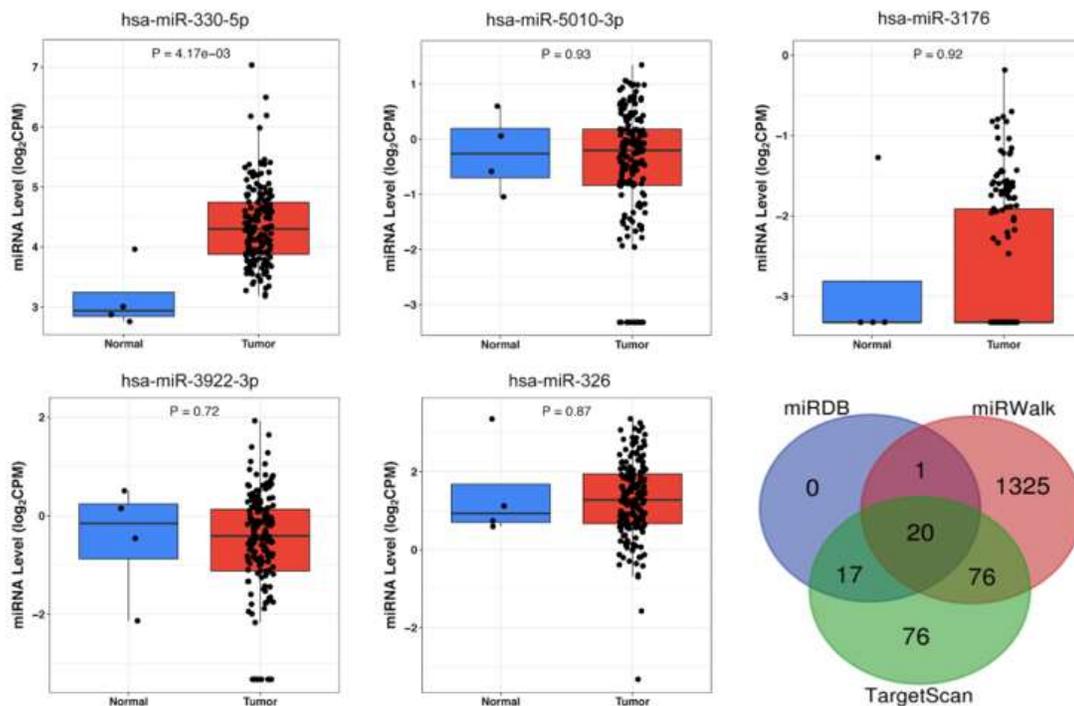


Fig. 7: miRNAs expression in PAAD and noncancerous samples through Cancer MIR Nome online database and miRNAs predicted to regulate MMP1. One of five miRNAs is over expressed in PAAD patients; red expresses cancer tissue and blue expresses healthy tissue; PAAD, pancreatic ductal adenocarcinoma; miRNAs, microRNAs.

Cancer MIR Nome is an extensive online database for interactive study and to visualize miRNA expression profiles derived from 33 TCGA studies and 40 publicly circulating miRNome datasets. The data analysis and visualization modules demonstrated that among the 20 miRNAs only 5 showed expression changes between PAAD and normal samples and one hsa-miR-330-5p expressed statistically significant differences.

DISCUSSION

PC is a fatal malignancy. Many variables may be associated with the bad outcome of PC, such as the rapid cancer development, the elevated propensity of metastasis, the lack of early diagnostic markers, and cancer resistance to chemotherapy and radiotherapy (Hosein *et al.*, 2020; Ho *et al.*, 2020; Hessmann *et al.*, 2020). Perioperative variables may be carcinogenesis-related, including surgical handling, anesthetics, and analgesics (Perry *et al.*, 2019; Dubowitz *et al.*, 2021).

Propofol is the most used intravenous anesthetic for surgery. Propofol suppresses PC development through various pathways. Chen *et al.* (Chen, 2017) revealed that inhibiting NMDA receptors enable propofol to attenuate PC malignancy. Wang *et al.* (Wang *et al.*, 2020) reported that propofol suppresses migration and enhances programmed cell death of PC cells by miR-34a-mediated E-cadherin. Propofol also suppresses PC proliferation and metastasis by upregulating miR-328 (Yu *et al.*, 2019), miR-34a (Wang *et al.*, 2020) and ADAM8 (Gao *et al.*, 2021). Moreover, Du *et al.* (Du *et al.*, 2013) showed that propofol inhibits NF-kappa B activity, resulting in programmed cell death and augments gemcitabine sensitivity in PC cells.

Ciprofol is a novel anesthetic agent similar to propofol. It is a short-acting alkylphenol intravenous anesthetic that can produce a rapid and stable anesthetic effect when administered. Ciprofol is widely used in inducing and maintaining general anesthesia, gastroenteroscopy and ICU sedation (Ding *et al.*, 2022; Qin *et al.*, 2022). Its mechanism of action mainly depends on the activation of γ -aminobutyric acid-A (GABAA) receptor-chloride ion complex. It inhibits central nervous system pathways by enhancing GABA-mediated chloride ion conductance (Long *et al.*, 2022). Ciprofol may have the same inhibitory effect on PC progression as propofol.

Herein, we evaluated the impacts of ciprofol on PC cells and discovered that the aggressiveness of PC cells was significantly inhibited by ciprofol treatment. Then, we investigated its molecular pathway. According to RNA-seq, several hub genes and pathways related to cell proliferation and migration were found. Propofol can act on MAPK (Kang *et al.*, 2020; Su *et al.*, 2020) and TNF signal pathways (Ding *et al.*, 2019) to regulate the

progression of different tumors. Similarly, we found that ciprofol was linked to ECM organization and the cell-substrate junction of cancer cells and also related to the same pathways propofol activated.

The analysis of the RNA-seq results revealed that matrix metalloproteinases (MMPs) may perform a key function in inhibiting ciprofol on PC. MMPs are zinc-dependent endopeptidases initially identified as proteases that target and break down extra cellular proteins. MMPs are essential for cell differentiation, proliferation, wound healing, apoptosis, and angiogenesis. Additionally, MMPs take part in cancer metastasis and tumor growth pathophysiology (Bassiouni *et al.*, 2021).

MMP1 expression in the early stages of several malignancies is correlated with a bad outcome. During the mitotic phase of the cell cycle, it accumulates intracellularly and localizes to the mitochondrial membrane and the nucleus. Intracellular MMP1 confers apoptosis resistance, which may explain the relationship between MMP1 and tumorigenesis and chemoresistance (Wang *et al.*, 2020). In PC, Huang *et al.* (Huang *et al.*, 2018) showed that MMP1 is a critical variable throughout the invasiveness of PC cells. Upregulation of MMP1 promoted PANC-1 and Mia PaCa-2 cell migration and invasiveness and MMP1 expression was upregulated in PAAD tissues. Moreover, MMP1 activated various signaling pathways, including MAPK (Cai *et al.*, 2021), NT3/TrkC (Xu *et al.*, 2022) and NF-kappa B (Qiu *et al.*, 2023) pathways, making it crucial in tumorigenesis.

We observed that miR-330-5p was strikingly related to MMP1 and was upregulated in the PAAD patients, according to the online database. MiRNAs are non-coding small RNAs that control multiple proteins expression in the biological process. miRNAs perform an essential function in initiating, developing and the metastasis of multiple cancers as potential biomarkers and prognostic agents (Rawat *et al.*, 2019). Wang *et al.* (Wang *et al.*, 2021) demonstrated that miR-330-5p mediates thyroid carcinoma (TC) development by targeting FOXE1. They discovered that miR-330-5p expression is overexpressed in TC tissue and cell lines and miR-330-5p downregulation inhibits TC cell aggressiveness. miR-330-5p negatively controls forkhead box E1 expression. Eneh *et al.* (Eneh *et al.*, 2020) found significant upregulation of miR-330-5p in colorectal cancer. Whether miR-330-5p is expressed in PC and its relationship with MMP1 will be further studied.

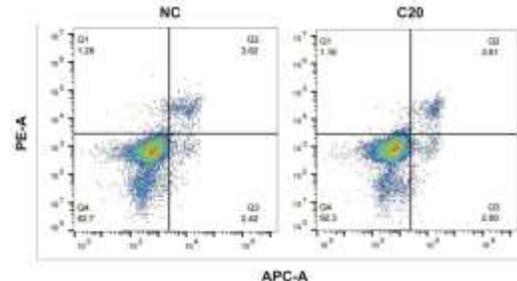
Although we verified MMP1 expression in PC cells, TRAF1 was also a hub correlation gene according to GEPIA results. TRAF1 is upregulated in several malignancies, such as renal cell carcinoma, gastric cancer, and hepatocellular carcinoma and TRAF1 attenuation promotes apoptosis in cellular models of tumors. TRAF1

regulates metabolic transformation during tumorigenesis; it mediates the proliferation and development of cancers by enhancing glycolytic metabolism and antagonizing the mitochondrial permeability transition (Chen *et al.*, 2022). TRAF1 may also be a potential target gene associated with ciprofol in PC.

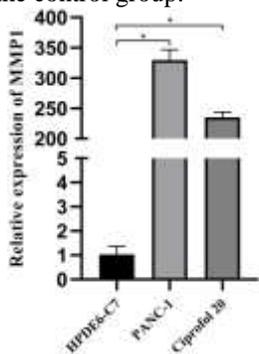
Herein, we investigated the impacts of ciprofol on the proliferation, metastasis, and apoptosis of PC cells and the underlying molecular pathways. We are the first to reveal the anti-tumor impact and potential mechanism of ciprofol on PC. There are certain limitations in this study. We used only one PC cell line, which needs to be verified in other PC cells. This research discovered that ciprofol unchanged apoptosis rate and cell cycle dispersion in 20µg/ml for 24h treatment. Higher concentration or longer time may have antiapoptosis potency.

CONCLUSION

We demonstrated that ciprofol inhibits PC cell proliferation, invasion and migration through different pathways. Experiments will be necessary to examine the posttranscriptional control of ciprofol to identify the complicated role and mechanism involved in controlling the target genes. This research showed the possible inhibition activity of ciprofol on PC cells and proposed a new therapeutic strategy for PC.



S. Fig. 1: Ciprofol did not induce cell apoptosis in PANC-1 cells of pancreatic cancer. The apoptotic level of PANC-1 cells treated with (C20) or without (NC) 20µg/mL ciprofol for 24 h. Data are presented as mean ± SD (at least three separate tests for cell line experiment). ^{ns}*p*>0.05 versus the control group.



S. Fig. 2: The expression of MMP1 genes in normal human pancreatic epithelial cells (HPDE6-C7) and

PANC-1 cells of PC. PANC-1 cells were treated with 0µg/mL, 20µg/mL ciprofol for 24 h. Data are presented as mean ± SD. * *p*<0.01 versus the control group.

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