Comprehensive Analysis of Clinical Prognosis and Molecular Immune Characterization of TPM4 in Pancreatic Cancer

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Research Article

Keywords: TPM4, pancreatic cancer, prognosis, Immune infiltrates, TME

Posted Date: March 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-326395/v1>

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Abstract

Pancreatic cancer (PC) is one of the most lethal human solid malignancies with devastating prognosis, making biomarker detection considerably important. Immune infiltrates in microenvironment is associated with patients' survival in PC. The role of TPM4 (Tropomyosin 4) gene in PC has not been reported. Our study first identifies TPM4 expression and its potential biological functions in PC. The potential oncogenic roles of TPM4 was examined using the datasets of TCGA (The cancer genome atlas) and GEO (Gene expression omnibus). We investigated the clinical significance and prognostic value of TPM4 gene based on The Gene Expression Profiling Interactive Analysis (GEPIA) and survival analysis. TIMER and TISIDB databases were used to analyze the correlations between TPM4 gene and tumor-infiltrating immune cells. We found that the expression level of TPM4 was upregulated in PC malignant tissues with the corresponding normal tissues as controls. High TPM4 expression was correlated with the worse clinicopathological features and poor prognosis in PC cohorts. The positive association between TPM4 expression and tumor-infiltrating immune cells was identified in tumor microenvironment (TME). Moreover, functional enrichment analysis suggested that TPM4 might participate in cell adhesion and promote tumor cell migration. This is the first comprehensive study to disclose that TPM4 may serve as a novel prognostic biomarker associating with immune infiltrates and provide a potential therapeutic target for the treatment of PC.

This study is not a clinical trial without the registration number.

Introduction

Pancreatic cancer (PC), one of the most fatal human malignancies with a 5-year survival rate of approximately 10%, shows a growing cause of cancer mortality in the United States ^[1]. According to the estimation from the American Cancer Society, approximately 57,600 new cases and 47,050 deaths of pancreatic cancer would occur in the United States in 2020, ranking second after colon cancer in digestive system neoplasms ^[2]. Even with the introduction of modern approaches to advanced diagnosis in recent years, only a minority of patients could be diagnosed in the early stage with surgically resectable status owing to little or no symptoms before developing in the advanced stage. Up until now, complete surgical resection remains still the unique curative therapeutic approach for PC, while the 5-year survival rates of patients undergoing surgery is 10–25% ^[1]. Recently, novel immunotherapy strategies currently have been tried to confer antigen specificity, enhance T cell effector function and neutralize immunosuppressive elements within the tumor microenvironment in the treatment for PC ^[3]. Besides, significant advances have been made in developing targeted therapies to improve the survival of PC patients ^[4]. Given the limited effectiveness of current treatments, it is urgent to explore a promising biomarker that make a significant contribution to the optimization of treatment.

Tropomyosin 4 (TPM4), a member of the tropomyosin family of actin-binding proteins, is thought to play a crucial role in the regulation of cytoskeleton functions and muscle contraction with other sarcomeric proteins such as actin, troponins and tropomodulin ^[5, 6]. It has been reported that TPM4 gene products

have confirmed postsynaptic localization and could participate in the regulating postsynaptic functions [7]. In the past decade, aberrant expression of TPM4 also has been involved in development and progression of several cancers, including lung cancer [8], hepatocellular carcinoma [9, 10], colon cancer [11], gastric cancer [12], breast cancer [13, 14], and esophageal squamous cell carcinoma [15]. Besides, TPM4 is significantly elevated in ovarian cancer patients compared with non-cancer controls, which is identified as a serological biomarker of ovarian cancer [16]. However, the correlation between TPM4 expression and its prognostic value in pancreatic cancer remain not to be explored.

In this study, we first used the TCGA (The cancer genome atlas) and GEO (Gene expression omnibus) databases to evaluate the association between TPM4 expression and pancreatic cancer patients' prognosis. In addition, the correlation of TPM4 mRNA levels with tumor-infiltrating immune cells was investigated. Our findings revealed the potential role of TPM4 in pancreatic cancer and would highlight a clearer understanding of the underlying mechanism between TPM4 and tumor-immune.

Materials And Methods

2.1. TPM4 gene expression analysis

The Oncomine database, a bioinformatics initiative including 86,733 samples and 715 gene expression datasets, is designed to collect, standardize, analyse, and deliver cancer transcriptome data to the biomedical research [17]. Therefore, we evaluated the association between TPM4 expression and the prognostic outcome in multiple types of tumors using this database with web server (<https://www.oncomine.org/resource/login.html>). We utilized the Genotype-Tissue Expression (GTEx) database (<https://www.gtexportal.org/home/-index.html>) and Cancer Cell Line Encyclopedia (CCLE) database (<https://portals.broadinstitute.org/ccle>) to obtain data on TPM4 gene expression in 31 normal tissues and 21 tumor cell lines respectively. Furthermore, we investigated the expression level of TPM4 gene between cancer and normal tissues in the integrated datasets combined TCGA with GTEx databases.

The Gene Expression Profiling Interactive Analysis (GEPIA), a web-based interactive database that compiles the standardized analysis of RNA-Seq data from 9736 tumors and 8587 normal samples based on TCGA and GTEx databases, has become an integral part of bioinformatic analysis (<http://gepia.cancer-pku.cn/>) [18]. Therefore, we employed GEPIA to profile the expression of TPM4 gene in different cancer stages. In addition, Sankey diagram was built based on the “ggalluval” package, which integrated the RNA-sequencing data and corresponding clinical information. In GEO cohorts (GSE15471, GSE16515, GSE23397 and GSE62165), we compared the TPM4 expression between normal and tumor tissues. Moreover, the distribution and subcellular localization of TPM4, as well as the expression in pancreatic cancer were observed by Immunohistochemistry (IHC) images using the Human Protein Atlas (THPA) (<https://www.proteinatlas.org/>).

2.2. Survival analysis

To reveal the prognostic value of TPM4 gene in patient's prognosis in PC, survival analysis such as OS (overall survival), DSS (disease specific survival), DFS (disease free survival), and PFS (progression free survival) was performed. RNA-sequencing data and corresponding clinical information of patients with PC were collected from [TCGA](#) datasets, which was visualized with gene distribution and Kaplan-Meier curves. The median expression of TPM4 divided the patients into low- and high-risk groups. *P*-values and hazard ratio (HR) with 95% confidence interval (CI) were generated by log-rank tests and univariate Cox proportional hazards regression.

2.3. Correlations between TPM4 expression and immune characteristics

TIMER2.0 (Tumor immune estimation resource, version 2), a comprehensive resource database, is designed for systematically analyzing the infiltration of 6 immune cells, including B cells, CD4⁺ T cells, CD8⁺ T cells, neutrophils, macrophages, and myeloid dendritic cells across diverse cancer types (<http://timer.cistrome.org/>) ^[19]. Spearman correlation analysis was performed to assess the correlation between TPM4 expression and immune infiltration. TISIDB, an integrated repository portal, plays a crucial role in seeking the interaction between tumor and immune system (<http://cis.hku.hk/TISIDB/>) ^[20]. To further elucidate the immune correlates of TPM4 in cancer, TISIDB tool was used. First, we created the landscape of correlation between TPM4 expression and 28 TILs across multiple cancer types via "lymphocyte" module. Specifically, we searched and screened the appropriate candidates with the statistical significance of Spearman's correlation.

In addition, we used ESTIMATE algorithm to analyze Immune Score and Stromal Score, which observed the relationship between TPM4 expression and tumor immune microenvironment in pancreatic cancer. In addition, Pearson correlation analysis was performed to evaluate the correlation of TPM4 expression with immune checkpoint gene levels. To further investigate the association between TPM4 and immune cell migration, we assessed chemokines/chemokine receptors via "chemokine" module based on TISIDB database.

2.4. Functional enrichment analysis

Limma package was used to explore the differential expression of mRNAs. The adjusted *P*-value was applied to correct the false positive results. Adjusted *P* < 0.05 and |log₂ (Fold Change)| > 1 were defined as the thresholds for the screening of differentially expressed genes (DEGs). To better understand the carcinogenesis of TPM4, "ClusterProfiler" package was employed to conduct Gene Ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) analysis. In the enrichment results, adjusted *P* < 0.05 is considered to be enriched to a meaningful pathway.

2.5. Protein–Protein Interaction analysis

We first investigated the protein–protein interaction (PPI) of TPM4-binding proteins via the STRING website (<https://string-db.org/>) with the main settings such as meaning of network edges ("evidence"), active interaction sources ("experiments"), minimum required interaction score ["Low confidence

(0.150)”, and max number of interactors to show (“no more than 50 interactors”) operated. Then, the interaction network of 50 TPM4-binding proteins with the experimental evidence identification was obtained. Jvenn, an interactive Venn diagram viewer^[21], was utilized to perform an intersection analysis to compare TPM4 expression-correlated DEGs and TPM4-interacted genes. Spearman’s correlation analysis was performed to describe the correlation between TPM4 expression and the common genes from intersection analysis. $P < 0.05$ was defined as statistically significant.

2.6. MMR gene mutation analysis.

More studies have indicated that the dysfunction of DNA mismatch repair system (MMRs) could be triggering tumorigenesis^[22]. We explored the mutation levels of MMR genes involving in MLH1, MSH2, MSH6, PMS2, and EPCAM from TCGA database. Pearson's correlation analysis was adopted to evaluate the relationship between TPM4 expression and MMR gene mutation levels.

2.7. Statistical analysis

Using Kruskal-Wallis test, we evaluated TPM4 expression levels in multiple tissues and cancer cell lines. The *t*-test was adopted to compare the different expression levels of TPM4 in tumor tissues and normal tissues. The Wilcox test was used to evaluate the significance of TPM4 expression in the two groups of different T-staging patients. According to different TPM4 expression levels, the survival analysis of patients was conducted by Kaplan-Meier curves. To calculate the HR and 95% CI in survival analysis, we used univariate Cox regression analysis. All R packages were performed using R software version v4.0.3, and a level of $P < 0.05$ was defined as statistical significance.

Results

3.1. The mRNA expression level of TPM4 in human cancers

To compare the expression of the tropomyosin family members including TPM1, TPM2, TPM3 and TPM4 in human cancers, we used the Oncomine database to analyze the mRNA levels in normal and tumor tissues. Specifically, this analysis indicated that TPM4 expression was higher in pancreatic cancer (Fig. 1a). Furthermore, we analyzed TPM4 expression in the 31 types of tissues through the GTEx dataset, which revealed TPM4 was generally lowly expressed in the pancreas tissues (Fig. S1a). Meanwhile, the expression of TPM4 was analyzed using the data of tumor cell lines downloaded from the CCLE database. The results indicated that TPM4 was expressed in various tumor cell lines (Fig. S1b). Next, we used the TCGA database to evaluate how TPM4 expression in multiple types of cancers (Fig. S1c). Considering pancreatic cancer with limited adjacent normal tissues in TCGA database, we compared the expression level of TPM4 gene in the integrated datasets combined TCGA with GTEx database. The analysis identified TPM4 was significantly upregulated in tumor tissues with the corresponding normal tissues as controls (Fig. 1b, c). Consistent with it, the upregulated TPM4 in PC tumor tissues was shown in the GSE15471, GSE16515, GSE23397 and GSE62165 cohorts (Fig. 2a-d).

We further evaluated the expression of TPM4 in different T-staging patients with PC, in which TPM4 was higher expressed in the T3/T4 than that of T1/T2 (Fig. 1d, $P < 0.05$). Besides, we found that TPM4 was also correlated with the pathological stages of patients with PC (Fig. 1e, $P < 0.05$). Sankey diagram could be used to show the distribution trend of the high and low expression of TPM4 gene in different ages, stages and other clinical features as well as the survival of patients with PC (Fig. 1f). In the end, we compared the protein level of TPM4 using the IHC staining by means of the THPA database. The protein expression level of TPM4 in PC tumor tissues was higher than that in normal tissues (Fig. 1g). These findings suggested that the upregulation of TPM4 may predict an advanced malignancy of PC.

3.2. Prognostic value of TPM4 in PC

To investigate the correlation of TPM4 expression with the prognosis of patients with pancreatic cancer, we assessed the distribution of TPM4 expression level and association with the survival for the patients using TCGA database. Notably, TPM4 expression was significantly correlated with patients' OS in PC. The cancer cases were divided into high- and low-expression groups by the median value of TPM4 expression. Comprehensive considering the risk curve and the survival status, we found that the fatality rate in the TPM4 high-expression group was significantly higher than that in the low-expression group (Fig. 3a). Specifically, Kaplan-Meier survival analysis indicated that high TPM4 expression was significantly linked with poor prognosis of patients with PC (HR = 1.695, 95% CI = 1.114–2.581, $P = 0.0138$) (Fig. 3b). To observe the predictive value of TPM4 mRNA levels for prognosis, we evaluated the expression of TPM4 to distinguish TPM4^{high} and TPM4^{low} patients by the receiver operating characteristic (ROC) curve. Evaluating the area under the curve (AUC) under the ROC curve was applied to predict the 1, 3, 5-year risk of PC patients (1-year, AUC = 0.605; 3-year, AUC = 0.669; 5-year, AUC = 0.794) (Fig. 3c). In addition, we analyzed the relationship between TPM4 expression and patients' DSS in PC. The results suggested that TPM4 expression impacted the survival status (Fig. 4a), DSS (HR = 1.801, 95% CI = 1.116–2.908, $P = 0.0161$) (Fig. 4b), and performed predictive effect on the risk of PC patients (1-year, AUC = 0.617; 3-year, AUC = 0.701; 5-year, AUC = 0.811) (Fig. 4c). Subsequently, the relationship between TPM4 expression and DFS (Fig. S2a-c) and PFS (Fig. S3a-c) were investigated, in which we found the same influence of TPM4 on poor prognosis. In conclusion, these consistent results from OS, DSS, DFS, and PFS analysis strongly revealed that TPM4 gene is significantly correlated with the prognosis of patients with PC.

3.3. Correlation of TPM4 expression with immune characteristics

TILs are an independent predictor in cancers [23, 24]. The association between TPM4 gene level and tumor-infiltrating immune cells across diverse cancer types based on the TIMER database, in which we found that TPM4 impacted tumor-infiltrating immune cells in PC. We first analyzed the percentage abundance of different types of tumor infiltrating immune cells in PAAD (Fig. 5a). Furthermore, we investigated the correlation of TPM4 expression with the tumor-infiltrating immune cells by establishing Immune cells score heatmap, which indicated that high TPM4 expression was significantly linked with immune infiltrates in PC (Fig. 5b). Specifically, as shown in Fig. 5c, TPM4 expression was negatively correlated

with the purity of tumor ($r = -0.174$, $P = 2.25e-02$). In addition, TPM4 expression was appreciably positively correlated with the infiltration of several immune cell types, including CD8⁺ T cells ($r = 0.411$, $P = 2.36e-08$), B cells ($r = 0.214$, $P = 5.00e-03$), macrophages ($r = 0.455$, $P = 4.10e-10$), neutrophils ($r = 0.469$, $P = 9.70e-11$), and myeloid dendritic cells ($r = 0.515$, $P = 6.08e-13$), while there was no marked infiltration with CD4⁺ T cells ($r = -0.064$, $P = 4.09e-01$) in PAAD. The Immune Score, Stromal Score and ESTIMATE Score were used to identify and quantify the immune and matrix components in PAAD. Results indicated that TPM4 expression was positively correlated with the Immune Score ($r = 0.188$, $P = 0.0122$), Stromal Score ($r = 0.46$, $P = 1.51e-10$) and ESTIMATE Score ($r = 0.33$, $P = 7.96e-06$) in PAAD (Fig. 5d). Next, we examined the relations between TPM4 expression and abundance of 28 TILs using the TISIDB database. As shown in Fig. 6a, the relationship between TPM4 expression and TILs in different types of cancer was exhibited. Specifically, in PAAD, TPM4 expression was significantly correlated with multiple types of TILs (Fig. 6b–r).

Immune checkpoint inhibitors (ICIs), a significantly novel strategy for tumor immunotherapy, has already gradually improved the outcomes of patients with many types of cancers [25, 26]. Subsequently, the correlation between the expression of TPM4 and that of over 40 common immune control genes was analyzed. Interestingly, in PAAD, TPM4 expression was associated with nearly 18 immune checkpoint markers, including CD274, CD276, CD44, CD80 and so on (Fig. 7a). It is important to emphasize that CD274 (PD-L1), a biomarker of response to immune-checkpoint inhibitors [26], performed significantly correlated with TPM4 expression in PAAD. Accordingly, these results strongly demonstrated that TPM4 gene may play a crucial role in tumor immunity. To further elucidate the association between TPM4 expression and immune cell migration, we comprehensively analyzed the connection with chemokines and chemokine receptors (Fig. 7b–f). The results have proven that TPM4 expression was positively correlated with immune cells-associated chemokines and chemokine receptors, such as CCL7 ($r = 0.348$, $P = 2.11e-06$), CCL13 ($r = 0.312$, $P = 2.32e-05$), CCL18 ($r = 0.312$, $P = 2.31e-05$), CXCL5 ($r = 0.336$, $P = 4.78e-06$), and CXCL8 ($r = 0.398$, $P = 4.57e-08$). Since those chemokines and chemokine receptors appeared to be upregulated with TPM4 expression level increased, high TPM4 expression may involve in the migration of immune cells to tumor microenvironment.

3.4. Functional inference of TPM4 in PAAD

Differentially expressed genes (DEGs), a popular method to explore the potential biological role by enrichment analysis, are applied for the study in disease. To further confirm the underlying biological function of TPM4 gene in PAAD, the transcriptome data from TCGA was analyzed by functional enrichment. According to the expression level of TPM4, the samples of pancreatic cancer were divided into two groups, including TPM4^{high} and TPM4^{low}. Next, we compared DEGs analysis between TPM4^{high} group and TPM4^{low} group with the criteria set of $|\log_2FC| > 1$, adjusted $P < 0.05$. As shown in the volcano plot, 381 genes were differentially expressed including 367 upregulated genes and 14 downregulated genes (Fig. 8a). Corresponding hierarchical clustering analysis of these DEGs was displayed by the heatmap (Fig. 8b). To further determine the potential function of TPM4, we attempted to perform a series

of enrichment analyses, including KEGG and GO. The results of KEGG pathway enrichment analysis of upregulated DEGs were mainly involved in small cell lung cancer, proteoglycans in cancer, protein digestion and absorption, PI3K–Akt signaling pathway, hypertrophic cardiomyopathy (HCM), human papillomavirus infection, focal adhesion, and ECM–receptor interaction (Fig. 8c). Moreover, we performed GO enrichment analysis of upregulated DEGs, which indicated that most of these genes were linked to the events such as regulation of cell–substrate adhesion, extracellular structure organization, extracellular matrix organization, connective tissue development, collagen metabolic process, collagen fibril organization, cell–substrate adhesion, and cell–matrix adhesion (Fig. 8d). Owing to a few number of downregulated genes, there seemed very little point in continuing with the enrichment analysis of those downregulated DEGs. The GO and KEGG analysis demonstrated that TPM4 might regulate the process of cell adhesion and metabolic process, which could provide a novel direction to the research on tumor cell migration.

To further investigate the internal mechanism of the TPM4 gene in tumorigenesis, the PPI network analysis was performed by utilizing the STRING database. Fig. 9a showed the visualizing interaction network of 50 TPM4-binding proteins with the experimental evidence identification. In addition, through comparing TPM4 expression-correlated DEGs with TPM4-interacted genes, we screened out the common members such as ACTA2, ACTG2, and TNNT1 (Fig. 9b). The detailed gene information mentioned has been provided in the Supporting Information materials. Moreover, the TPM4 expression level was remarkably positively correlated with that of ACTA2 ($r = 0.692$, $P = 7.13e-27$), ACTG2 ($r = 0.487$, $P = 4.71e-12$), and TNNT1 ($r = 0.335$, $P = 4.63e-06$) (Fig. 9c).

3.5. Correlation of TPM4 expression with MMR gene mutation levels in human Pan-Cancer.

MMRs is involve in the DNA damage repair. To investigate the potential role of TPM4 in tumorigenesis, the correlation between TPM4 expression and MMR gene mutation levels was analyzed. The landscape of correlation between TPM4 expression and 5 MMR genes, including MLH1, MSH2, MSH6, PMS2, and EPCAM in human cancers was obtained (Fig. S4). Notably, results indicated a positive correlation between TPM4 expression and these MMR genes in PAAD.

Discussion

Despite the main treatment combined surgery with chemotherapy and radiotherapy has conducted, there is still a dismal prognosis in patients with pancreatic cancer. In recent years, cancer immunotherapy has shifted the paradigm for the treatment of cancer, which is gradually gained a recognition for a promising strategy to treat some cancers ^[27]. Currently, many researches with regard to immunotherapeutic strategies in pancreatic cancer mainly focus on immune checkpoint inhibitors, cancer vaccines, adoptive cell transfer, and combinations with other immunotherapeutic agents, chemoradiotherapy or other molecularly targeted agents ^[28]. Notably, immune checkpoint inhibitors (ICIs) therapy, a burgeoning treatment strategy including anti-PD-1 and anti-PD-L1 antibody drugs, has occupied an important position in the field of cancer immunotherapy, which demonstrates obvious advantages in widespread

applicability across cancer types and excellent clinical response when treatment is effective [29, 30]. However, only some patients present a good response to this treatment. Therefore, it is urgent to explore a novel immune-infiltrates associated biomarker predicting patients' prognosis and identify the potential molecular mechanisms underlying the response to immunotherapy.

Considering the limited researches had been performed with regard to TPM4 gene in cancer, we determined to perform a comprehensive and integrated bioinformatics analysis to seek its biological functions and potential regulatory pathways in pancreatic cancer. In this study, we first attempted to identify the expression and prognostic value of TPM4 gene in cancer, and found that TPM4 was upregulated in tumor tissues and high TPM4 expression was closely associated with poorer OS, DSS, DFS, and PFS in pancreatic cancer. Simultaneously, high TPM4 expression was linked to the worse clinicopathological features in PC cohorts. These findings strongly suggested that TPM4 may serve as an oncogene and a prognostic biomarker in PC.

Tumor microenvironment comprises a series of different non-tumor components such as cancer-associated fibroblasts (CAFs), tumor-infiltrating immune cells, endothelial cells and neurons. Moreover, abundant extracellular matrix (ECM) components play a crucial role in creating a highly dynamic TME with complex interactions among the multiple internal components that promote tumor progression and therapeutic resistance [31]. With the constant understanding and acquaintance of tumor immune microenvironment deepening among researchers, there is a great development potential in the ability to predict and guide immunotherapeutic responsiveness [32]. Our results demonstrated that the positive association between TPM4 expression and tumor-infiltrating immune cells was identified in TME according to TIMER and TISIDB database. Moreover, TPM4 expression was significantly correlated with the Immune Score, Stromal Score, and ESTIMATE Score in pancreatic cancer. There are many factors that can affect the normal immune function of tumor infiltrating immune cells in TME. Studies have found that all the components of TME conducted to the proliferation and metastasis of cancer by generating growth factors, chemokines, matrix-degrading enzymes and supporting tumor cells [33]. Moreover, in the complex TME, tumor-suppressor released by cancer cells, stromal fibroblasts and other cells plays a crucial role in contributing to the dismal prognosis and little improved outcomes of the patients with current anticancer immunotherapies by weakening the antitumor activities of immune cells and generating an immunosuppressive [34].

Although effective immune responses can exert the anti-tumor efficiency, cancer cells have evolved multiple mechanisms, involving in the dysfunction of antigen presentation, the upregulation of negative regulatory pathways, and the recruitment of immunosuppressive cells, to promote tumor immune escape leading to unexpected antitumor immune reaction [35]. Moreover, it has been demonstrated that neoantigen expression accelerates progression and insensitive immunoreaction in pancreatic cancer, due to a lack of Th17 response and low Th1 and CD8⁺ T cell levels, which elucidate a mechanism for the obstacle of T cell-mediated immunotherapy [36]. Some studies have found that pancreatic cancer patients with DNA mismatch repair deficiency (MMRD) have particular clinical, pathological and genomic

characteristics^[37]. According to the guideline, MMRD is suggested to be tested in the patients with pancreatic cancer before the application of immune checkpoint inhibitors^[38]. In this study, we revealed that TPM4 expression was significantly associated with the mutation levels of 5 MMR genes in pancreatic cancer.

To further elucidate the underlying biological function of TPM4 gene, we identified 381 DEGs by comparing the two groups based on TPM4 expression and performed GO and KEGG functional enrichment analysis. The results suggested that TPM4 was mainly related to cell adhesion, cell matrix's metabolism and PI3K–Akt signaling pathway. Another study has discovered that the combination of immunotherapy and PI3K-AKT pathway inhibitors display promising effect on the improvement of anti-PD-1 therapy outcomes^[39]. These findings revealed that TPM4 may play a role in influencing TME and regulating cell migration. Consequently, TME-associated researches and novel stroma-targeted approaches may contribute to improve poor prognosis of patients with pancreatic cancer.

In conclusion, the findings of our study uncovered that TPM4 could exert predictable role in prognosis and correlate with immune infiltration levels in pancreatic cancer. Therefore, TPM4 may serve as a novel prognostic biomarker and provide an opportunity to drive the development of new immunotherapeutic strategies.

Abbreviations

AUC, area under the curve; CCLE, cancer cell line encyclopedia; CAFs, cancer-associated fibroblasts; CI, confidence interval; DFS, disease free survival; DSS, disease specific survival; DEGs, differentially expressed genes; ECM, extracellular matrix; FC, fold change; FDR, false discovery rate; GEO, gene expression omnibus; GEPIA, gene expression profiling interactive analysis; GO, gene ontology; GTEx, genotype-tissue expression; HR, hazard ratio; IHC, Immunohistochemistry; ICIs, immune checkpoint inhibitors; KEGG, Kyoto encyclopedia of genes and genomes; MMRs, mismatch repair system; MMRD, mismatch repair deficiency; OS, overall survival; PFS, progression free survival; PC, pancreatic cancer; PAAD, pancreatic adenocarcinoma; PPI, protein–protein interaction; ROC, receiver operating characteristic; TPM4, Tropomyosin 4; TCGA, the cancer genome atlas; THPA, the human protein atlas; TIMER, tumor immune estimation resource; TILs, tumor infiltrating lymphocytes; TME, tumor microenvironment.

Declarations

Ethics approval

This article is not involved in any studies with human participants or animals performed by any of the authors.

Consent to participate

Not applicable.

Consent for publication

All authors consent to the publication of this study.

Availability of data and material

All data is available under reasonable request.

Competing interests

The authors have declared no competing interests.

Funding

Not applicable.

Authors' contributions

Xue Zhou and Ning Wang contributed to the study conception, design, and visualization. XiaoWei Zhu and Junchao Yao performed the data analysis and figure generation. Xue Wang contributed to collection and integration of data. Xue Zhou wrote a draft and Ning Wang revised the manuscript.

Acknowledgements

We acknowledge the Oncomine, TCGA, GEO, GTEx, CCLE, THPA, TIMER, TISIDB, GEPIA, and STRING databases for free use.

Code availability

Not applicable.

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Figures

Figure 1

The expression level of TPM4 gene and association with clinical and pathological characteristics in PC patients. (a) TPM4 expression in different types of tumor tissues and normal tissues based on the Oncomine database. (P value is 0.05, fold change is 2.) (b) TPM4 expression in different cancer types based on the GTEx database and TCGA database. (c) TPM4 expression in PC matched TCGA normal and GTEx data based on the GEPIA database. (d) TPM4 expression in different T-staging patients. (e) TPM4

expression were analyzed in different pathological stages (stage I, stage II, stage III, and stage IV) based on the TCGA database. (f) TPM4 expression association with different clinical characteristic rely on Sankey diagram shown. (g) Representative immunohistochemistry images and detailed information on the expression of TPM4 in PC tumor tissues and normal tissues based on the THPA database. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 2

The aberrant expression of TPM4 based on the GEO database. TPM4 mRNA levels in PC tumor tissues and normal tissues in the (a) GSE15471, (b) GSE16515, (c) GSE23397, and (d) GSE62165 datasets. ** $P < 0.01$, **** $P < 0.0001$.

Figure 3

Prognostic analysis of TPM4 expression on OS in PAAD based on the TCGA database. (a) TPM4 expression distribution, survival status and heatmap of the TPM4 expression profiles. (b) Survival curve of TPM4 expression. (c) ROC curve of TPM4 expression.

Figure 4

Prognostic analysis of TPM4 expression on DSS in PAAD based on the TCGA database. (a) TPM4 expression distribution, survival status and heatmap of the TPM4 expression profiles. (b) Survival curve of TPM4 expression. (c) ROC curve of TPM4 expression.

Figure 5

Correlation analysis of TPM4 expression with TILs and Immune Score/Stromal Score in PAAD. (a) The percentage abundance of tumor-infiltrating immune cells. (b) Immune cells score heatmap associated with TPM4 expression. (c) Correlation analysis of TPM4 expression with immune infiltration levels of CD8+ T cells, CD4+ T cells, B cells, macrophage cells, neutrophil cells, and myeloid dendritic cells. (d) Correlation between TPM4 expression and Immune Score, Stromal Score, and ESTIMATE Score. * $P < 0.05$, *** $P < 0.001$.

mast, mem_B, NK, NKT, pDC, tcm_CD8, tcm_CD4, Th1, Tgd, Th2, and Treg in PAAD, and was negatively related with infiltrating levels of eosinophil in PAAD.

a

b

c

d

e

f

Figure 7

Correlation analysis of TPM4 expression with immune checkpoint genes and chemokines/chemokine receptors. (a) Correlation analysis of TPM4 expression levels with over 40 common immune checkpoint gene levels in PAAD. (b-f) TPM4 expression was positively closely related with CCL7, CCL13, CCL18, CXCL5, and CXCL8 in PAAD.

Figure 8

Enrichment analysis of TPM4 expression-correlated DEGs in PAAD. (a) Volcano plot of DEGs between samples with high TPM4 expression and low TPM4 expression. (b) Clustering analysis heatmap of TPM4 expression-correlated DEGs. (c) KEGG enrichment and (d) GO enrichment analysis by TPM4 expression-correlated upregulated DEGs.

Figure 9

PPI network analysis of TPM4-related genes. (a) The visualizing interaction network of TPM4-binding proteins was obtained base on STRING database. (b) An intersection analysis of TPM4 expression-correlated DEGs and TPM4-interacted genes was performed. (c) Correlation analysis between TPM4 expression and screened common genes, including ACTA2, ACTG2, and TNNT1.

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