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Elevated Transient Receptor Potential Melastatin 8 (*TRPM8*) Expression Is Correlated with Poor Prognosis in Pancreatic Cancer

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Background: The transient receptor potential melastatin 8 (*TRPM8*) was found to be expressed abnormally in a variety of tumors and is associated with unfavorable prognosis in human cancers. However, its clinical significance in pancreatic cancer (PC) is mostly unknown.

Material/Methods: qRT-PCR was performed to measure the expression of *TRPM8* in 110 pairs of PC tissues and the adjacent non-cancerous tissues. The association of *TRPM8* expression with the clinical characters of PC patients was analyzed using the chi-square test. Furthermore, the prognostic value of *TRPM8* was determined with Kaplan-Meier survival curve and Cox regression analysis.

Results: We found that the expression level of *TRPM8* was significantly elevated in PC tissues compared to the non-cancerous controls ($P < 0.001$). In addition, a close relationship was observed between elevated *TRPM8* expression with large tumor size ($P = 0.001$), advanced TNM ($P = 0.013$), and distant metastasis ($P = 0.034$). Survival analysis suggested that patients with high *TRPM8* expression has worse OS ($P = 0.001$) and DFS ($P < 0.001$) than those with low *TRPM8* expression. Moreover, *TRPM8* was confirmed as a valuable prognostic biomarker for OS (HR=1.913; 95% CI: 1.020–3.589; $P = 0.043$) or DFS (HR=2.374; 95% CI: 1.269–4.443; $P = 0.007$) of PC patients.

Conclusions: This study shows that *TRPM8* expression is significantly up-regulated in PC and it might be a useful prognostic factor for patients with PC.

MeSH Keywords: **Biological Markers • Pancreatic Neoplasms • Prognosis • Transient Receptor Potential Channels**

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Background

Pancreatic cancer (PC) remains one of most lethal malignant tumors; it is responsible for approximately 227 000 deaths worldwide every year due to its high recurrence and mortality rates [1–3]. Recently, the mortality rate of PC has been increasing and the average age of patients has decreased [4]. Surgical resection remains the only potentially curative treatment for PC [5,6]. However, due to the lack of specific clinical symptoms at early stages, most cases are diagnosed in advanced stages and are ineligible for potentially curative resection; therefore, the prognosis is poor and the 5-year survival rate is only 3–6% [7,8]. Several clinicopathologic parameters have been identified as prognostic factors of PC, including neural invasion, lymph node metastasis, CA19-9 level, and TNM [9–12]. Moreover, a variety of biological prognostic factors have been reported in the development and progression of PC [13], but there currently is no satisfactory way to effectively improve the survival of PC patients.

The family of transient receptor potential (TRP) channels are essential mediators of signaling pathways involved in cellular functions and associated with progression and treatment of various cancers [14]. As a member of the TRP family, transient receptor potential melastatin 8 (*TRPM8*) is a Ca^{2+} -permeable cation channel [15] and is required for the transduction of moderately cold temperatures [16,17]. Initially, human *TRPM8* was known as *trp-p8* and has been identified to have a high expression level in prostate cancer [18]. Moreover, increased expression of *TRPM8* was found in prostate cancer cells and it emerged as a promising prognostic marker in prostate cancer [19]. In fact, more and more reports have suggested that *TRPM8* plays vital functions in carcinogenesis of many cancers, including melanoma [20], bladder cancer [21], oral cancer [22], lung cancer, colon cancer, and breast cancer [18]. More importantly, studies on the relationship between *TRPM8* and tumors reveal that high expression of *TRPM8* appears to be associated with poor prognosis [23,24]. Hence, *TRPM8* had been reported to be a valuable prognostic biomarker and might be a potential treatment target [25]. It has reported that *TRPM8* expression is elevated in PC, and the aberrantly over-expressed *TRPM8* is required for cellular proliferation and invasion [26,27]. However, its clinical significance in prognosis for PC has not been previously reported. The present study investigated the potential of *TRPM8* as a prognostic biomarker in PC patients.

Material and Methods

The collection of patients and samples

The present study was approved by the Ethics Committees of the General Hospital of the PLA. A total of 110 fresh PC tissues and

corresponding adjacent non-cancerous tissues were obtained from patients who were pathologically diagnosed with PC in the General Hospital of the PLA. All patients provided written informed consent and none of them had received any related anti-tumor therapies (e.g., chemotherapy, radiotherapy, or hormone therapy) before surgery. The TNM stage was classified according to the International Association of Cancer. For the expression of CA19-9 and CEA in serum, blood samples were randomly obtained before surgery, and serum was obtained by routine centrifuging. The expressions of CA19-9 and CEA were determined by double-antibody sandwich ELISA (electrochemiluminescence immunoassay). All patients had complete 3-year follow-up. Clinicopathological data of all patients are summarized in Table 1.

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

All the RNA was exacted from the collected PC tissues and adjacent normal specimens with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. The purity of RNA was determined by NanoDrop ND-1000 (Wilmington, DE, USA). First-strand complementary DNA (cDNA) was transcribed using the PrimeScript RT reagent kit (TaKaRa, Dalian, China). The qRT-PCR was performed using SYBR Premix Ex Taq (Takara, Dalian, China). The relative expression of *TRPM8* was normalized to *GAPDH* and quantified by $2^{-\Delta\Delta\text{CT}}$ method. All experiments were performed in triplicate.

Statistical analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA) were used to perform statistical analyses. The *TRPM8* expression differences of PC tissues with adjacent normal specimens were measured by *t* test. The chi-square test was used to analyze the relationship between *TRPM8* expression and the clinical features of PC patients. Kaplan-Meier analysis with log-rank test was used for survival analysis. The prognostic value of *TRPM8* mRNA in pancreatic cancer was determined using multivariate Cox regression analysis after adjusting the baseline characteristics, including age, sex, tumor size, metastasis, differentiation, tumor location, CA19-9, and CEA. $P < 0.05$ was considered to be statistically significant.

Results

TRPM8 expression was up-regulated in PC tissues

To illustrate the abnormal expression of *TRPM8* in PC, we investigated its mRNA expression level in PC tissues and adjacent non-cancerous tissues by use of qRT-PCR. We observed that the expression of *TRPM8* was obviously higher in PC tissues than that in adjacent non-cancerous tissues (mean $\pm$ SD: 1.45 ± 0.55 versus 0.85 ± 0.44 , $P < 0.001$; Figure 1).

Table 1. The relationship between *TRPM8* level and the clinicopathological characteristics of PC patients.

Variables	N	<i>TRPM8</i> level		P value
		High (57)	Low (53)	
Age				0.249
≥55	54	31	23	
<55	56	26	30	
Gender				0.279
Male	70	39	31	
Female	40	18	22	
Tumor size				0.001
≥2 cm	58	39	19	
<2 cm	52	18	34	
TNM				0.013
III, IV	55	35	20	
I, II	55	22	33	
Distant metastasis				0.034
Positive	53	33	20	
Negative	57	24	33	
Tumor differentiation				0.177
High/moderate	57	26	31	
Poor	53	31	22	
Tumor location				0.053
Body/tail	52	32	20	
Head	58	25	33	
CA19-9 (U/mL)				0.053
≥37	52	32	20	
<37	58	25	33	
CEA (ng/mL)				0.086
≥4.7	55	33	22	
<4.7	55	24	31	

Relationship of *TRPM8* expression with clinical parameters of PC patients

To further determine the clinical significance of *TRPM8* expression in PC, the relationship of *TRPM8* expression with clinical parameters was investigated. As shown in Table 1, based on the median expression level of *TRPM8* mRNA in PC tissues (1.23), the participants were divided into 2 groups: the high-expression group (n=57), and the low-expression group (n=53). The results showed that the high expression of

TRPM8 was significantly associated with tumor size ($P=0.001$), TNM ($P=0.013$), and distant metastasis ($P=0.034$). However, no close relationship was found between *TRPM8* expression and age, sex, tumor differentiation, tumor location, CA19-9, or CEA (all, $P>0.05$).

The prognostic value of *TRPM8* expression in PC

The results of Kaplan-Meier curves showed that the survival time of patients in the high-*TRPM8* expression group was

Figure 1. Analysis of *TRPM8* expression in PC tissues by qRT-PCR. Relative expression of *TRPM8* in PC tissues was significantly increased compared with that in adjacent non-cancerous controls ($P < 0.001$).

shorter than those in the low-*TRPM8* expression group (OS, $P = 0.001$; DFS, $P < 0.001$; Figure 2A, 2B). In univariate analysis for OS, the results suggested that several clinical parameters were closely related with poor survival, including tumor size, advanced TNM stage, distant metastasis, tumor located in body/tail, high CA19-9, high CEA, and high *TRPM8* (all, $P < 0.05$). Moreover, further analysis of multivariate revealed that *TRPM8* (HR=1.913; 95% CI: 1.020–3.589; $P = 0.043$) along with TNM stage (HR=2.141; 95% CI: 1.074–4.269; $P = 0.031$) and CA19-9 (HR=1.938; 95% CI: 1.074–3.498; $P = 0.028$) were potential valuable prognostic biomarkers for OS of PC patients (Table 2). Similarly, the univariate analysis for DFS demonstrated that male sex, large tumor size, advanced TNM, distant metastasis, tumor located in body/tail, high CA19-9, CEA, and *TRPM8* (all, $P < 0.05$) were significantly associated with poor DFS. According to the multivariate analysis, *TRPM8* (HR=2.374;

95% CI: 1.269–4.443; $P = 0.007$), TNM stage (HR=2.039; 95% CI: 1.044–3.984; $P = 0.037$), and CA19-9 (HR=2.018; 95% CI: 1.118–3.642; $P = 0.020$) all have very good prognostic value for DFS of PC patients (Table 3).

Discussion

In the present study, our data further verified the findings of previous studies showing that *TRPM8* expression is elevated in PC tissues compared to that in adjacent non-cancerous tissues. In addition, our data demonstrate for the first time that high *TRPM8* expression is obviously associated with poor OS and DFS in PC patients, and it could be a valuable prognostic biomarker for patients with PC.

As a Ca^{2+} -permeable cation channel, *TRPM8* has been increasingly reported in recent years. *TRPM8* was initially thought to be over-expressed in prostate cancer [18]; subsequently, an increasing number of studies demonstrated the consistent profile of *TRPM8* expression in other human tumors such as urothelial carcinoma of the bladder [24], osteosarcoma [28], and breast cancer [29]. In PC, Yee et al. indicated that the expression of *TRPM8* is increased in pancreatic adenocarcinoma and it plays important role in cellular proliferation [26]. Consistent with the previous study, our data also revealed that *TRPM8* mRNA levels are obviously elevated in PC tissues compared to the adjacent non-cancerous tissues. All these data show that *TRPM8* may be a tumor promoter in the development of PC. Therefore, to assess the clinical significance of *TRPM8* in PC progression, we evaluated its association with the clinicopathological characteristics. The results showed significant correlations between *TRPM8* expression and tumor size, TNM stage, and distant metastasis.

Figure 2. Survival analysis. The patients with high *TRPM8* expression had worse overall survival (OS, $P = 0.001$; **A**) and disease-free survival (DFS, $P < 0.001$; **B**) than did patients with low *TRPM8* expression.

Table 2. Univariate and multivariate analysis of variables associated with OS in patients with PC.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age	1.395 (0.834–2.334)	0.205	–	–
Gender	1.904 (1.028–3.526)	0.040	–	–
Tumor size	2.657 (1.487–4.750)	0.001	–	–
TNM	3.334 (1.789–6.213)	<0.001	2.141 (1.074–4.269)	0.031
Distant metastasis	2.311 (1.327–4.023)	0.003	–	–
Tumor differentiation	1.507 (0.896–2.535)	0.122	–	–
Tumor location	1.943 (1.146–3.295)	0.014	–	–
CA19-9 (U/mL)	2.566 (1.460–4.510)	0.001	1.938 (1.074–3.498)	0.028
CEA (ng/mL)	2.185 (1.274–3.749)	0.005	–	–
<i>TRPM8</i> levels	2.680 (1.485–4.837)	0.001	1.913 (1.020–3.589)	0.043

Table 3. Univariate and multivariate analysis of variables associated with DFS in patients with PC.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age	1.465 (0.876–2.452)	0.146	–	–
Gender	1.967 (1.062–3.642)	0.031	–	–
Tumor size	2.869 (1.604–5.129)	<0.001	–	–
TNM	3.355 (1.827–6.161)	<0.001	2.039 (1.044–3.984)	0.037
Distant metastasis	2.447 (1.406–4.260)	0.002	–	–
Tumor differentiation	1.539 (0.915–2.587)	0.104	–	–
Tumor location	2.052 (1.210–3.481)	0.008	–	–
CA19-9 (U/mL)	2.706 (1.540–4.756)	0.001	2.018 (1.118–3.642)	0.020
CEA (ng/mL)	2.236 (1.303–3.836)	0.003	–	–
<i>TRPM8</i> levels	3.246 (1.797–5.863)	<0.001	2.374 (1.269–4.443)	0.007

A growing body of evidence supports the idea that *TRPM8* mRNA expression can be used as a prognostic marker and as a tool for the design of novel cancer therapies [23,30,31]. For example, Zhao et al. showed that both the mRNA and protein expression levels of *TRPM8* were high in osteosarcoma patients and the over-expression of *TRPM8* was a valuable prognostic biomarker for patient survival; therefore, it may be a novel potential molecular target for the treatment of osteosarcoma patients [23]. Although the previous study has supported exploration of *TRPM8* as a biomarker and target of PC [32], its role in prognosis of PC is still unclear. In the present study we found that patients in the high-*TRPM8* expression group had worse outcomes than those with low

expression, and *TRPM8* might be a potential valuable prognosis biomarker for PC patients.

Previous reports have indicated that *TRPM8* affects the development of human tumors by regulating cell proliferation, invasiveness, and adhesion [16,33]. Moreover, the expression of *TRPM8* is involved in a variety of signal channels of cells, such as the activation of phospholipase C and phosphoinositides [34]. However, the mechanism underlying the over-expression of *TRPM8* in PC cells remains unclear. The expression profiles of *TRPM8* mRNA were reported to be significantly associated with Ca²⁺ channel activity and the Ca²⁺ signaling pathway is involved in both cell survival and death through

the transfection of Ca^{2+} from the endoplasmic reticulum (ER) to the mitochondria [35]. The transfection process may result in alterations in multiple biological processes, such as energy production, and reactive oxygen species (ROS) production, thus significantly influencing cell survival status [36,37]. We found that the alterations in Ca^{2+} -permeable channel induced by over-expression of *TRPM8* played contributory roles in cellular proliferation, thus contributing to pancreatic tumor growth and metastasis. *TRPM8* might be a potential biomarker for the treatment and therapy of pancreatic adenocarcinoma [26]. Therefore, in the future, more attention should be

focused on the role of *TRPM8* expression in the generation of Ca^{2+} release channels in PC cells.

Conclusions

In conclusion, the present study demonstrates that *TRPM8* levels are significantly increased in PC tissues. Moreover, high *TRPM8* expression level correlates with worse OS and DFS of PC; therefore, it could be an effective prognosis factor for PC patients.

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