Original Article

Upregulated expression of *PTTG1* is associated with progression of pancreatic cancer

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Background: Pancreatic cancer (PC) is an aggressive disease with a very poor prognosis. The insidious onset, rapid progression, and resistance to conventional therapies mark the imperious need for novel biomarkers and therapeutic targets. The pituitary tumor transforming gene 1 (*PTTG1*), implicated in tumorigenesis and cellular transformation, has been studied in various cancers, however, its role and mechanisms in PC remain to be elucidated for better understanding the disease pathology and in enhancing patient management strategies.

Methods: The present study examined the *PTTG1* messenger RNA (mRNA) expression levels and clinical significance through meta-analysis based on The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. Immunohistochemistry (IHC) was used to measure *PTTG1* protein levels in PC and adjacent non-cancerous tissues. A correlation was observed between *PTTG1* expression and some clinical characteristics based on the TCGA and IHC data. Univariate and multivariate Cox regressions were used to identify independent prognostic factors. Kaplan-Meier (KM) survival analysis was performed. The co-expressed genes of *PTTG1* were determined by integrating online tools, and the enrichment analyses were performed to determine *PTTG1*-related pathways and hub co-expressed genes.

Results: *PTTG1* was highly expressed in PC tissues based on the TCGA, GEO, and IHC data. The combined standard mean difference (SMD) values of *PTTG1* expression based on TCGA and GEO databases was 1.02 [95% confidence interval (CI): 0.74–1.30]. The area under the curve (AUC) based on the summary receiver operating characteristic (sROC) curve was 0.93 (95% CI: 0.90–0.95). *PTTG1* overexpression was remarkably correlated with an inferior overall survival (OS). A total of 367 genes were identified as co-expressed genes of *PTTG1* in PC and were mainly involved in the cell cycle pathway. The four identified core genes were *CDK1*, *CCNA2*, *CDC20*, and *MAD2L1*.

Conclusions: The upregulated expression of *PTTG1* plays an essential role in PC's progression as a biomarker.

Keywords: Pancreatic cancer (PC); pituitary tumor transforming gene 1 (*PTTG1*); bioinformatics; prognosis; immunohistochemistry (IHC)

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Introduction

Pancreatic cancer (PC), as one of the most aggressive human solid malignancies, is the 4th-highest cause of cancer morbidity and mortality in the USA and the 7th worldwide. Patients with PC, including in China, face a very high risk of mortality and extremely poor prognosis, and the incidence of PC has displayed a clear ascendant trend throughout the years. In America, for the patients with PC, overall survival (OS) is dismal and the 5-year survival rate of approximately 9% has not shown significant improvement compared with other cancer types. In addition, PC is anticipated to become the 2nd or 3rd most common cause of cancer deaths in high-income countries in the next years (1). However, PC is often silent as most patients remain asymptomatic in its early stages and are initially diagnosed at an advanced stage, for which few effective therapies exist (2-5). Currently, therapeutic strategies such as surgical excision and chemotherapy are the main treatment options for patients, but they remain unsatisfactory due to the relatively few operation opportunities, drug resistance, and cancer recurrence (6,7). Molecular markers that could be used to accurately predict the course of the disease or response to therapy have not yet been applied in the treatment of PC. Therefore, molecular biomarkers and targeted therapy may bring new hope for early diagnosis and management of PC (7). For instance, Du *et al.* utilized the overexpression of *SGLT-2* to predict the prognosis of the patients with pancreatic ductal adenocarcinoma (8). There is a critical need at this juncture for new strategies to explore the molecular mechanism of PC and to improve its diagnosis and treatment.

Pituitary tumor transforming gene 1 (*PTTG1*), a recently characterized protooncogene, resides at human chromosome 5q33.3 and encodes the mammalian protein securin, originally isolated from rat pituitary tumor cells (9). *PTTG1* has an essential role in cell-cycle regulation and sister chromatid separation during mitosis (10) and its activation has been associated with the regulation of growth and progression in many types of cancer cells (11). Accumulating evidence suggests that *PTTG1* is a potential biomarker for cancer malignancy and a cell-cycle regulatory protein involved in a variety of cellular processes (12). A published study has shown that *PTTG1* in hepatocellular carcinoma may be an independent prognostic indicator and potential therapeutic target by upregulating c-myc (13). One previous study has shown that *PTTG1* expression has an association with the expression of *bFGF* and *VEGF*, therefore contributing to cell proliferation and migration (14). In addition, aberrant expression of *PTTG1* is highly immunogenic and is an important target for immunotherapy of multiple myeloma (15). Previous data reported that altered levels of *PTTG1* are expressed in breast cancer cells, suggesting that *PTTG1* has a role in breast tumorigenesis (16). Similarly, a recent paper showed that *PTTG1* is known to be down-regulated in thyroid cancer cells (17). *PTTG1* has been verified to be abundantly expressed in many tumors, such as glioma (18), lung cancer (19), laryngeal cancer (20), and prostate cancer (21).

For the present *PTTG1* is ascertained to be highly correlated with various aspects of PC, including gender, clinical stage, and prognosis. Long *et al.* found that higher expression of *PTTG1* was associated with higher clinical stages and worse prognosis of PC, the potential mechanism of which was the enhanced OAd5 transduction into PC cells by increasing CXADR expression on the cell surface (22). In a study of human PC tissues, the scholars discovered that *PTTG1* was highly expressed in PC, and its expression was related to the gender of PC patients (23). However, no significant correlation was found between *PTTG1* expression and perineural infiltration as well as age, tumor sizes, pathological styles, and distant metastases in PC patients. Since *PTTG1* plays an essential role in PC progression and in the present study, we analyzed the expression of *PTTG1* messenger RNA (mRNA) and protein

Highlight box

Key findings

- This study demonstrates that pituitary tumor transforming gene 1 (*PTTG1*) was highly expressed in pancreatic cancer (PC) tissues based on The Cancer Genome Atlas, Gene Expression Omnibus and immunohistochemistry databases. Meanwhile, we also demonstrate that *PTTG1* may serve as a driving gene associated with the occurrence and progression of PC.

What is known and what is new?

- Research has confirmed that *PTTG1* participates in the occurrence and development of many tumors, such as glioma, lung cancer, laryngeal cancer, and prostate cancer.
- The upregulated expression of *PTTG1* plays an essential role in PC's progression as a biomarker.

What is the implication, and what should change now?

- Our finding may contribute to prolong patients' survival time with PC.

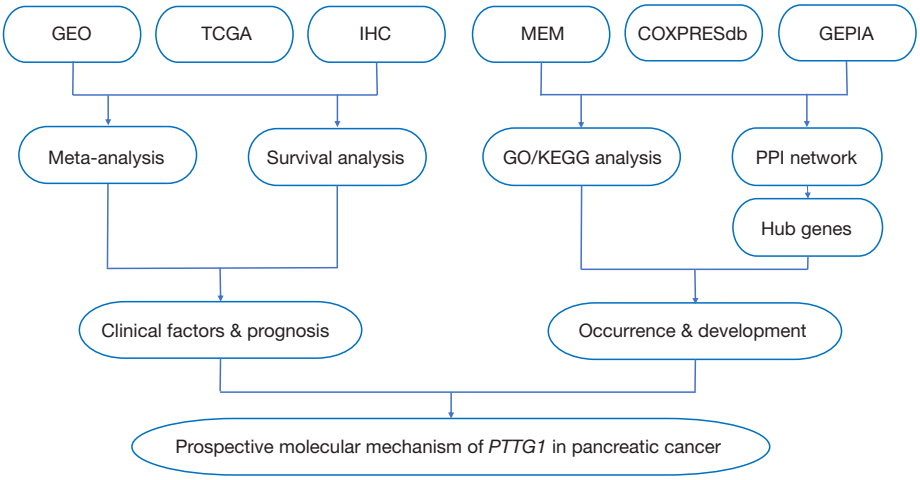

Figure 1 Flow chart for the research. GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas; IHC, immunohistochemistry; MEM, Multi Experiment Matrix; GEPIA, Gene Expression Profiling Interactive Analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; *PTTG1*, pituitary tumor transforming gene 1.

and their relation to progression in PC to demonstrate the role of *PTTG1* in the development of PC and its value as a molecular target for cancer therapy. We present this article in accordance with the REMARK reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-979/rc).

Methods

Study design

Hence, a new mining method of high-throughput analysis based on sequence data such as microarrays, RNA-sequencing (RNA-seq), and all available published documents was presented to evaluate the potential of the *PTTG1* gene in early diagnosis and prompt treatment. The mining of various databases, and bioinformatics analyses are required to verify the different expression levels, clinical value, and potential pathological role of *PTTG1* in PC. Additionally, further validation of the protein expression level of *PTTG1* was conducted using in-house immunohistochemistry (IHC). Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and a protein-protein interaction (PPI) network were constructed and analyzed to harvest the enrichment functions, potential pathways, and hub genes. In addition, comprehensive meta-analyses of hazard ratio (HR) and Kaplan-Meier (KM) survival were performed to examine the potential prognostic value of *PTTG1* expression in patients with PC. The study design is presented in *Figure 1*.

Data extraction of PTTG1 expression from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases

Initially, PC-related RNA-seq data were searched in the GEO (https://www.ncbi.nlm.nih.gov/gds/) database using the search strategy 'pancreas' OR 'pancreatic', AND 'carcinoma', 'adenocarcinoma', 'cancer', 'neoplasm', 'tumor', 'tumour', 'malignan*', 'neoplas*', 'PDAC', 'or', 'PAAD' OR 'PC'. 'Homo sapiens' was used to limit the search range. The criteria for the chip filter conditions were as follows: (I) human pancreatic tissue; (II) comparative RNA expression data chip with PC tissue and paracancer or normal pancreatic tissue; and (III) patients without adjuvant therapies, including radiotherapy and chemotherapy. The exclusion criteria were as follows: (I) samples based on cell line; (II) methylation; (III) samples that have been treated with adjuvant therapies; and (IV) cancer samples without a comparison of paracancer or normal tissue. The mRNA expression data associated with PC of TCGA was extracted from the University of California Santa Cruz (UCSC) Xena (https://xena.ucsc.edu/). Therefore, we obtained the mRNA profiles of 179 PC tissues and four non-cancerous tissues.

In-house IHC

The tissue microarray that included 79 cases of PC tissues

and 73 adjacent non-cancerous tissues (HPanA170Su04) was purchased from Shanghai Outdo Biotech Company (Shanghai, China) and some clinical information for each sample, such as age, gender, clinical stage, surgery and OS were also provided. OS was defined as the time interval from surgery to cancer-related death or the last follow-up. In the IHC analysis, *PTTG1* was detected with anti-securin antibody (at a 1/100 dilution; JB37-37; HUABIO, Woburn, MA, USA). The *PTTG1* expression intensity for each sample was evaluated based on the score, and the score was calculated as the product of the proportion of stained cells among all cells (0, <5%; 1, 5–10%; 2, 11–50%; 3, 51–75%; 4, >75%) and the staining degree of the positive cells (0, no staining; 1, light yellow or yellow; 2, brown; 3, dark brown). Finally, a total expression score was given ranging from 0 to 12 (23). Based on the analysis results by X-tile software (Yale School of Medicine, New Haven, CT, USA), *PTTG1* protein expression was regarded as negative expression in PC tissues when the score was 0–6, and as positive expression when the score was ≥6. The scoring of IHC staining was performed by two experienced pathologists without knowing the patient's clinical information. The study was approved by the Ethics Committee of Shanghai Outdo Biotech Company (No. YB M-05-02) and carried out in accordance with the Declaration of Helsinki (as revised in 2013).

Acquisition of co-expressed genes of PTTG1 in PC

The Multi Experiment Matrix (MEM; https://biit.cs.ut.ee/mem/index.cgi) website is a web-based tool employed in multi experiment gene expression query. The COXPRESdb (https://coxpresdb.jp/) is a database providing co-expression relationships for 11 animal species such as human and mouse (24). Putative co-expressed genes of *PTTG1* were determined based on the MEM and COXPRESdb. In MEM, a P value of less than 0.05 was considered statistically significant. In COXPRESdb, the upper limit number was set to output 2,000 genes. The Gene Expression Profiling Interactive Analysis database (GEPIA; http://gepia.cancer-pku.cn/) is a cancer data mining website, based on the RNA sequencing data expression of 9,736 tumors and 8,587 normal samples from TCGA and the Genotype-Tissue Expression (GTEx) projects. The differentially expressed genes (DEGs) of PC were further studied when the $|\log_2(\text{fold change})| \geq 1.5$ and adjusted P value <0.05 in the GEPIA database. Intersecting genes of the three dataset parts were treated as co-expressed genes of *PTTG1* in PC (25).

Bioinformatics analysis of overlapping genes

The Database for Annotation, Visualization and Integrated Discovery tool (DAVID 6.8; https://david.ncifcrf.gov/) was used to perform GO functional annotation and KEGG biological pathway analyses by using the overlapped genes of *PTTG1*. Statistical significance was indicated when the pathways and enrichment significance level were set as a P value <0.05. GO is an ontology used extensively in this field of bioinformatics for annotating genes, gene products, and sequences. It covers three aspects of biology: biological process (BP), cellular component (CC), and molecular function (MF) (26). The top 10 significant function enrichment analyses included 10 BP, CC, and MF entries as well as 10 KEGG pathways, which were visualized using the 'GOplot' package of R software v.3.5.2 to reveal possible enrichment of candidate target genes. A PPI network was established and forecast by the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://string-db.org/). Based on the degree of nodes, hub co-expressed genes of *PTTG1* were identified. Hub genes encoded protein expression and IHC staining in PC and normal pancreatic tissues were searched in The Human Protein Atlas (HPA; https://www.proteinatlas.org/) database to present the expression pattern.

Statistical analysis

In the statistical analysis, continuous variable results of *PTTG1* expression were presented as means ± standard deviation (SD). The measurement data of normal distribution were compared by *t*-test between the two groups and analysis of variance between multiple groups; The measurement data with non-normal distribution were compared by Mann-Whitey *U* test between the two groups and Kruskal-Walls *H* test between multiple groups. Additionally, receiver operating characteristic (ROC) curve analyses were constructed to compare the predictive performance of *PTTG1* in the study groups. ROC curves were plotted with sensitivity *vs.* 1 – specificity and were then used to obtain the cut-off points for optimal sensitivity and specificity. The area under the curve (AUC) was performed to assess the overall discriminative capability of the variables. The best cut-off value was

determined as the point with a maximum Youden index. The diagnostic value parameters of true positivity (TP), false positivity (FP), false negativity (FN), and true negativity (TN) cases were calculated from the ROC curve. Further, we summarized these analyses and drew a summary ROC (sROC) curve to investigate the accuracy of *PTTG1* in discriminating between cancerous and normal tissue. The statistical analysis was carried out using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Additionally, we conducted this meta-analysis to explore the association between *PTTG1* and the risk of PC. Stata 12.0 (StataCorp., College Station, TX, USA) was utilized to perform the forest plots with a standard mean difference (SMD) and 95% confidence interval (CI), and funnel plots based on TCGA and GEO data. A funnel plot was carried out to evaluate the publication bias. The heterogeneity of the meta-analysis was evaluated with Higgins I^2. When the $P \geq 0.05$ or $I^2 \leq 50\%$, it was deemed there was less or no heterogeneity, and the fixed effects model was used. A random effect model should be selected if $P<0.05$ and $I^2>50\%$. A value of $P<0.05$ (two-tailed) was considered statistically significant in all analyses. GraphPad Prism v.8.0 software (GraphPad Software, San Diego, CA, USA) was used to obtain violin plots and ROC curves with the RNA-seq and microarray data.

Evaluation of the prognostic value of PTTG1 in PC

Cox regression was employed to calculate the HR and 95% CIs for PC. The prognostic data downloaded from TCGA and GEO were analyzed using SPSS 22.0 to estimate HR and 95% CIs. An observed HR >1 indicated that patients with high *PTTG1* expression were more likely to have an unfavorable prognosis. A comprehensive meta-analysis was conducted on the HR to evaluate the efficiency of *PTTG1* in PC prognosis. The prognostic data from TCGA and GEO were analyzed with the Cox regression model to estimate the effect of *PTTG1* on survival time. A funnel plot was performed to evaluate publication bias by using SelnHR as the abscissa and lnHR as the ordinate. We then conducted the sensitivity analyses using Stata 12.0. In addition, the KM plotter survival analysis tool (https://kmplot.com/) with detailed survival including gene chip and RNA-seq data from GEO, European Genome-phenome Archive (EGA), and TCGA databases, was used to validate the effect of *PTTG1* in PC. In this research, the expressions and prognostic values of *PTTG1* were carried out through GEPIA and KM online datasets.

Results

PTTG1 expression in PC from the GEO, TCGA, and IHC databases

A total of 29 GEO microarray datasets containing *PTTG1* were included in the current study, namely, GSE14245, GSE11838, GSE15471, GSE16515, GSE19279, GSE22780, GSE28735, GSE32676, GSE32688, GSE36076, GSE41368, GSE43288, GSE46234, GSE49515, GSE55643, GSE56560, GSE58561, GSE60646, GSE60980, GSE62165, GSE62452, GSE63158, GSE71729, GSE71989, GSE74629, GSE91035, GSE101448, GSE101462, and GSE107610. The final analysis included 1,056 PC samples and 394 non-cancer samples. In the overall data analysis of 29 GEO microarrays, the results showed that 19 of the selected *PTTG1* microarrays (GSE15471, GSE16515, GSE28735, GSE32676, GSE32688, GSE41368, GSE43288, GSE55643, GSE56560, GSE60646, GSE60980, GSE62165, GSE62452, GSE63158, GSE71729, GSE71989, GSE91035, GSE101462, and GSE107610) had remarkably increased expression in cancer tissues compared to normal tissues. The mRNA expression data of TCGA associated with PC were downloaded from the UCSC Xena database. We extracted the mRNA profiles of 179 PC tissues and four non-cancerous tissue expression data together with corresponding clinical parameters. For the IHC data, *PTTG1* was found to be upregulated in 79 PC tissues (6.47±3.042) compared to 73 normal tissues (4.67±2.404, $P<0.001$). The expression of *PTTG1* in the GEO microarrays and the TCGA and IHC databases is shown in *Tables 1,2*.

Based on the obtained RNA-seq and microarray data, GraphPad Prism v.8.0 was used for statistical analysis and violin plots. The software was applied to compare the expression levels of *PTTG1* in PC and non-cancerous tissues ($P<0.05$, *Figures 2,3*). ROC curves were also generated (*Figures 4-6*). In the overall data analysis of 29 GEO chips, the results revealed that 19 chips had significant upregulation in PC tissues, compared with normal tissues. The box chart showed the mRNA expression of the *PTTG1* gene in the PC tissues (179 cases) and non-PC tissues (171 cases) in GEPIA. The results indicated that the expression level of *PTTG1* in PC tissues was significantly higher than that in non-PC tissues ($P<0.05$, *Figure 7A*). GEPIA analysis of high and low expression levels of *PTTG1* in PC on the pathological stage of patients is shown in *Figure 7B*. *PTTG1* expression levels did not

Table 1 *PTTG1* expression profile based on IHC data, GEO datasets, and TCGA sequencing data

Datasets	Platform	Year	Country	Patients			Normal			*t* value	P value
				Number	Mean	SD	Number	Mean	SD		
GSE14245	GPL570	2008	USA	12	4.5883	4.23926	12	13.0092	13.35287	2.082	0.057
GSE11838	GPL6977	2002	USA	28	7.5244	9.60781	4	2.1813	0.41225	–1.097	0.282
GSE15471	GPL570	2009	Romania	36	8.2642	0.85627	36	7.2728	1.04296	–4.408	<0.001
GSE16515	GPL570	2009	USA	36	296.8483	293.835	16	79.1056	71.5034	–2.911	0.005
GSE19279	GPL96	2013	United Kingdom	4	6.178	0.54797	3	5.4217	0.13092	–2.290	0.071
GSE22780	GPL570	2011	USA	8	754.8165	336.3105	8	624.235	489.5187	–0.622	0.544
GSE28735	GPL6244	2012	USA	45	4.9654	0.5298	45	4.3872	0.53832	–5.135	<0.001
GSE32676	GPL570	2011	USA	25	8.0265	1.38649	7	6.253	2.08149	–2.675	0.012
GSE32688	GPL570	2011	USA	25	8.0265	1.38649	7	6.253	2.08149	–2.675	0.012
GSE36076	GPL570	2014	Singapore	3	8.1048	1.33284	10	8.1048	0.29178	0.000	1.000
GSE41368	GPL6244	2013	Italy	6	88.5152	31.78445	6	37.3604	7.93625	–3.825	0.010
GSE43288	GPL96	2013	United Kingdom	4	6.5067	0.43875	3	5.7663	0.1229	–2.781	0.039
GSE46234	GPL570	2017	Norway	4	2,544.08	2,180.275	4	687.6562	719.012	–1.617	0.157
GSE49515	GPL570	2013	Singapore	3	8.1048	1.33284	10	8.1048	0.29178	0.000	1.000
GSE55643	GPL6480	2014	United Kingdom	45	12.181	1.2603	8	10.9756	1.70234	–2.363	0.022
GSE56560	GPL5175	2014	United Kingdom	28	128.3821	53.96653	6	59.7369	13.82657	–3.060	0.004
GSE58561	GPL14550	2014	Norway	3	12.3301	0.73458	2	12.2394	0.30142	–0.159	0.884
GSE60646	GPL5175	2015	USA	10	5.821	1.28669	10	4.426	0.95763	–2.750	0.013
GSE60980	GPL14550	2015	Norway	49	10.7902	0.92524	12	9.0403	1.5351	–3.784	0.002
GSE62165	GPL13667	2016	Belgium	118	7.6285	1.01979	13	5.1483	1.17441	–8.199	<0.001
GSE62452	GPL6244	2016	USA	69	4.8242	0.51968	61	4.3376	0.4681	–5.616	<0.001
GSE63158	GPL5175	2014	United Kingdom	28	128.3821	53.96653	6	59.7369	13.82657	–3.060	0.004
GSE71729	GPL20769	2015	USA	145	7.1439	0.7425	46	6.4856	0.56642	–5.521	<0.001
GSE71989	GPL570	2015	USA	13	9.4416	0.8487	8	8.0801	0.2303	–5.466	<0.001
GSE74629	GPL10558	2015	Spain	36	7.0844	0.08333	14	7.0775	0.06365	–0.278	0.782
GSE91035	GPL22763	2016	USA	25	10.2488	1.12223	8	7.5965	0.39864	–10.007	<0.001
GSE101448	GPL10558	2018	Germany	24	7.9148	0.34089	19	8.1349	0.5504	1.610	0.115
GSE101462	GPL10558	2018	Germany	6	6.5341	0.15836	4	6.2645	0.04729	–3.250	0.012
GSE107610	GPL15207	2018	Japan	39	10.56	0.65089	2	9.3773	1.16649	–2.438	0.019
TCGA				179	8.0081	1.01655	4	7.083	0.7361	–1.807	0.072
IHC				79	6.47	3.042	73	4.67	2.404	4.056	<0.001

PTTG1, pituitary tumor transforming gene 1; IHC, immunohistochemistry; GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas; SD, standard deviation.

Table 2 Potential of *PTTG1* to serve as a biomarker for identifying PC tissues and normal tissue

Datasets	Sensitivity (%)	Specificity (%)	TP	FP	FN	TN
GSE14245	100.00	25.00	12	9	0	3
GSE11838	75.00	100.00	21	0	7	4
GSE15471	100.00	50.00	36	18	0	18
GSE16515	88.90	81.20	32	3	4	13
GSE19279	75.00	100.00	3	0	1	3
GSE22780	75.00	75.00	6	2	2	6
GSE28735	88.90	62.20	40	17	5	28
GSE32676	92.00	71.40	23	2	2	5
GSE32688	92.00	71.40	23	2	2	5
GSE36076	33.30	100.00	1	0	2	10
GSE41368	100.00	100.00	6	0	0	6
GSE43288	100.00	100.00	4	0	0	3
GSE46234	75.00	100.00	3	0	1	4
GSE49515	33.30	100.00	1	0	2	10
GSE55643	84.40	75.00	38	2	7	6
GSE56560	75.00	100.00	21	0	7	6
GSE58561	33.30	100.00	1	0	2	2
GSE60646	90.00	90.00	9	1	1	9
GSE60980	98.00	75.00	48	3	1	9
GSE62165	98.30	84.60	116	2	2	11
GSE62452	60.90	86.90	42	8	27	53
GSE63158	85.70	100.00	24	0	4	6
GSE71729	41.40	97.80	60	1	85	45
GSE71989	84.60	100.00	11	0	2	8
GSE74629	38.90	78.60	14	3	22	11
GSE91035	96.00	100.00	24	0	1	8
GSE101448	100.00	10.50	24	17	0	2
GSE101462	83.30	100.00	5	0	1	4
GSE107610	71.80	100.00	28	0	11	2
TCGA	82.70	75.00	148	1	31	3
IHC	92.41	44.58	73	40	6	33

PTTG1, pituitary tumor transforming gene 1; PC, pancreatic cancer; TP, true positive; FP, false positive; FN, false negative; TN, true negative; TCGA, The Cancer Genome Atlas; IHC, immunohistochemistry.

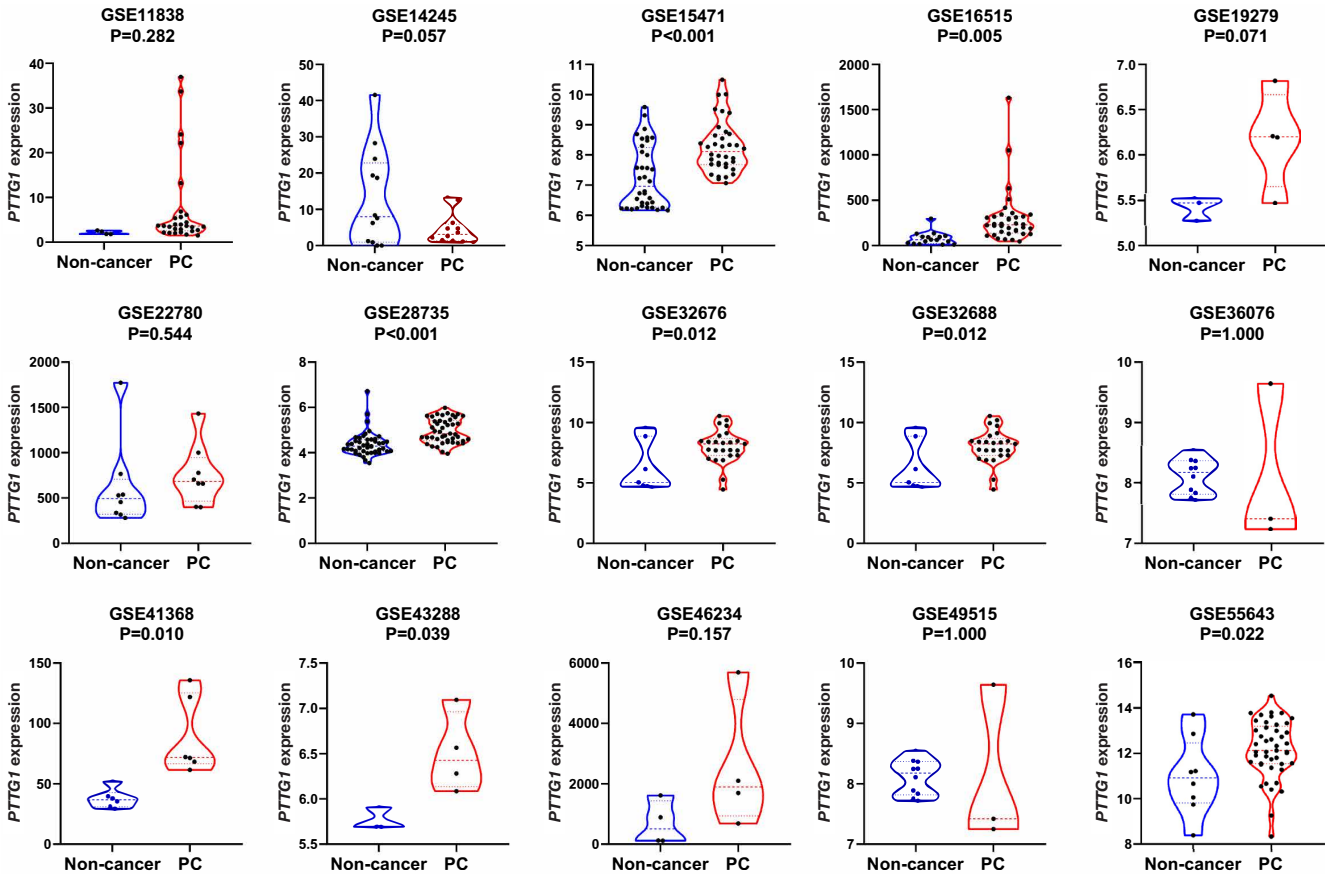

Figure 2 Differential expression of *PTTG1* in PC and non-cancer tissues from GEO datasets and TCGA sequencing data. *PTTG1*, pituitary tumor transforming gene 1; PC, pancreatic cancer; GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas.

significantly differ between the different pathological stages (P=0.07).

Correlation between PTTG1 expression and clinicopathological parameters

The Cox regression analysis was applied to analyze the prognostic factors. According to the IHC data, the univariate analysis indicated that there was no significant relationship between *PTTG1* expression and OS (P=0.317), as shown in *Table 3*. Other clinical parameters including gender (HR: 0.48, 95% CI: 0.30–0.76, P=0.002), size (HR: 1.91, 95% CI: 1.20–3.02, P=0.006), T stage (HR: 2.13, 95% CI: 1.33–3.40, P=0.002), N stage (HR: 1.64, 95% CI: 1.14–2.34, P=0.007), and M stage (HR: 5.61, 95% CI: 3.22–9.76, P<0.001) were also associated with poorer OS. Multivariate analysis showed that gender (HR: 0.36, 95% CI: 0.21–0.60, P<0.001) and M staging (HR: 4.71, 95% CI: 2.47–9.01, P<0.001) were independently associated with OS. In addition, the association between *PTTG1* and some clinical parameters was also calculated using TCGA data, and the results indicated that the expression of *PTTG1* was associated with the histological grade (*Table 4*, *F*=4.020, P=0.009). As the grade of PC increased, *PTTG1* expression also increased.

Meta-analysis of GEO and TCGA

We used Stata 12.0 for the meta-analysis of continuous variables to conduct a holistic evaluation of *PTTG1* expression levels. A total of 1,056 PC cases and 394 non-cancerous tissue cases were collected in our study from 29 GEO microarrays and TCGA sequencing data. *PTTG1* in PC tissues was significantly higher than that in non-cancerous controls (SMD: 1.02, 95% CI: 0.74–1.30, *Figure 8A*). Due to the high heterogeneity in this meta-

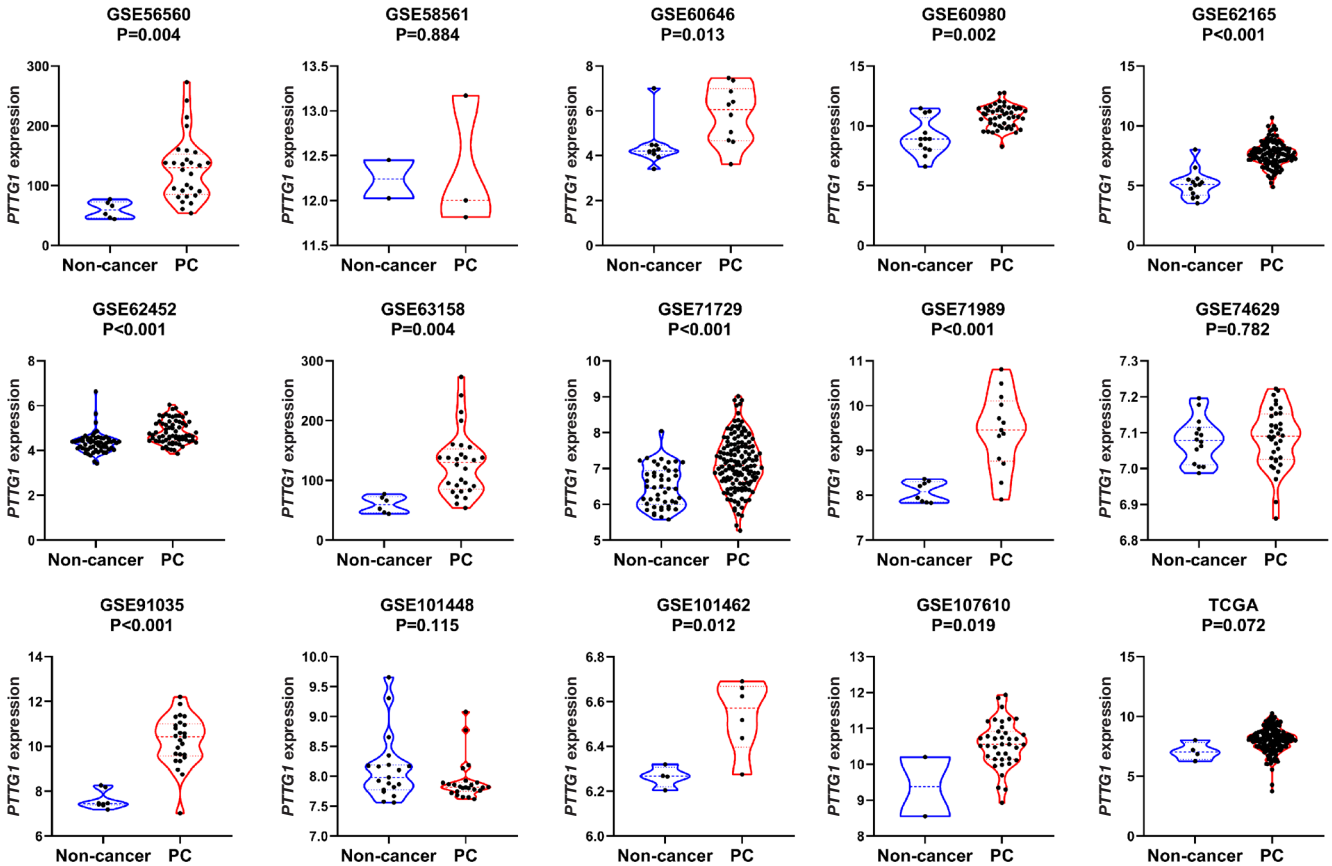

Figure 3 Differential expression of *PTTG1* in PC and non-cancer tissues from GEO datasets and TCGA sequencing data. *PTTG1*, pituitary tumor transforming gene 1; PC, pancreatic cancer; GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas.

analysis (I^2=71.4%, P<0.001), a random effects model was used for the analysis. As shown by the sensitivity analysis in *Figure 8B*, there was no significant heterogeneity in the study. At the same time, funnel plots (*Figure 8C*) did not find publication bias. The results of the diagnostic analysis of GEO microarrays and TCGA sequencing data showed that the pooled specificity and sensitivity of *PTTG1* for the diagnosis of PC were 0.86 (95% CI: 0.77–0.92) and 0.87 (95% CI: 0.77–0.93), respectively, sROC (AUC) =0.93 (95% CI: 0.90–0.95) (*Figure 8D*).

The prognostic value of PTTG1 in PC

Concerning the prognostic value, an elevated *PTTG1* level was remarkably correlated with worse OS (HR: 1.36, 95% CI: 1.07–1.75, *Figure 9A*) and with unobserved heterogeneity (I^2=0.0%, P=0.852) when employing the fixed-effect model. Furthermore, a sensitivity analysis was conducted, and the result indicated the pooled HR was stable (*Figure 9B*). As shown in *Figure 9C*, Begg's regression plot revealed no statistically significant publication bias in the eligible studies. Additionally, GEPIA showed a correlation between survival and *PTTG1* gene expression levels in PC patients. The OS (HR: 2.0, P<0.001, *Figure 10A*) and disease-free survival (DFS; HR: 1.7, P=0.013, *Figure 10B*) of PC patients with high expression of *PTTG1* (n=89) was significantly lower than that of patients with low expression of *PTTG1* (n=89). However, KM survival curves showed that there was no significance between *PTTG1* mRNA expression with the OS of PC patients (HR: 1.254, P=0.3104, *Figure 10C*) based on the IHC data.

Genes co-expressed with PTTG1

We collected 1,574 related genes from the MEM database

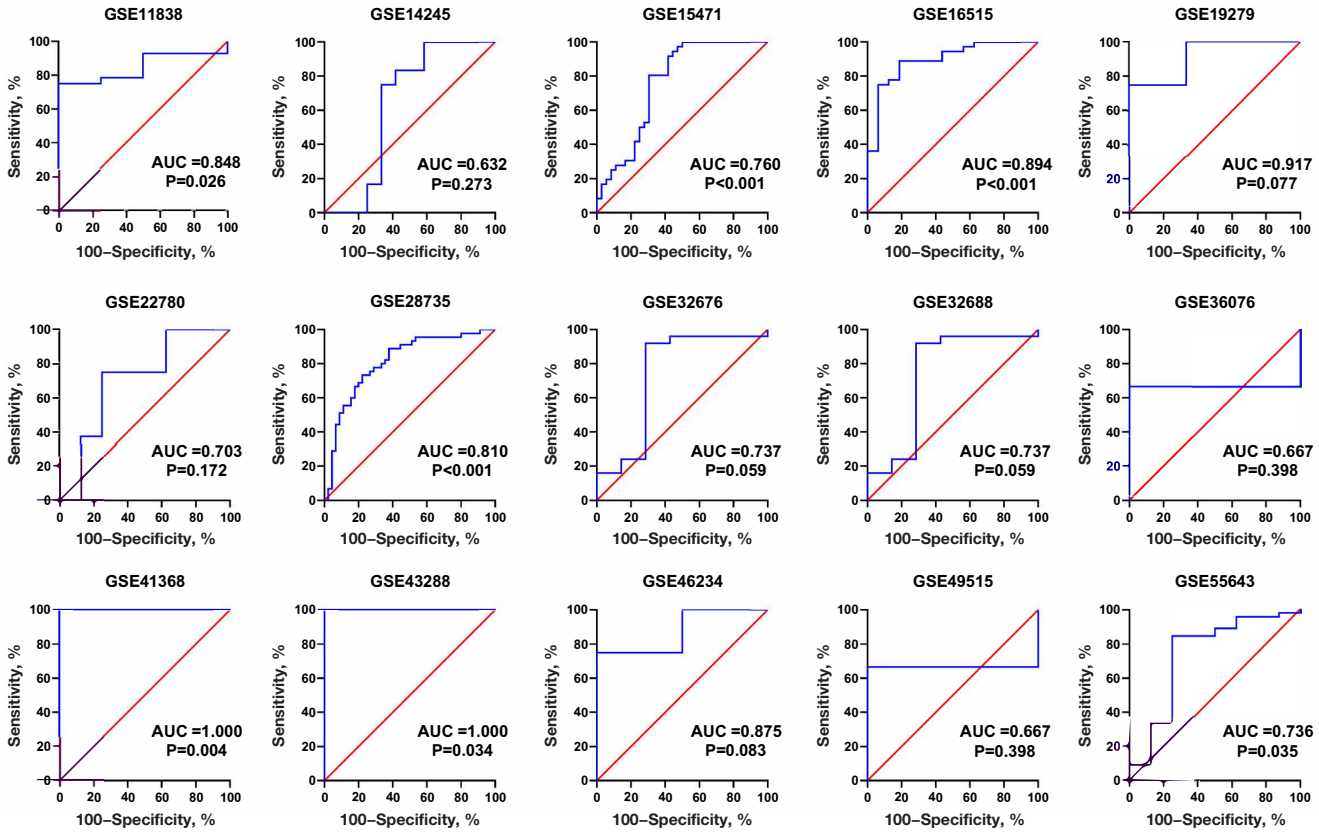

Figure 4 ROC curve of *PTTG1* expression in PC. AUC, area under the curve; ROC, receiver operating characteristic; *PTTG1*, pituitary tumor transforming gene 1; PC, pancreatic cancer.

and identified 2,000 genes from COXPRESdb. From GEPIA, the 5,076 DEGs between PC and non-cancer tissues were selected. We eventually identified 367 overlapping genes (*Figure 11A*).

Bioinformatics analysis

To explore the mechanisms and pathways of *PTTG1*, the 367 overlapping genes were analyzed by using the DAVID and STRING online tools. According to the GO enrichment analysis, in terms of BP, the top 3 most significant processes were cell division, anaphase-promoting complex-dependent catabolic process, and negative regulation of ubiquitin-protein ligase activity involved in the mitotic cell cycle. As for the analysis of CC, the topmost significant annotations were nucleoplasm, nucleus, and cytosol. In the annotations of MF, the top 3 most significant functions were protein binding, poly(A) RNA binding, and threonine-type endopeptidase activity (*Table 2*, *Figure 11B-11D*). The results of the KEGG pathway analysis indicated that DEGs were centralized in these pathways, such as cell cycle, proteasome, and spliceosome (*Table 5*, *Figure 11E*). We placed the top 367 overlapping genes into STRING and generated the PPI network (*Figure 12*). The PPI network analysis of *PTTG1* targets indicated that four hub genes (*CDK1*, *CCNA2*, *CDC20*, and *MAD2L1*) were found at the highest level.

Protein levels of the hub genes from the HPA database

We put the four hub genes into the HPA database to understand the protein expression pattern of these genes: *CDK1* (antibody HPA003387), *CDC20* (antibody CAB004525), *CCNA2* (antibody CAB000114), and *MAD2L1* (antibody HPA003348). *CDK1* exhibited low staining and weak intensity in normal pancreatic tissues and showed medium staining and moderate intensity in PC tissues. *CCNA2* displayed undetected staining and negative

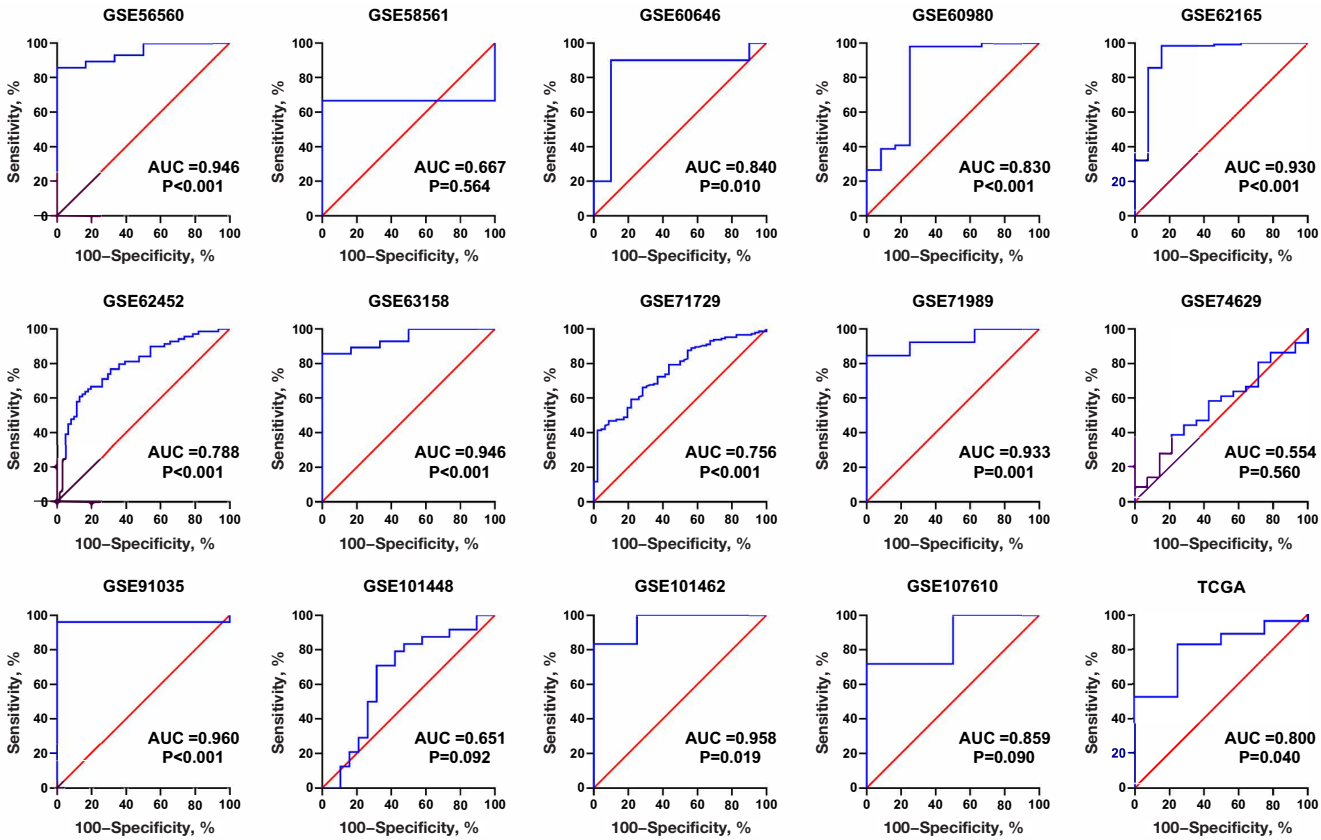

Figure 5 ROC curve of *PTTG1* expression in PC. AUC, area under the curve; TCGA, The Cancer Genome Atlas; ROC, receiver operating characteristic; *PTTG1*, pituitary tumor transforming gene 1; PC, pancreatic cancer.

intensity in normal pancreatic tissues, whereas it displayed medium staining and strong intensity in PC tissues. *CDC20* showed undetected staining and negative intensity in normal pancreatic tissues, and high staining and strong intensity in PC tissues. *MAD2L1* presented low staining and weak intensity in normal pancreatic tissues while recording low staining and moderate intensity in PC tissues.

Discussion

PC is the 4th most common cause of cancer death and is the 12th most common cause of cancer (27). To make things worse, diagnosis of advanced stage, recurrence, and metastasis shatter the treatment window of opportunity for PC patients (28). The tumor microenvironment (TME) in PC is known to influence tumor progression and can be influenced by different tumor characteristics, leading to diverse mechanisms of immune evasion (29). Current therapeutic strategies targeting the TME of PC have been designed to modulate the cancer-associated fibroblast and immune compartments. These strategies include the use of CD40 agonistic monoclonal antibodies, chemotherapy, and immune checkpoint inhibitors (30). As an essential factor influencing TME, *PTTG1* has been associated with tumorigenesis and poor prognosis in a variety of endocrine-related tumors including breast cancer (16) and thyroid cancer (17), as well as nonendocrine-related cancers involving the central nervous, pulmonary, and gastrointestinal systems such as hepatocellular carcinoma (13), multiple myeloma (15), and glioma (18). Though the overexpression of *PTTG1* is controversial in promoting or inhibiting cell proliferation in various kinds of tumors, *PTTG1* is confirmed to be tumorigenic via cell transforming by the induction of chromosomal instability and aneuploidy (31). Moreover, overexpressed PTTG1 may act as a paracrine/autocrine activator, enhancing expression of growth factors that in turn further sustain tumor growth and contribute to the tumorigenic microenvironment.

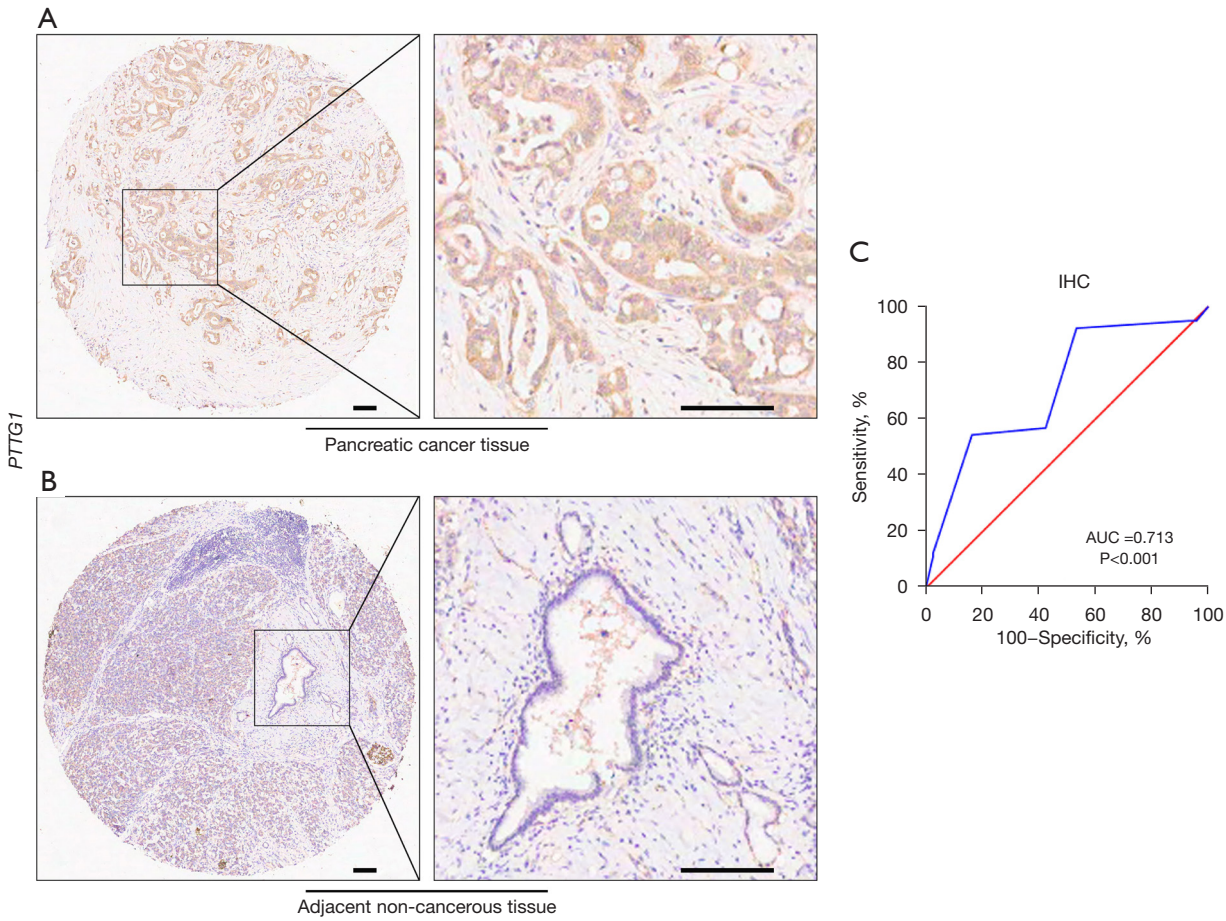

Figure 6 Expression of *PTTG1* protein in PC and adjacent non-cancerous tissues by IHC. (A) *PTTG1* protein expression in PC tissue. Scale bars, 100 and 400 μm. (B) *PTTG1* protein expression in adjacent non-cancerous tissue. Scale bars, 100 and 400 μm. (C) ROC curve of *PTTG1* expression in PC. *PTTG1*, pituitary tumor transforming gene 1; IHC, immunohistochemistry; AUC, area under the curve; PC, pancreatic cancer; ROC, receiver operating characteristic.

Interestingly, *PTTG1* is highly expressed in PC, and the positive expression of *PTTG1* is related to the gender of PC patients (23). The discovery of molecular markers can improve diagnosis, evaluation of prognosis, and individualized treatment, including molecularly targeted therapies. To the best of our knowledge, few studies to date have investigated the relationship between *PTTG1* regulation and PC development. On the molecular level, the precise mechanism has not yet been fully clarified, and intensive scientific research is currently underway to identify potential therapeutic targets for further therapies. Thus, our study sought to validate how *PTTG1* was expressed in PC and explored the potential molecular mechanism and clinical pathological features. In the current study, we observed that the expression of *PTTG1* in PC was dramatically up-regulated compared with non-tumor tissues. We first assessed *PTTG1* expression in PC from GEO and TCGA databases, and it was observed that *PTTG1* expression was significantly upregulated in PC tissues compared to non-cancerous tissues. Then, we validated this finding by IHC. We also performed a meta-analysis to exhaustively evaluate the prognostic role of *PTTG1* expression in patients with PC and the results further verified that *PTTG1* is an independent prognostic marker. To verify overlapping genes from the perspective of the underlying biological mechanism, we used the DAVID database to perform the GO functional analysis and KEGG pathway analyses. Functional analysis of the overlapping genes revealed a significant enrichment in BP that included cell division, anaphase-promoting complex-

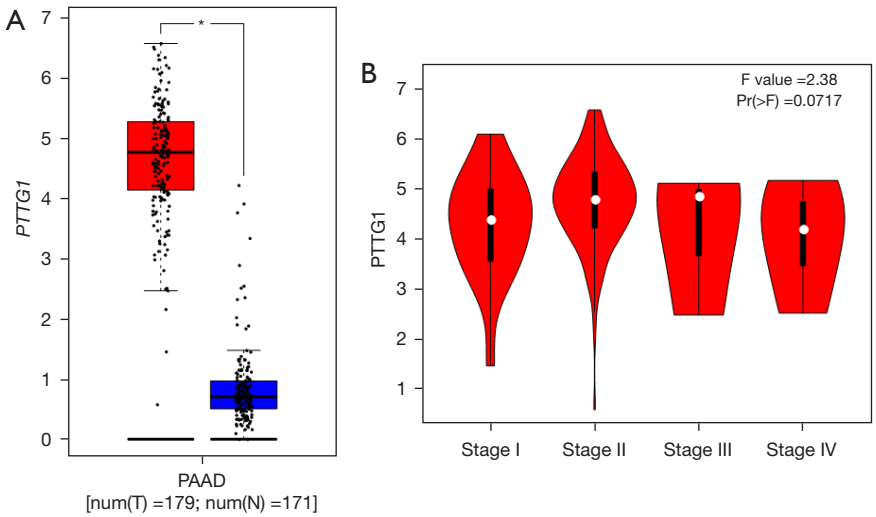

Figure 7 The expression level of *PTTG1* from GEPIA. (A) Cancer (179 cases) and non-cancer (171 cases) tissue. Red represents cancer tissue, blue represents non-cancer tissue, and black spots represent individual cases. (B) Arithmetic mean expression level of *PTTG1* in different clinical stages of PAAD patients. *, P<0.05. PAAD, pancreatic ductal adenocarcinoma; T, cancer tissue; N, non-cancer tissue; *PTTG1*, pituitary tumor transforming gene 1; GEPIA, Gene Expression Profiling Interactive Analysis.

Table 3 Univariate/multivariate Cox regression analysis based on IHC data

Characteristics	Univariate Cox regression		Multivariate Cox regression	
	HR (95% CI)	P value	HR (95% CI)	P value
Gender (male *vs.* female)	0.48 (0.30–0.76)	0.002	0.36 (0.21–0.60)	<0.001
Age (≥60 *vs.* <60 years)	0.87 (0.56–1.37)	0.554		
CEA (≥5 *vs.* <5 ng/mL)	1.59 (0.97–2.60)	0.064	1.34 (0.77–2.33)	0.301
CA199 (≥37 *vs.* <37 U/mL)	0.97 (0.56–1.70)	0.926		
Size (≥4 *vs.* <4 cm)	1.91 (1.20–3.02)	0.006	1.55 (0.63–3.77)	0.337
T stage (T3&T4 *vs.* T1&T2)	2.13 (1.33–3.40)	0.002	1.29 (0.56–2.99)	0.551
N stage (N2 *vs.* N1 *vs.* N0)	1.64 (1.14–2.34)	0.007	0.87 (0.54–1.41)	0.576
M stage (M1 *vs.* M0)	5.61 (3.22–9.76)	<0.001	4.71 (2.47–9.01)	<0.001
Venous invasion (present *vs.* absent)	1.37 (0.87–2.15)	0.176		
Nervous invasion (present *vs.* absent)	1.25 (0.78–2.01)	0.357		
PTTG1 (high *vs.* low)	0.79 (0.50–1.25)	0.317		

IHC, immunohistochemistry; HR, hazard ratio; CI, confidence interval; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 199; T, tumor; N, node; M, metastasis; *PTTG1*, pituitary tumor transforming gene 1.

dependent catabolic process, and negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle and in the MF that included protein binding, poly(A) RNA binding, and threonine-type endopeptidase activity. In the KEGG pathway network, we found that *PTTG1* was involved in regulating multiple cancer-related pathways, including cell cycle, DNA replication, and spliceosome. The results suggested that the cell cycle might be actively involved in oncogenesis and progression. Deregulation of the cell cycle induces uncontrolled cell growth and loss of

Table 4 Association between *PTTG1* expression and some clinical pathological parameters based on TCGA data

Clinicopathological parameters	Number	*PTTG1* expression[†]	*H*/*Z*/*F* value	P value
Age			–0.072	0.943
<60 years	55	8.096 (7.419–8.552)		
≥60 years	124	8.045 (7.475–8.616)		
Gender			–0.036	0.971
Female	80	8.040 (7.479–8.616)		
Male	99	8.120 (7.453–8.585)		
Tumor size			–1.503	0.133
<4 cm	102	8.008 (7.435–8.466)		
≥4 cm	77	8.169 (7.510–8.870)		
Histological grade			4.020	0.009
G1	32	7.484±1.343		
G2	96	8.082±0.877		
G3	48	8.169±0.937		
G4	3	8.672±0.968		
T stage			4.054	0.256
T1	7	7.873 (6.710–8.310)		
T2	24	7.816 (7.095–8.631)		
T3	143	8.096 (7.508–8.617)		
T4	5	8.348 (6.288–9.229)		
N stage			1.837	0.399
N0	51	8.070 (7.249–8.794)		
N1–N1b	124	8.063 (7.482–8.477)		
NX	4	7.220 (6.150–8.509)		
M stage			3.282	0.194
M0	80	8.091 (7.461–8.670)		
M1	5	7.503 (6.249–7.942)		
MX	94	8.047 (7.474–8.625)		
Tumor stage			6.891	0.075
I–IB	21	7.794 (6.828–8.401)		
IIA–IIB	147	8.096 (7.508–8.666)		
III	6	8.571 (6.413–9.191)		
IV	5	7.503 (6.249–7.942)		

[†], data are presented as HR (95% CI) or mean ± SD. *PTTG1*, pituitary tumor transforming gene 1; TCGA, The Cancer Genome Atlas; G, grade; T, tumor; N, node; M, metastasis; HR, hazard ratio; CI, confidence interval; SD, standard deviation.

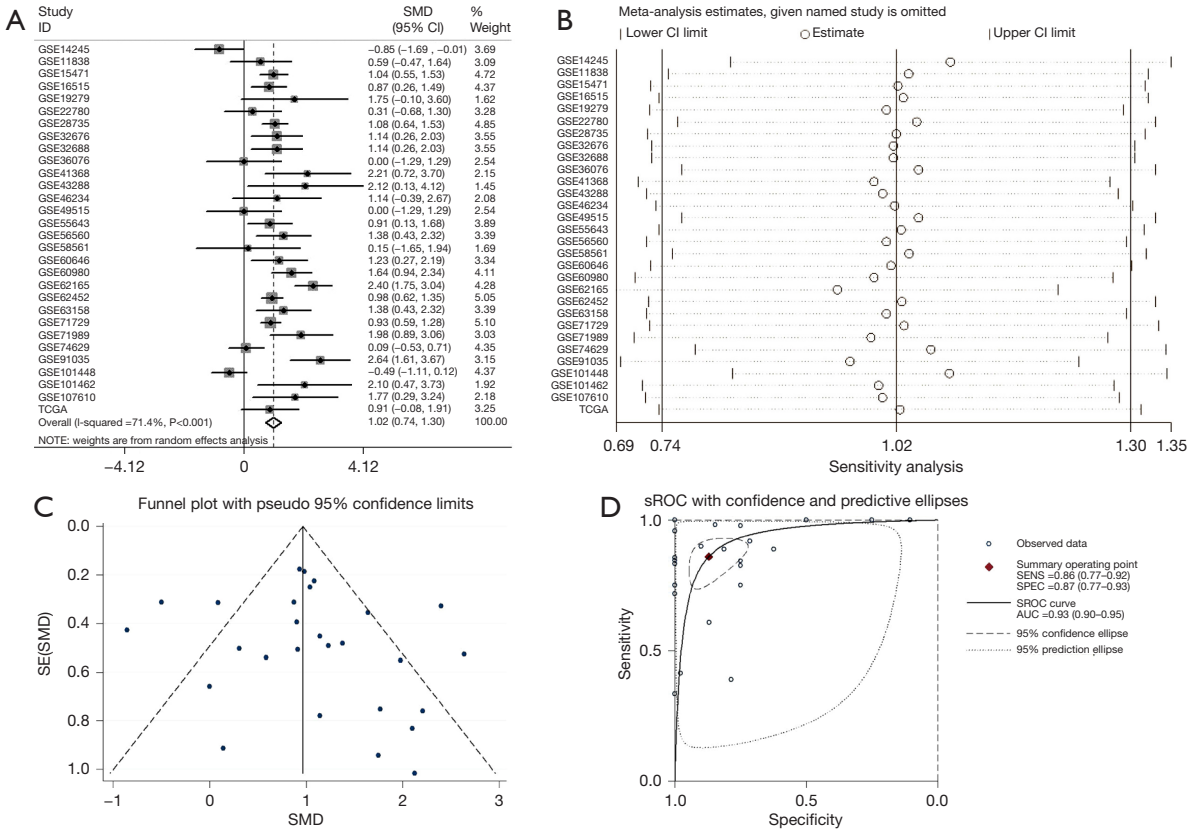

Figure 8 Meta-analysis of GEO microarrays and TCGA database. (A) Forest plot of *PTTG1* expression data from GEO microarrays and TCGA database. The pooled SMD of *PTTG1* was 1.02 (95% CI: 0.74–1.30) by the random effects model. The I^2 value was 71.4% (P<0.001). (B) Sensitivity analysis of GEO microarrays and TCGA database. (C) Funnel plot was used to show the publication bias of GEO microarrays and the TCGA database. (D) sROC curve (AUC) of *PTTG1* in the diagnosis of PC data from the GEO microarrays and TCGA database. The AUC was 0.93 (95% CI: 0.90–0.95). SMD, standard mean difference; CI, confidence interval; TCGA, The Cancer Genome Atlas; SE, standard error; sROC, summary receiver operating characteristic; SENS, sensitivity; SPEC, specificity; AUC, area under the curve; GEO, Gene Expression Omnibus; *PTTG1*, pituitary tumor transforming gene 1; PC, pancreatic cancer.

cell cycle checkpoint control accelerates genetic instability, which are key characteristics of cancer (32). Uncontrolled cell division is the primary key in the progression of tumors and it has previously been shown that *IRAK1* overexpression promotes endometrial carcinoma tumorigenesis by activating the mitotic cell cycle and cell division pathways (33). It has also been reported that *PTTG1* is highly involved in many other cellular processes, such as regulation of the cell cycle, growth, DNA repair, and even cell senescence (34).

Xu *et al.* revealed that dysregulation of cell cycle control is a hallmark of melanoma tumorigenesis (35). Oi *et al.* reported that *LTA4H* is an important regulator of the cell cycle at the G0/G1 phase in skin cancer acting by negatively regulating p27 expression (36). Cao *et al.* investigated the promotion effect of *DDX21* on malignant growth accompanied by an effect on the cell cycle and found that *DDX21* stimulated the growth of gastric cancer cells by promoting G1/S conversion (37). Zhu *et al.* showed that *SNAP23* inhibited the proliferation and progression of cervical cancer and induced cell cycle G2/M arrest by upregulating p21cip1 and downregulating CyclinB1 (38). A recent study demonstrated that long non-coding RNA *EPIC1* promotes the growth of PC cells by regulating the cell cycle via interacting with *YAP1* (39). Similarly, our enrichment analysis showed that *PTTG1* and its co-expressed genes were involved in the cell cycle pathway and played an integral role in PC. This prompted the hypothesis that abnormal *PTTG1* expression may play a distinct role in the occurrence, progression, and prognosis

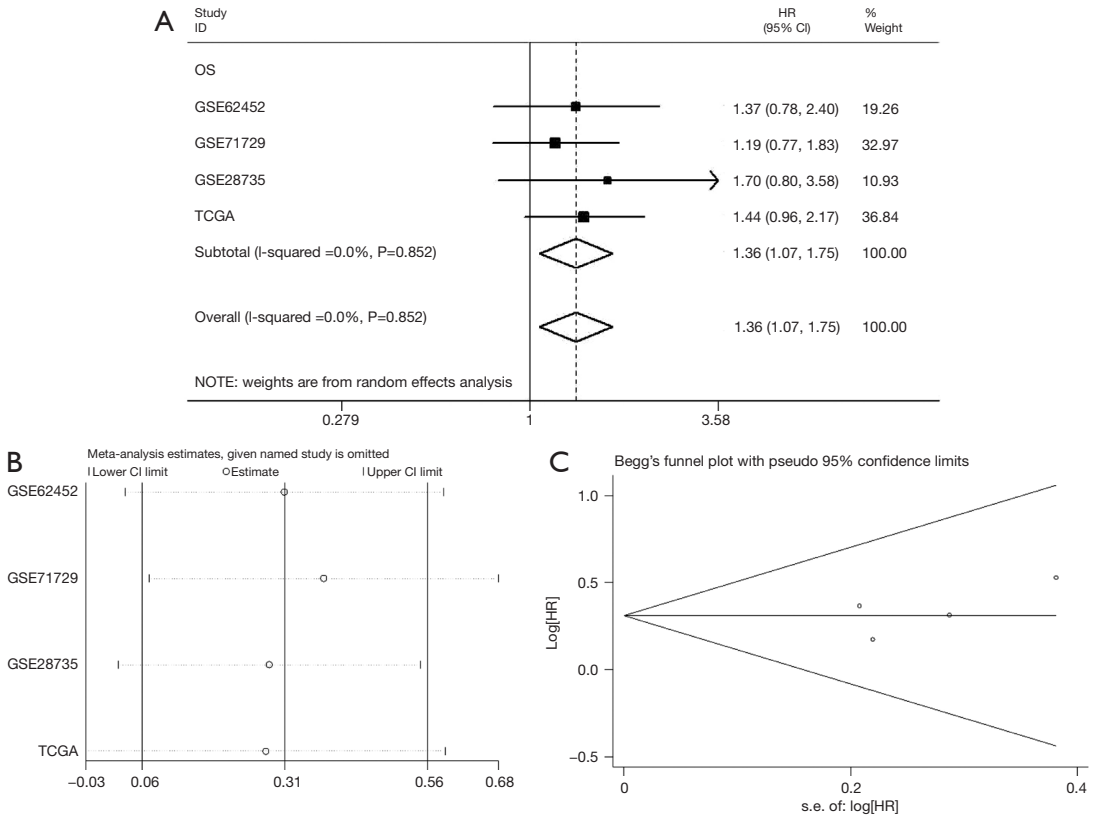

Figure 9 The meta-analysis of OS outcomes. (A) Forest plot of the pooled HR for OS reflecting the relationship between *PTTG1* expression and PC patients. A fixed effects model was used to combine data. HR >1 and corresponding 95% CI was not covered 1 implied adverse prognosis with increased *PTTG1*. (B) The sensitivity test is based on the data of *PTTG1* expression in PC. (C) Begg's funnel plot for visual detection of potential publication bias test on studies assessing *PTTG1* overexpression. HR, hazard ratio; CI, confidence interval; OS, overall survival; TCGA, The Cancer Genome Atlas; s.e., standard error; *PTTG1*, pituitary tumor transforming gene 1; PC, pancreatic cancer.

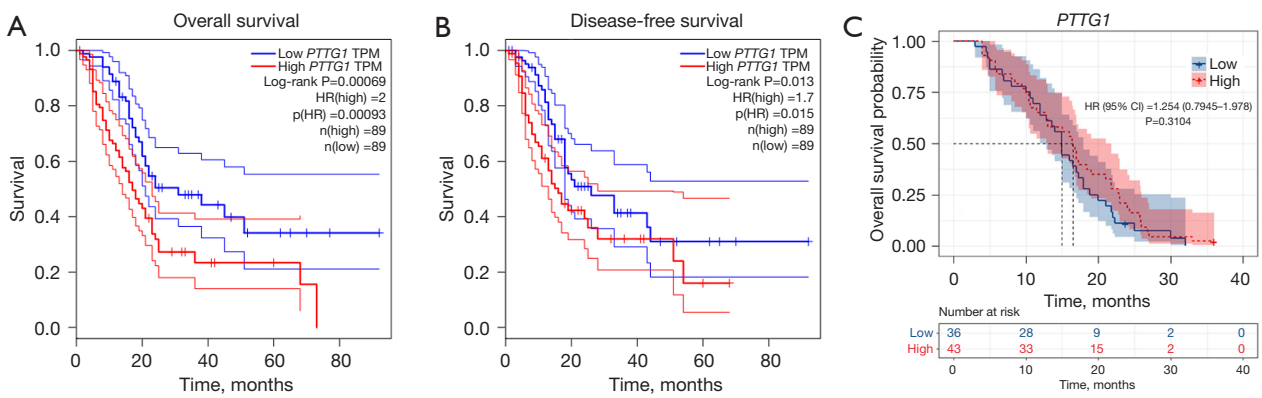

Figure 10 Survival curves of patients with PC according to *PTTG1* levels based on GEPIA. Red lines represent the survival time of patients with cancer with high *PTTG1* expression levels. Blue lines represent the survival time of patients with cancer with low *PTTG1* expression levels. (A) OS of patients with PC based on *PTTG1* levels provided by GEPIA; (B) DFS of patients with PC based on *PTTG1* levels provided by GEPIA. (C) Survival curves of patients with PC based on IHC. *PTTG1*, pituitary tumor transforming gene 1; TPM, transcripts per million; HR, hazard ratio; CI, confidence interval; PC, pancreatic cancer; GEPIA, Gene Expression Profiling Interactive Analysis; OS, overall survival; DFS, disease-free survival; IHC, immunohistochemistry.

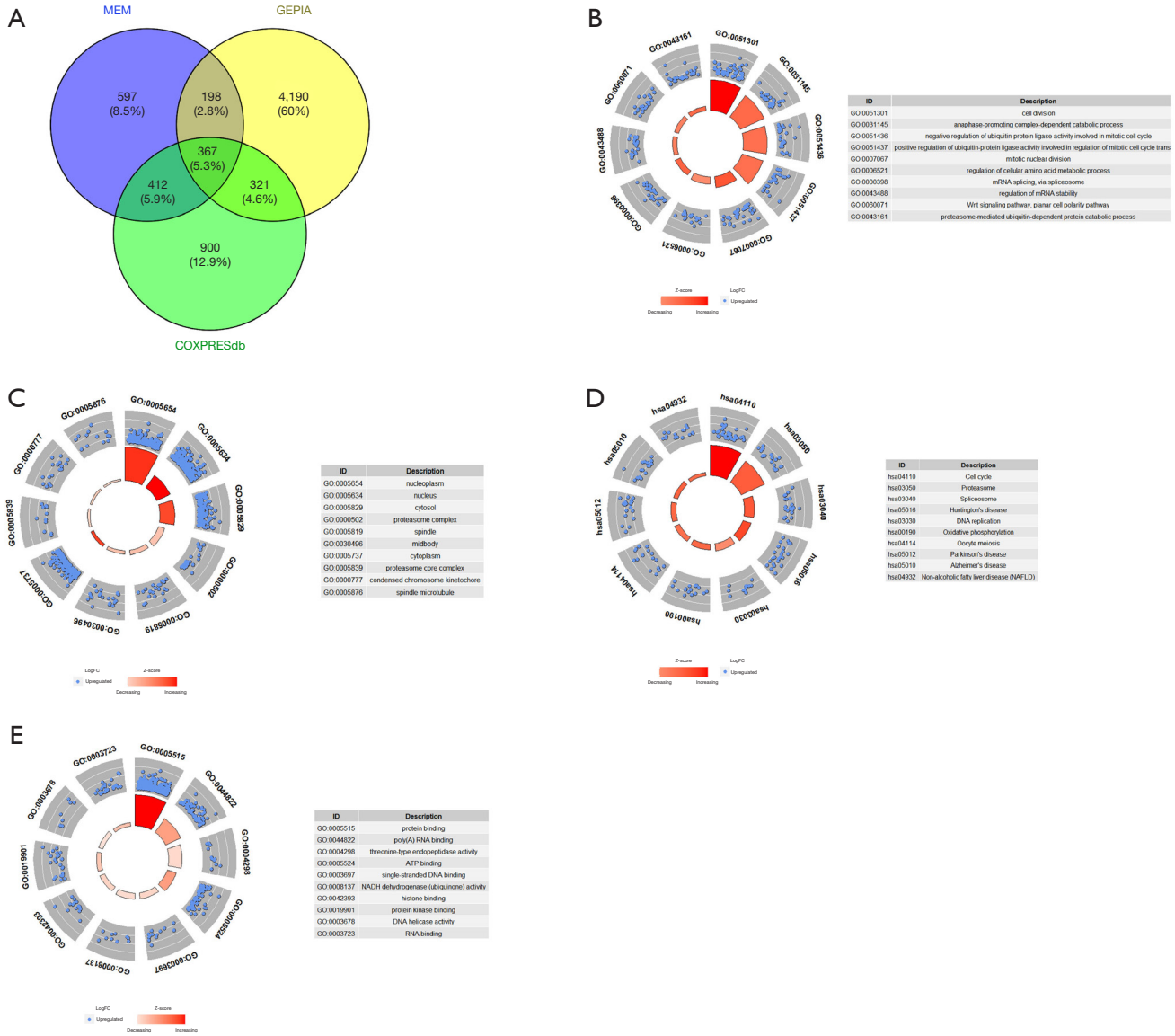

Figure 11 GO terms and KEGG pathway analysis for the DEGs. (A) Three hundred and sixty-seven overlapping genes; (B-D) GO terms in the categories: (B) BP, (C) CC, and (D) MF; (E) KEGG pathway analysis. MEM, Multi Experiment Matrix; GEPIA, Gene Expression Profiling Interactive Analysis; GO, Gene Ontology; FC, fold change; mRNA, messenger RNA; hsa, homo sapiens; NAFLD, non-alcoholic fatty liver disease; ATP, adenosine triphosphate; NADH, Nicotinamide adenine dinucleotide; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes, BP, biological process; CC, cellular component; MF, molecular function.

of PC. Taken together, cell cycle regulation is closely associated with tumorigenicity; this perspective could be exploited to develop new treatment strategies. The findings of the current research can provide a new orientation for deepening our understanding of PC. According to our research, a total of four hub genes (*CDK1*, *CCNA2*, *CDC20*, and *MAD2L1*) were identified, as determined from co-expression and PPI network analysis.

CDK1 (also known as *CDC2*), is a protein-coding gene and is crucial for a transition during the G1/S and G2/M phase of the eukaryotic cell cycle (40). In addition, entering into mitosis is primarily governed by the activity of *CDK1* (41). Dysregulation of the cell cycle is an uncontrolled proliferative signal that acts as a marker

Table 5 The 10 most significant items of the GO and KEGG analyses based on 367 targets of *PTTG1*

Geneset	Description	Count	P value
BP			
GO:0051301	Cell division	55	4.61E−31
GO:0031145	Anaphase-promoting complex-dependent catabolic process	30	1.24E−28
GO:0051436	Negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	28	3.18E−27
GO:0051437	Positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition	28	2.79E−26
GO:0007067	Mitotic nuclear division	37	4.93E−20
GO:0006521	Regulation of cellular amino acid metabolic process	19	7.31E−18
GO:0000398	mRNA splicing, via spliceosome	32	6.50E−17
GO:0043488	Regulation of mRNA stability	23	2.49E−16
GO:0060071	Wnt signaling pathway, planar cell polarity pathway	22	2.85E−16
GO:0043161	Proteasome-mediated ubiquitin-dependent protein catabolic process	30	3.63E−16
CC			
GO:0005654	Nucleoplasm	184	2.29E−57
GO:0005634	Nucleus	211	3.60E−30
GO:0005829	Cytosol	159	3.74E−30
GO:0000502	Proteasome complex	20	2.43E−18
GO:0005819	Spindle	23	2.24E−15
GO:0030496	Midbody	22	9.55E−14
GO:0005737	Cytoplasm	168	4.57E−13
GO:0005839	Proteasome core complex	11	2.32E−12
GO:0000777	Condensed chromosome kinetochore	17	1.28E−11
GO:0005876	Spindle microtubule	13	3.52E−11
MF			
GO:0005515	Protein binding	277	1.45E−24
GO:0044822	Poly(A) RNA binding	67	1.90E−14
GO:0004298	Threonine-type endopeptidase activity	11	4.16E−12
GO:0005524	ATP binding	68	1.77E−09
GO:0003697	Single-stranded DNA binding	13	5.45E−07
GO:0008137	NADH dehydrogenase (ubiquinone) activity	10	5.78E−07
GO:0042393	Histone binding	14	1.71E−06
GO:0019901	Protein kinase binding	24	4.84E−06
GO:0003678	DNA helicase activity	7	7.97E−06
GO:0003723	RNA binding	28	3.68E−05

Table 5 *(continued)*

Table 5 *(continued)*

Geneset	Description	Count	P value
KEGG			
hsa04110	Cell cycle	32	1.69E−20
hsa03050	Proteasome	20	5.45E−18
hsa03040	Spliceosome	21	2.86E−09
hsa05016	Huntington's disease	24	1.62E−08
hsa03030	DNA replication	11	7.54E−08
hsa00190	Oxidative phosphorylation	18	5.25E−07
hsa04114	Oocyte meiosis	16	1.22E−06
hsa05012	Parkinson's disease	18	1.34E−06
hsa05010	Alzheimer's disease	19	3.31E−06
hsa04932	NAFLD	17	1.37E−05

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; *PTTG1*, pituitary tumor transforming gene 1; BP, biological process; mRNA, messenger RNA; CC, cellular component; MF, molecular function; ATP, adenosine triphosphate; NADH, Nicotinamide adenine dinucleotide; hsa, homo sapiens; NAFLD, non-alcoholic fatty liver disease.

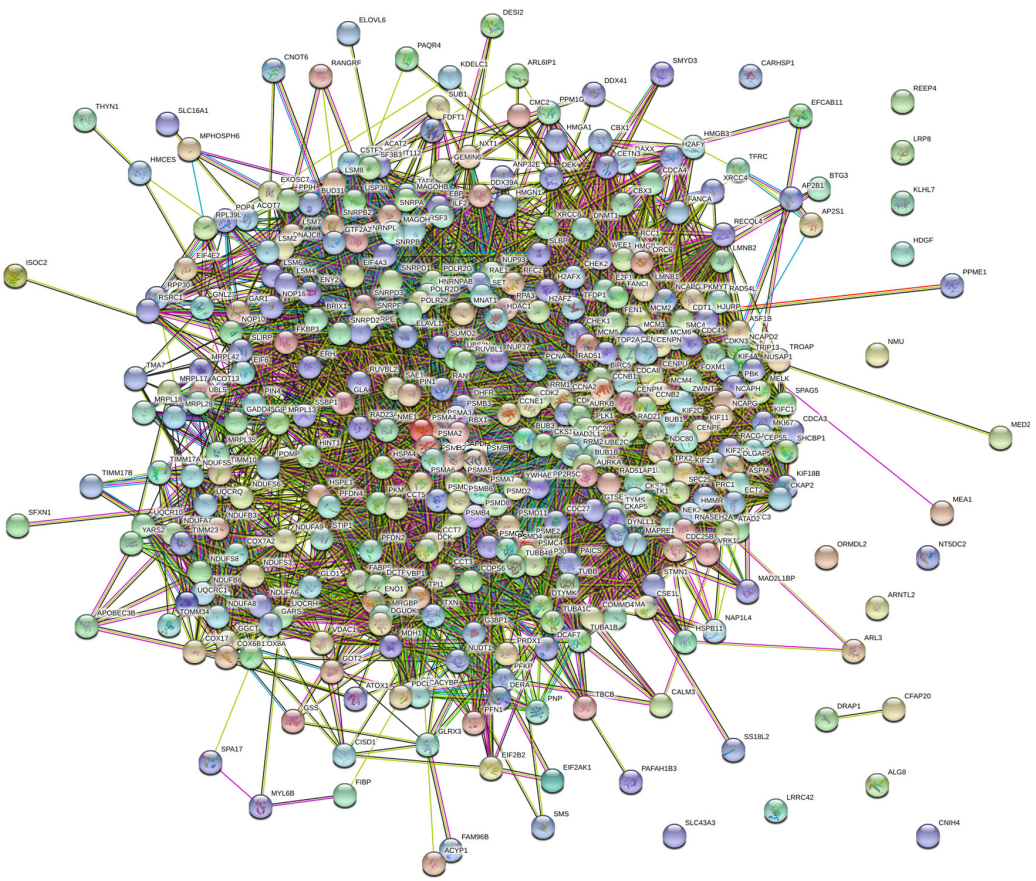

Figure 12 The PPI network of the significant overlapping genes. PPI, protein-protein interaction.

for cancer (42). Meanwhile, *CDK1* has been reported to phosphorylate YAP at multiple sites such as sites T119 and S289 at the G2/M phase of the cell cycle, promoting invasion and migration in cancer cells (43). Moreover, *CDK1* expression has also been described in pancreatic ductal adenocarcinoma (44), epithelial ovarian cancer (45), hepatocellular carcinoma (46), and melanoma (47).

The protein coding gene *CCNA2* resides on chromosome four which promotes G1/S and G2/M phase transition by modulating the cell cycle (48). Specifically, one previous study has found that the accumulation of *CCNA2* in the S phase is up-regulated in the S, G2, and early M phases (49). Similarly, *CCNA2*, belonging to the highly conserved cyclin family, is identified as a regulator of CDK kinases (50). Deficiency and overabundance of *CCNA2* have been observed in human cancers where it may affect cell proliferation, tumorigenesis (51), and poor clinical prognosis (52). Mounting evidence has revealed that the expression of *CCNA2* is abnormally increased in a variety of cancers, including esophageal squamous cell carcinoma (53), colon cancer (54), hepatocellular carcinoma (55), PC (56), osteosarcoma (57), and cervical carcinoma (58).

CDC20, located in chromosome 1 (59), plays a critical role in cell cycle progression as an essential activator of the anaphase-promoting complex/cyclosome (APC/C) that controls the protein overexpression levels of vital regulators of mitosis and DNA replication (60,61). In addition to its main functions in mitosis, recent research had linked *CDC20*-APC/C to various cellular processes beyond the cell cycle such as stem cell expansion, neurogenesis, apoptosis, and epigenetic regulation (62). Up-regulated *CDC20* along with clinicopathological parameters has been detected and verified in several cancers. We suspect that inhibiting *CDC20* will play an important role in anticancer treatment (63). In addition, there is evidence that *CDC20* is a carcinogenesis factor associated with cell growth, motility, apoptosis, and metastasis (64). A study has shown that increased expression of *CDC20* eliminated the cytotoxic functions induced by curcumin and enhanced cellular proliferation and invasion in PC cells (65).

MAD2L1, a member of the spindle assembly mitotic checkpoint, is gene that assures the proper segregation of the sister chromatids at the metaphase plate during cell division to maintain genomic stability (66). Dysregulation of *MAD2L1* has been shown to result in chromosomal instability and aneuploidy, which ultimately culminate in tumor development (67). *MAD2L1* as a potential target for the treatment of several tumors plays a critical role in breast cancer, lung cancer, liver cancer, and gastric cancer (68). Li *et al.* demonstrated that a significant up-regulation of *MAD2L1* in lung adenocarcinoma tissues could promote cell proliferation, migration, and invasion and its high expression is correlated with poor OS in lung adenocarcinoma patients (69). Lu *et al.* reported that MAD2L1 was associated with poor OS and event-free survival in rhabdomyosarcoma (70). Also, Wang *et al.* found that *MAD2L1* was up-regulated in gastric cancer, and the miR-30a-3p can down-regulate the *MAD2L1* expression to suppress the proliferation of gastric cancer cells and regulate the cell cycle (71).

In summary, *PTTG1* may serve as a novel prognostic biomarker and a therapeutic target for patients with PC. However, there were still some limitations in this study that should be acknowledged. Although expression of *PTTG1* was observed to be significantly upregulated in PC tissue after validation in GEO, TCGA, and IHC databases, the association of *PTTG1* and its prognosis showed variation between public databases and real-world data. In the future, more laboratory experiments and clinical trials need to be performed to further validate the findings in this study. To substantiate *PTTG1* as an independent prognostic factor, comprehensive research is needed. With multivariate analysis, we will conduct prospective trials or retrospective studies to accurately assess the prognostic value of *PTTG1* independent of other confounding factors. Mendelian randomization analysis can also be applied for proving the causal relationship between *PTTG1* and poor prognosis of PC.

Conclusions

PTTG1 may serve as a driving gene associated with the occurrence and progression of PC.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-979/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-979/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was performed in line with the principles of the Declaration of Helsinki (as revised in 2013). Approval was granted by the Ethics Committee of Shanghai Outdo Biotech Company (No. YB M-05-02).

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