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Bioinformatics-Based Analysis of SORT1 on the Pathogenesis of Pancreatic Cancer

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ABSTRACT

There are few effective treatment options for patients with pancreatic cancer (PC), and SORT1 has been implicated in many cancers. However, its role in pancreatic cancer is still unclear. This study aimed to investigate the diagnostic and prognostic value of SORT1 in pancreatic cancer and the mechanisms influencing its biological function in this disease. Immunohistochemical testing confirmed that SORT1 is upregulated in pancreatic cancer, and its expression in the nucleus (NSE) has significant diagnostic value.Univariate and multivariate analyses revealed that NSE was an independent prognostic factor in PC patients. Multiple bioinformatic analyses of pancreatic cancer revealed SORT1 may promote pancreatic cancer by ubiquitinating genes in the TP53 pathway and by interacting with MMP9, elucidating a novel mechanism for the pathogenesis of pancreatic cancer.These phenomena were also confirmed via in vitro cell experiments, where proliferation, invasion and migration of PC cells were inhibited following SORT1 suppression.In conclusion, SORT1 is a promising biomarker for the diagnosis, treatment and prognosis of pancreatic cancer, and its mechanism of action may be related to the SORT1 / TP53 / MMP 9 pathway.

INTRODUCTION

As the 7th leading cause of cancer mortality, pancreatic cancer (PC) remains one of the most lethal malignancies. Furthermore, PC prevalence and mortality trends are increasing, particularly in women and those over 50 years of age Huang et al. (2001). The incidence of PC is projected to increase to 18.6 per 100,000 by 2050 et al. (2020). Many clinical trials are now investigating the efficacy of immunotherapeutic strategies for PC, but this is far from meeting clinical need Schizas et al. (2020). In the family of neurotrophic factors, SORT1 has been implicated in breast Rhost et al.(2018), lung Al-Akhrass et al.(2017) and prostate cancer Tanimoto et al.(2015).F. Gao et al et al.(2020) found that SORT1 levels were increased in PC cells, and inhibition of SORT1 decreased the adhesion and invasion of PC cells, but did not affect cell survival and viability. Given the therapeutic potential of SORT1 inhibition, our aim in this study was to investigate the role of SORT1 in pancreatic cancer and its relationship to pancreatic cancer pathogenesis.

MATERIALS AND METHODS

Chemicals and reagents

The PC tissue microarray (hpan-ade120sur-01) was purchased from SHANGHAI OUTDO BIOTECH CO.,LTD. (https://www.superchip.com.cn). R&D Biotechnology (MN, USA) provided the goat polyclonal human SORT1 IgG antibody (AF3154). Rabbit anti-goat antibodies, 3,3'-diaminobenzidine (DAB) reagent and PC cell lines (Capan1 and Bxpc3) were obtained from North Sichuan Medical College Image Processing Lab. (IMDM, SH30228.01) and Roswell Park Memorial Institute (RPMI) 1640 media (SH30809.01) were purchased by HyClone (Logan, UT, USA). Tianhang Biotechnology (Zhejiang, China) provided fetal bovine serum (11011-8611). Trypsin EDTA (PYG0015) and CCK-8 reagents were purchased from Boster Biological Technology (Wuhan, China). The siRNA (SR304211) was synthesised by OriGene (MD, USA). Rfect (11013) and TRIzol (DP405-02) were purchased from Baidai Biotechnology (Changzhou, China) and Tiangen Biotech (Beijing, China), respectively. Tianmo Technology (Beijing, China) provided the total RNA extraction kit (TR201-100). The kit for reverse transcription (K1622) was purchased from Thermo Fisher Scientific (MA, USA).The Bestar® SybrGreen qPCR Mastermix (DBI-2043) was provided by DBI.Bioscience (Shanghai, China). The primer sequences was designed by Shenggong bioengineering (Shanghai) Co. , Ltd. Matrigel was purchased from BD Biosciences (Beijing, China). MIP-3α (10485-H07E) was obtained by Sino Biological Solution Specialist.

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RIPA lysate (P0013B) was purchased from Beyotime Biotechnology (Shanghai, China). SORT1 antigen (AF3154) affinity-purified polyclonal goat IgG was purchased from R&D Bio-techne. The secondary antibodies using HRP-conjugated AffiniPure goat anti-rabbit IgG (BA1060) were purchased from Boster Biological technology Co., Ltd. microplate reader is Bio-rad iMark from USA.

Immunohistochemistry (IHC)

Tissue microarray containing 60 tumor tissues of pancreatic cancer patients (including 5 tissues with AJCC stage I, 42 tissues with AJCC stage II,4 tissues with AJCC stage IV and 9 tissues no AJCC stage) and 55 normal pancreatic tissues. A full description of the prototype IHC assay is provided in the supplementary methods of G. Akturk Yeh et al. (2019). TPS et al(2022) were used as the quantitative procedure. TPS = (the number of viable tumour cells positive for SORT1/total number of viable tumour cells)×100%.0 score (negative), 1 score (1-25%), 2 scores (26%-50%), 3 scores (51-75%), 4 scores (76%-100%). The median (90%) was used as the standard, and a score below or at 90% was defined as low expression and above 90% as high expression.

Bioinformatics Analysis

Gene Cards (http://www.genecards.org)and GEPIA2(http://gepia2.cancer-pku.cn/) were used to investigate the different expression profiles in pancreatic cancer. The Retrieval of Interacting Genes STRING (http://cn.string-db.org)was used to investigate gene profiles associated with SORT1. The gene expression levels were shown using p-value < 0.05 and |log2 (Fold change)| ≥ 1. Heat map clustering, the GO function and KEGG pathway analysis were executed through the bioinformatics platform. Interactions and functions of genes were executed by online analysis tools GeneMANIA(http://genemania.org/).UALCAN(http://ualcan.path.uab.edu/index.html) was executed to select cell lines CAPAN1 and BXPC3 in pancreatic cancer for in vitro experiments.

Cell culture and siRNA transfection

Capan1 cells were grown in IMDM supplemented with 20% fetal bovine serum (FBS),and Bxpc-3 cells were grown in RPMI-1640 media supplemented with 10%FBS in a humidified atmosphere containing 5% CO2 at 37 °C.The siRNA/Rfect (50 pmol :10µl) mixture was mixed with 2 ml of complete medium overnight. The target sequences were

siRNA-A: GCAGAGCUAGAUAUAGCAC,

siRNA-B: CGCAAGGACAGGGUACAAACUUAGC,

siRNA-C: AGACGUAGGAAACUCAAUAUUCUTC

The experiment was tested three times. Transfection effect of SORT1 knockdown was evaluated by RT-PCR.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Cell collections were made at 24h (Bxpc3) and 48h (Capan1) after siRNA transfection. Following the manufacturer's instructions, total RNA was extracted. Total RNA was reverse transcribed to cDNA using a reverse transcription kit, and the total RNA concentration and OD value were measured. Bestar® SybrGreen qPCR Master mix was used for qPCR reactions (20 µl final volume). Table I shows the primer sequences. Reaction conditions for RT-PCR were denaturation at 95°C for 5 minutes, 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 40 seconds for 35 cycles. The results were analysed using 2-ΔΔct. Each experiment is repeated 3 times. We chose GAPDH as the internal control for normalization in this experiment.

Cell Proliferation, Migration and Invasion Experiment

Proliferative capacity was assessed by the CCK-8 assay. Control and siRNA-transfected cells were seeded at a density of 5000 cells in 96-well plates. CCK-8 reagent was prepared in fresh medium (100 µL medium containing 10 µL CCK-8 solution) and assayed on Capan1 cells at 48-, 72- and 96-hours post-transfection and on Bxpc3 cells at 24-, 48- and 72-hours post-transfection. After addition of CCK-8, cells were incubated for 2 hours and optical density (OD) was measured at 450 nm using a microplate reader. Inhibition was calculated according to the following formula: Inhibition = [(ODsiRNA-ODwhite)/(ODMock-ODwhite)] × 100%. Three replicates are available for each result.

Migration was calculated by scratch assay. Controls as well as siRNA-transfected cells were planted in 12-well plates. Straight scratches were produced by a 200 ul pipette tip after cultured cell connectivity reached more than 90%.Serum-free medium was added after scratching, and after 0 and 12 hours of incubation, wound closure was observed and counted.

Each result was repeated three times. We performed the results analysis after opening the picture with Image J software, and randomly delimit 6 to 8 horizontal lines to calculate the mean of the cell distance. Cell migration rate (wound healing rate)=(mean initial intercell distance-mean intercell distance at time 0,12,24h) / mean initial intercell distance.

Invasion analysis was performed in Transwell chambers on the upper surface of the 8-um pore size membrane. Two transfected cell lines were cultured in Medium with 10% FBS in upper chamber.

Table 1: The primer sequence of related molecule

Gene name	Primer sequence	Base number	Tm	Product location
SORT1	Forward primers AAGTCTTTGGACCGACATCTCT	22	60.8	751-772
	Reverse primers AGCACGCTTGTTATGTAGACG	21	60.4	845-825
GAPDH (Internal Control Gene)	Forward primers GGAGCGAGATCCCTCCAAAAT	21	61.6	108-128
	Reverse primers GGCTGTTGTCATACTTCTCATGG	23	60.9	304-282
TP53	Forward primers CAGCACATGACGGAGGTTGT	20	62.4	382-401
	Reverse primers TCATCCAAATACTCCACACGC	21	60.1	506-486
MMP9	Forward primers AGACCTGGGCAGATTCCAAAC	21	62	306-326
	Reverse primers CGGCAAGTCTTCCGAGTAGT	20	61.3	399-380

Cell Proliferation, Migration and Invasion Experiment

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Two transfected cell lines were cultured in Medium with 10% FBS in upper chamber. Medium with 20% FBS was placed in the lower chamber. After incubation for 72 h, the cells that invaded into the lower surface was fixed, stained and counted. There were three replicates for each result.

Statistical analysis

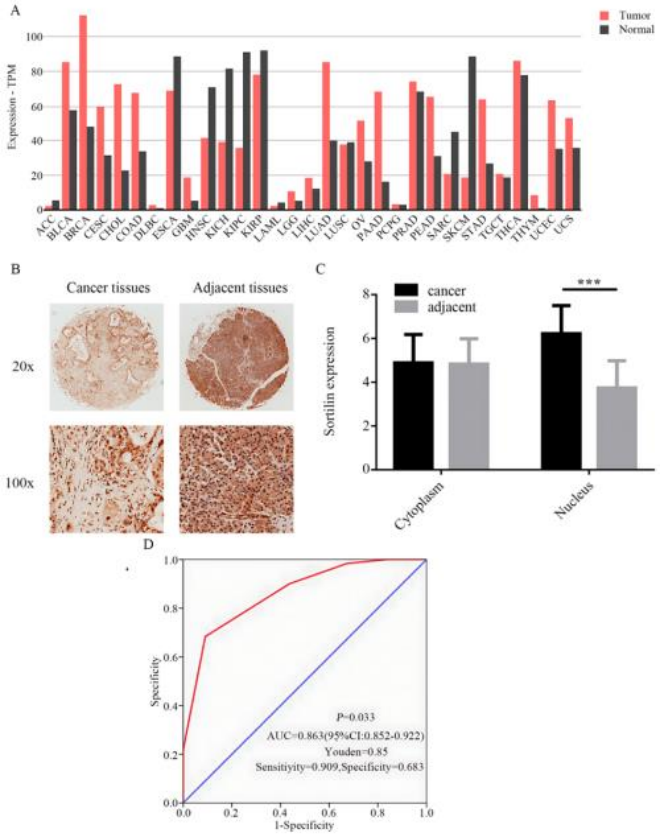
Statistics were conducted with SPSS 23.0 (SPSS Inc., NY, USA). Measures were expressed as mean ± standard deviation (SD). Enumeration data and categorical 2 or Fisher exact test was used for analysis. The Kaplan-Meier method was used to calculate median and mean survival time. Statistically significant difference was considered when *:P<0.05, **:P<0.01, ***:P < 0.001 and ****:P < 0.0001 between groups. IHC data were analyzed using receiver operating characteristic (ROC) curve and binary logistic regression.The correlation analysis was not previously included in the article and is now added in TableIIusing the Kendall's 2-tailed test.We used log-rank for survival , and Chi-square test of Cox proportional risk model for multiple comparisons groups.The post hoc test that was used after one-way ANOVA is Student–Newman–Keuls test.

Results

SORT1 correlate with PC and predicts poor prognosis in PC patients

To evaluate the association of SORT1 in PC, we firstly analyzed the gene expression profile of SORT1 in human tumor tissues using GEPIA2.The mRNA expression level of SORT1 in Liver hepatocellular carcinoma (LIHC), ovary (OV), pancreas(PAAD),Skin Cutaneous Melanoma (SKCM) and Stomach adenocarcinoma (STAD) displayed higher expression levels (Figure 1A).

Figure 1: Differential expression and diagnostic value of SORT1



Pan-cancer analysis of the expression profile of the SORT1 gene using GEPIA2 based on RNA-Seq data from the TCGA database.B.Pancreatic cancer tissue chips were analyzed by IHC staining,magnification for the panel is 20X and 100X respectively;C.The expression level of sortilin in the cytoplasm and nucleus by a total score standard;D.ROC curve analysis was performed to evaluate the specificity (68.3%)and sensitivity (90.9%)of SORT1.Area under curve, AUC = 0.863; 95% confidence interval, CI: 0.852 – 0.922.The cut-off point was 0.85, calculated by the Youden Index.

In order to verify this phenomenon, After dividing all specimens into high expression group and low expression group based on the median of IHC score, the relationship between SORT1 and tumor characteristics was assessed. The results not only demonstrated the upregulation of SORT1 in PC but also revealed the differential expression of SORT1 in PC tissues was mainly from the nucleus. SORT1 expression in the nucleus (NSE) was 20%, while the expression in the cytoplasm was not statistically significant(Fig.1B and C, Table S1).And the significant relationship between NSE expression and Lymphatic metastasis (Table II).

Collectively, these data confirmed the tight association of SORT1 with carcinogenesis in pancreatic cancer.In addition, we also used a binary logistic regression (BLR) model to predict the diagnostic value of SORT1 for pancreatic cancer. The results showed that the accuracy rate was 86.3%, especially that NSE had good diagnostic value for pancreatic cancer (Figure 1D).

Prognostic analysis of clinicopathological indicators in pancreatic cancer

The prognostic value of different Clinicopathological indicators for PC patients was obtained by Kaplan-Meier analysis.As shown in the results, NSE, M stage, clinical stage correlated with the prognosis of PC, with high levels of NSE (Table S2,Fig. 2G,P=0.010), distant metastasis (Fig.2H, P=0.022) and high clinical stage(Fig.2I,P=

0.030) having shorter overall survival, while multivariate (Table S3,Fig. 2J) showed NSE (HR: 0.403, p=0.015) and M stage (HR: 0.255, p=0.017) as independent prognostic factors for PC patients.Taken together, NSE predicted poor overall survival, revealing that it may be a promising prognostic biomarker for pancreatic cancer patients.

Figure 2: Univariate and multivariate analysis of NSE and patient survival

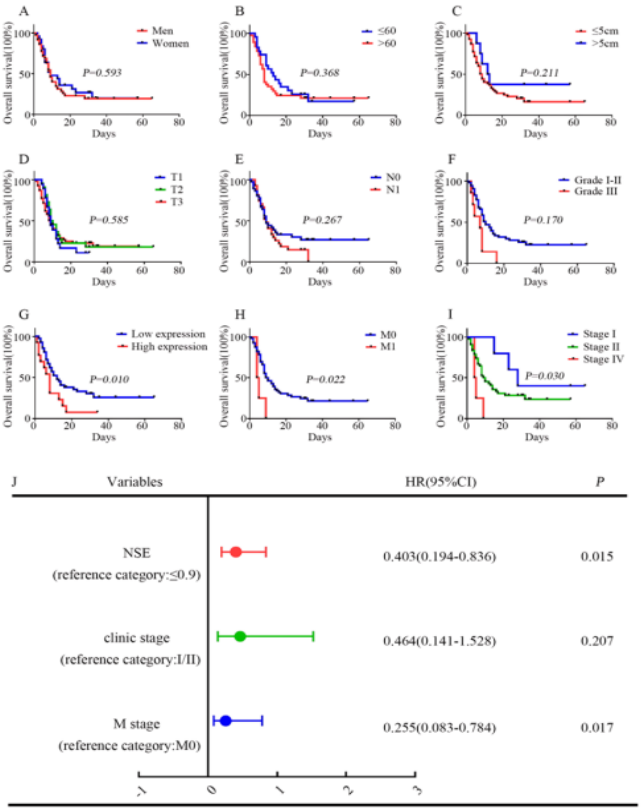


Table 2: Correlation between NSE and clinicopathological features of 60 PC patients

Features	No.of patients	NSE Expression		Correlation Coefficient	*p*
		Low	High		
Age(years)					
≤60	24	18	6	0.179	0.076
>60	36	26	10		
Sex					
men	35	25	10	0.07	0.559
women	25	19	6		
Tumor size					
≤5cm	52	39	13	0.102	0.335
>5cm	8	5	3		
Pathological grade					
I/II	31	21	10	0.171	0.157
III	29	23	6		
T stage（Deficiency:16）					
T1	2	1	1	0.075	0.587
T2	6	4	2		
T3	36	27	9		
N stage (Lymphatic metastasis)					
N0	33	23	10	-0.254	0.04
N1	27	18	9		
M stage (Distant metastasis)					
MO	56	41	15	-0.145	0.229
M1	4	3	1		
Clinical stage（Deficiency:9）					
I	5	3	2	-0.03	0.814
II	42	31	11		
IV	4	3	1		

A,B, C, D, E,F are the overall survival rate among gender(p=0.593), age(p=0.368),tumor size(p=0.211),T stage(p=0.585), N stage(p=0.267) and pathological grade(p=0.170) respectively; G,H,I are the overall survival rate among nucleus sortilin expression(p=0.010),M stage(p=0.002),clinical stage(p=0.030) respectively;J.Multivariate analysis of the prognostic value of SORT1 and M stage for PC.

Coexpression genes(CGs) and functional analysis of SORT1 in PC

114 genes related to SORT1 in pancreatic cancer were obtained from GEPIA2, Genecards and STRING (Fig. 3A). Figure 3B is a heat map of the 114 co-expressed genes, indicating better clustering of the samples and higher confidence levels.GO annotation and KEGG pathway analysis were performed on these genes using the Bioinformatics platform. An examination of the top 10 significant pathways identified by KEGG analysis revealed that co-expressed genes were mainly enriched in cell cycle,pancreatic cancer and TP53 signaling pathways, with TP53 present in all 10 of these pathways (Table III, Fig. 3C).GO analysis confirmed that SORT1 is predominantly localized to the transcriptional regulatory complex and functions as a ubiquitin-like protein ligase involved in TP53 mediator signaling (Fig. 3D).

Functional enrichment analyses

We constructed a gene–gene interaction network for CGs to analyze the function of these genes using the GeneMANIA database. The hub node representing CGs was surrounded by 20 nodes representing genes that were significantly correlated with CGs(Fig. 4).

Validation of the effects of SORT1 knockdown on proliferation, migration and invasion in pancreatic cancer cell lines

Forty-six cell lines associated with pancreatic cancer were analysed, of which 24 showed cell death and 22 showed positive cell values.

Table 3: KEGG pathway analysis of 114 CGs

Description	P value	Gene ID	Count
hsa04110: Cell cycle	2.43548×10-24	TP53/RB1/CDK4/CDKN1B/RBX1/CCND1/ SKP1/SMAD2/CDKN1A/HDAC1/GSK3B/ CDC6/CDC25B/CCNB1/CCNE1/CDK6/ CDK2/CCNA2/SFN/HDAC2/CDKN2A/ PRKDC/CHEK1/CHEK2/CCNH	25
hsa05212: Pancreatic cancer	2.82511×10-16	TP53/STAT3/RB1/RELA/CDK4/CCND1/ SMAD2/CDKN1A/CDK6/ERBB2/CHUK/ BCL2L1/CDKN2A/MAPK8/RAD51/NFKB1	16
hsa04115: TP53 signaling pathway	3.80669×10-15	TP53/CDK4/CCND1/CDKN1A/PTEN/ RRM2B/CCNB1/CCNE1/CDK6/CDK2/ BCL2L1/SFN/CDKN2A/CHEK1/CHEK2	15
hsa05220: Chronic myeloid leukemia	7.22528×10-15	TP53/RB1/RELA/CDK4/CDKN1B/CCND1/ CDKN1A/HDAC1/NFKBIA/CDK6/ CHUK/BCL2L1/HDAC2/ CDKN2A/NFKB1	15
hsa05166: Human T-cell leukemia virus 1 infection	7.96416×10-15	RELB/TP53/XPO1/RB1/RELA/CDK4/ CCND1/SMAD2/CDKN1A/PTEN/NFKBIA/ CCNE1/CDK2/CCNA2/CHUK/BCL2L1/ CDKN2A/MAPK8/CHEK1/ KAT5/CHEK2/NFKB1	22
hsa05169: Epstein-Barr virus infection	1.35053×10-14	RELB/TP53/STAT3/RB1/RELA/ CDK4/CDKN1B/CCND1/ CDKN1A/HDAC1/ NFKBIA/MAPK14/CCNE1/ CDK6/CDK2/CCNA2/ CHUK/HDAC2/MAPK8/NFKB1/TLR2	21
hsa04218: Cellular senescence	1.52807×10-14	TP53/RB1/RELA/CDK4/CCND1/SMAD2/ CDKN1A/PTEN/MAPK14/CCNB1/CCNE1/ NBN/CDK6/CDK2/CCNA2/CDKN2A/ CHEK1/CHEK2/NFKB1	19
hsa05215: Prostate cancer	1.69455×10-14	TP53/RB1/RELA/HSP90AA1/CDKN1B /CCND1/CDKN1A/PTEN/ GSK3B/NFKBIA/ CCNE1/CDK2/ERBB2/CHUK/NFKB1/MMP9	16
hsa05222: Small cell lung cancer	1.42971×10-13	TP53/RB1/RELA/CDK4/CDKN1B/ CCND1/CDKN1A/PTEN/NFKBIA/ CCNE1/CDK6/CDK2/ CHUK/BCL2L1/NFKB1	15
hsa05203: Viral carcinogenesis	1.92716×10-13	TP53/STAT3/RB1/RELA/CDK4/ CDKN1B/CCND1/CDKN1A/HDAC1/NFKBIA/ CCNE1/CDK6/CDK2/ CCNA2/HDAC2/CDKN2A/ CHEK1/NFKB1/UBE3A/GTF2H1	20

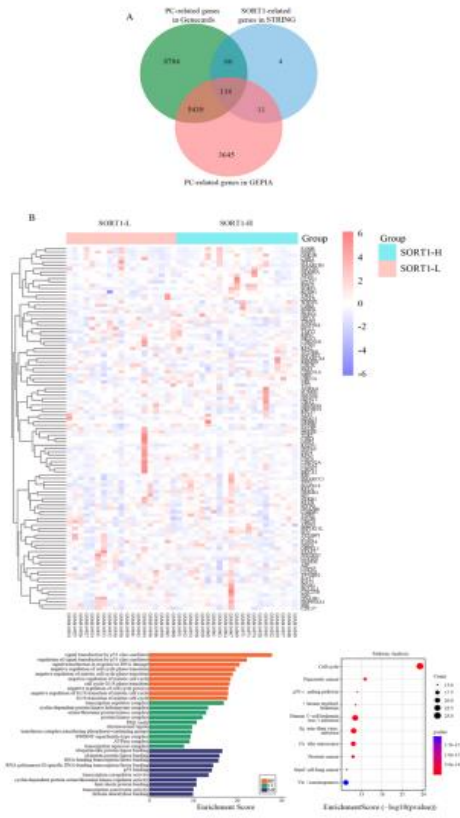
and we selected BXPC3 and CAPAN1 cells for the study(Fig.5A, $p<0.005$).After transfection with siRNA, SORT1 was significantly downregulated in both cell lines (Table S4, Fig. 5B, $p < 0.001$). CCK-8,wound healing and transwell assays indicated that SORT1 knockdown significantly reduced the proliferation, migration and invasion ability of pancreatic cancer cells (Table S5, Fig. 5C, D, E). These results demonstrated that reducing SORT1 expression helps suppress pancreatic cancer progression. All experiments were performed in triplicates.

The expression of TP53 and MMP9 mRNA was examined following siRNA transfection

The expression of TP53 and MMP9 mRNAs was determined after siRNA transfection. The results showed that there was a decrease in the mRNA levels of and MMP9 and an increase in the mRNA levels of TP53 in Capan1 cells and Bxpc3 cells. There were no significant differences between the blank, negative control and mock groups(Table S6 and Table S7, Fig. 6).This suggested that SORT1 may impact pancreatic cancer cell migration, invasion and proliferation through effects on TP53 and

MMP9 expression.SORT1 inhibition may affect pancreatic cancer cell migration, invasion and proliferation by upregulating TP53 and downregulating MMP9.All experiments were performed in triplicates.

Figure 3: Coexpression genes(CGs) and functional analysis of SORT1 in PC



Venn diagram of CGs;B.Heat map of the CGs ;C. KEGG pathway enrichment analysis of the 114 co-expression genes.;D.GO enrichment in biological processes, molecular function and cellular components for the 114 CGs.

Figure 4 : The gene–gene interaction network for SRGs was analyzed using the GeneMANIA database.

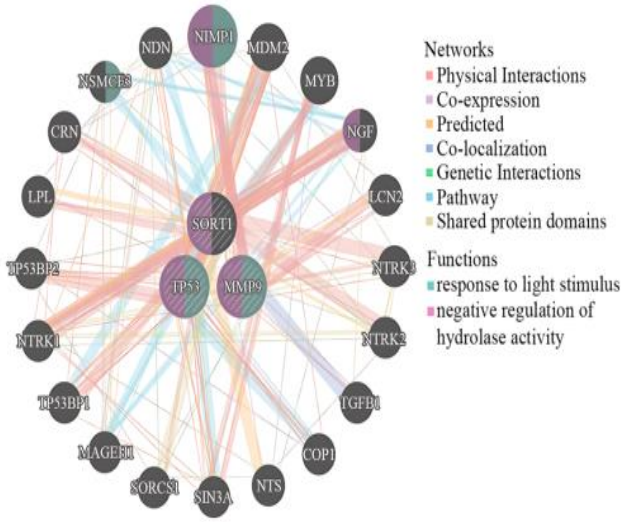
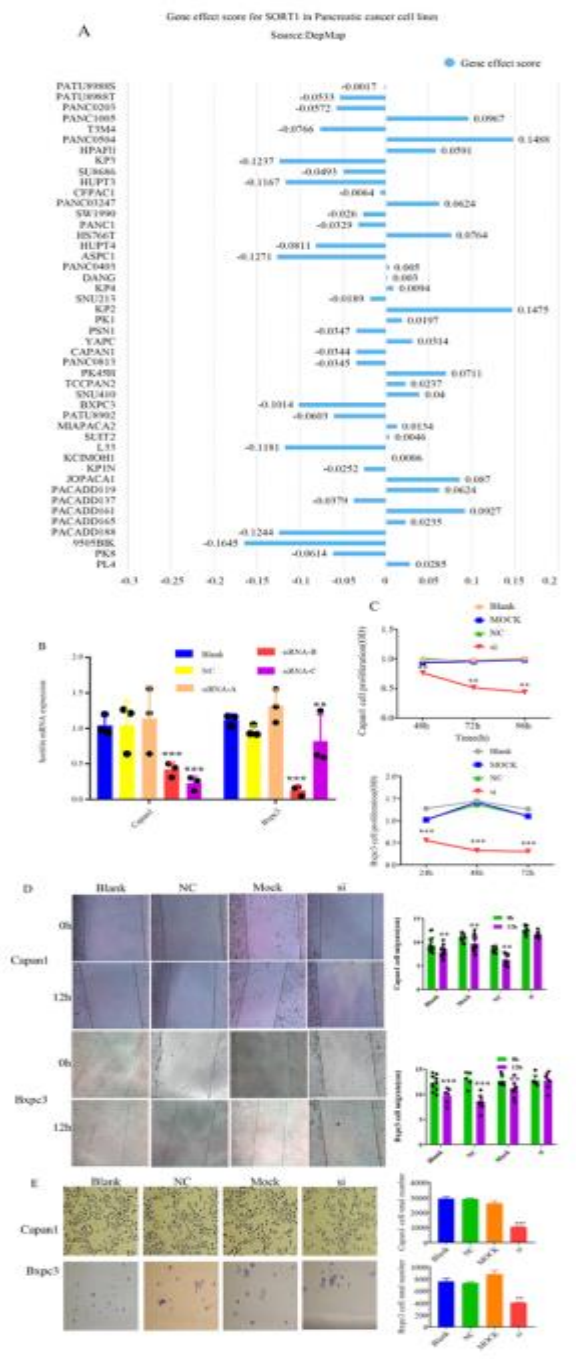


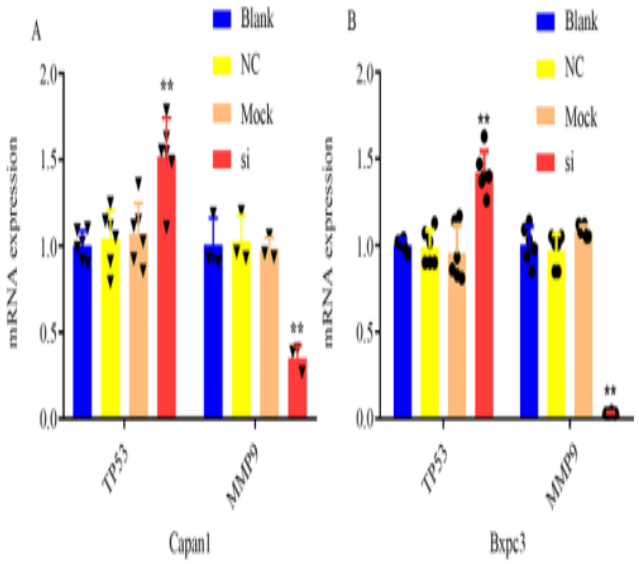
Figure 5: SORT1 expression in cell lines and knockdown inhibits proliferation, migration and invasion in PC cells.



Gene effect score for SORT1 in Pancreatic cancer cell lines;B.RT-PCR verification of sortilin knock down level.Five groups were set up: blank control, negative control(siRNA negative control), siRNA-A, siRNA-B and siRNA-C. After 48 and 24 hours of transfection,it was found that siRNA-B($P<0.001$) and siRNA-C($P<0.001$) knocked down sortilin in both cells;C.PC cells proliferation after knocking down sortilin. Capan1 cell proliferation($P<0.01$) decreased in the knockdown group with an inhibition rate of 71.21% after 48 hours of transfection.

Bxpc3 cell proliferation(P<0.001) decreased in the knockdown group with an inhibition rate of 52.28% after 24 hours of transfection.D. PC cells Migration of two cell lines after knocking down sortilin.After 12 hours of transfection, the cells in the blank, negative and mock groups showed varying degrees of migration, with no significant migration in the transfected groups(P<0.01);E.Invasion of two cell lines after knockdown sortilin. After SORT1 inhibition, the invasiveness of Capan1 cells in the inhibition group (si) was significantly lower than that in the blank group (si:404 ± 5 vs. blank:758 ± 40,p<0.001), and the number of invading Bxpc3 cells in the inhibition group (si) was also significantly lower than that in the blank group (si:1044 ± 38 vs. blank:2925 ± 93,p<0.001).

Figure 6: mRNA expression of TP53 and MMP9 in pancreatic cancer cells



A.There was no statistical significance in the blank group, negative control (p=0.474) group and Mock group(p=0.970), and there was statistical significance in the blank group and knockdown group(p=0.002) in TP53.There was no statistical significance in the blank group, negative control (p=0.400) group and mock group(p=0.600), and there was statistical significance in the blank group and knockdown group(p=0.0001) in MMP9;

B.There was no statistical significance in the blank group, negative control (p=0.351) group and mock group(p=0.101), and there was statistical significance in the blank group and knockdown group(p=0.006) in TP53.There was no statistical significance in the blank group, negative control (p=0.896) group and mock group(p=0.143), and there was statistical significance in the blank group and knockdown group(p=0.002) in MMP9.

DISCUSSIONS

Pan-cancer analysis of SORT1 in the GEPIA database revealed it was indeed upregulated in pancreatic cancer.IHC verification found SORT1 had good predictive and diagnostic value, consistent with F. Gao et al.(2020). Based on this, we further determined the diagnostic value of NSE, with a diagnostic threshold around 0.85 and sensitivity and specificity of 90.9% and 68.3%, respectively.GO and KEGG analyses revealed SORT1 may act as a ubiquitin-like protein ligase in pancreatic cancer, linking ubiquitin-like proteins Lambert et al.(2018) to the TP53 signaling pathway and transcriptionally regulating Yeh et al.(2019) related genes, resulting in aberrant expression.Approximately 114 genes were aberrantly expressed during this process.It is well known that ubiquitination is essential for a variety of cellular processes by the dynamic regulation of proteins related to cell growth, proliferation, and survival. Dysregulation of ubiquitin-mediated proteasomal degradation has become an important mechanism in the pathogenesis of multiple cancers Han et al.(2022), Cuneo et al.(2019).

Of these 114 CGs,we focused on TP53 and MMP9 to investigate how SORT 1 affects the pathogenesis of pancreatic cancer. First, we analyzed 46 pancreatic cancer-related cell lines and selected BXPC3 and CAPAN1 cells for the study. It is well known that TP53 plays an important role in promoting apoptosis and can cooperate with other signaling molecules to inhibit tumor Wang et al.(2018). In the study conducted by Ruggeri B and Khan AA et al,TP53 is abnormally expressed in pancreatic cancer cells. In this in vitro cell experiment, we knocked down SORT1 and observed that TP53 expression was up-regulated in the cells of the two strains, and the proliferation, migration and invasion ability of pancreatic cancer cells were reduced to varying degrees. Our study adds that one of the reasons for the abnormal expression of TP53 in pancreatic cancer cells may arise from the regulation of SORT 1. Of course, this also needs more research evidence to support it, so this is the direction of our next research study.According to the GO and KEGG analysis,we sepeculated that upregulated SORT1 in pancreatic cancer may lead to TP53 loss through the ubiquitination process Yi et al.(2019), thus promoting further cancer development—a notion that may provide new insights into pancreatic cancer pathogenesis.Of course, this also needs more research evidence to support it.

Interestingly, although MMP9 did not show direct links with SORT1 in the bioinformatics analysis, MMP9 was significantly downregulated and pancreatic cancer cell invasion capacity decreased in the in vitro assays following SORT1 inhibition. MMP9 Ren et al.(2018) is a well-known matrix metalloproteinase that degrades

the extracellular matrix and remodels it Deng et al.(2019), Jiang et al.(2019), Jing et al.(2019).Although extracellular matrix proliferation and extensive fibrosis are closely linked to pancreatic cancer growth, proliferation and metastasis Dumaresq-Doiron et al.(2013), more data is needed to elucidate this phenomenon.

In conclusion, we identified a novel mechanism by which SORT1 promotes pancreatic cancer proliferation through effects on ubiquitinated TP53 and MMP9 expression.These findings suggest that novel therapeutic strategies targeting SORT1 may prove beneficial for pancreatic cancer treatment. However, further data and more extensive analysis are needed to determine if SORT1 plays a similar role across other pancreatic cancer cell lines.

DECLARATIONS

Ethical Approval

The studies involving human participants were reviewed and approved by the Ethics Committee of North Sichuan Medical College.

Authors' contributions

Di Xu designed the work and wrote the manuscript and performed the statistical analysis. Rong Cai and Shangqing Liu participated in data analysis ,the discussion and language editing.Di Xu, Rong Cai and Shangqing Liu collected clinical information and validated the data. Both Di Xu and Rong Cai saw and verified all the raw data.Shangqing Liu reviewed the manuscript. Rong Cai contributed equally to this work.All authors read and approved the final manuscript.

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Availability of data and materials

Applicable.Based on patient privacy protection, the original data may not be fully made available, so may be made available as appropriate upon request.

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Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors affirm that they do not have any identifiable competing financial interests or personal affiliations that may have influenced or biased the outcomes presented in this research paper.

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