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 Ca2+-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 immunonassay). 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-ΔΔCT 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 ±SD: 1.45±0.55 versus 0.85±0.44, P<0.001; Figure 1).

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

| Variables         | N  | TRPM8 level | P value |
|-------------------|----|-------------|---------|
|                   |    | High (57)   | Low (53) |         |
| Age               |    |             |         |
| ≥55               | 54 | 31          | 23      | 0.249   |
| <55               | 56 | 26          | 30      |         |
| Gender            |    |             |         |
| Male              | 70 | 39          | 31      | 0.279   |
| Female            | 40 | 18          | 22      |         |
| Tumor size        |    |             |         |
| ≥2 cm             | 58 | 39          | 19      | 0.001   |
| <2 cm             | 52 | 18          | 34      |         |
| TNM               |    |             |         |
| III, IV           | 55 | 35          | 20      | 0.013   |
| I, II             | 55 | 22          | 33      |         |
| Distant metastasis|    |             |         |
| Positive          | 53 | 33          | 20      | 0.034   |
| Negative          | 57 | 24          | 33      |         |
| Tumor differentiation|   |             |         |
| High/moderate     | 57 | 26          | 31      | 0.177   |
| Poor              | 53 | 21          | 32      |         |
| Tumor location    |    |             |         |
| Body/tail         | 52 | 32          | 20      | 0.053   |
| Head              | 58 | 25          | 33      |         |
| CA19-9 (U/mL)     |    |             |         |
| ≥37               | 52 | 32          | 20      | 0.053   |
| <37               | 58 | 25          | 33      |         |
| CEA (ng/mL)       |    |             |         |
| ≥4.7              | 55 | 33          | 22      | 0.086   |
| <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
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 = 2.018; 95% CI: 1.118–3.642; \(P = 0.020\)) all have very good prognostic value for DFS 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 Ca2+-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.
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

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

| Variables           | 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   | –             | –       |
| TRPM8 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           | 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   | –             | –       |
| TRPM8 levels        | 3.246 (1.797–5.863) | <0.001  | 2.374 (1.269–4.443) | 0.007  |
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.

References:

1. Siegel RL, Miller KD, Jemal A: Cancer statistics, 2016. Cancer J Clin, 2016; 66: 7–30
2. Bocek S, Heinemann V: Improving post-surgical management of resected pancreatic cancer. Lancet, 2017; 390: 847–48
3. Kleeft J, Michl P: Targeted therapy of pancreatic cancer: Biomarkers are needed. Lancet Oncol, 2017; 18: 421–22
4. Schellhaas B, Vitali F, Wildner D et al: Dynamics of Fukuoka criteria and patient management in pancreatic intraductal papillary mucinous neoplasms (IPMNs) during follow-up. Med Sci Monit, 2017; 23: 1483–92
5. Ma N, Wang Z, Zhao J et al: Improved survival in patients with resected pancreatic carcinoma using postoperative intensity-modulated radiotherapy and regional intra-arterial infusion chemotherapy. Med Sci Monit, 2017; 23: 2315–23
6. Zhou B, Zhan C, Wu J et al: Prognostic significance of preoperative neutrophil-to-lymphocyte ratio in surgically resectable pancreatic neuroendocrine tumors. Med Sci Monit, 2017; 23: 5754–88
7. Hu RI, Ma JY, Hu G: Lymphocyte-to-monocyte ratio in pancreatic cancer: Prognostic significance and meta-analysis. Clin Chim Acta, 2018; 481: 144–46
8. Siegel R, Naishadham D, Jemal A: Cancer statistics, 2012. Cancer J Clin, 2012; 62: 10–29
9. Chattejee D, Katz MH, Rashid A et al: Perineural and intraneural invasion in posttherapy pancreaticoduodenectomy specimens predicts poor prognosis in patients with pancreatic ductal adenocarcinoma. Am J Surg Pathol, 2012; 36: 409–17
10. Robinson SM, Rahman A, Haugk B et al: Metastatic lymph node ratio as an important prognostic factor in pancreatic ductal adenocarcinoma. Eur J Surg Oncol, 2012; 38: 333–39
11. Hallemeier CL, Botros M, Corsini MM et al: Preoperative CA 19-9 level is an important prognostic factor in patients with unresectable pancreatic cancer. Pancreas. 2010; 39: 1247–53
12. Han J, Chen Y, Shuai S et al: Prognostic factors for survival in patients with resectable pancreatic cancer. Pancreas. 2010; 39: 1247–53
13. Jia S, Zhou L, Shen T et al: Down-expression of CD36 in pancreatic adenocarcinoma and its correlation with clinicopathological features and prognosis. J Cancer, 2018; 9: 578–83
14. Chen J, Luan Y, Yu R et al: Transient receptor potential (TRP) channels, promising potential diagnostic and therapeutic tools for cancer. Biosci Trends, 2014; 8: 1–10
15. Montell C: A mint of mutations in TRPM8 leads to cool results. Nat Neurosci, 2006; 9: 466–68
16. Bautista DM, Siemons J, Glazer JM et al: The menthol receptor TRPM8 is the principal detector of environmental cold. Nature, 2007; 448: 204–8
17. Knowlton WM, McKey DD: TRPM8: From cold to cancer, peppermint to pain. Curr Pharm Biotechnol, 2011; 12: 68–77
18. Tsvanelov L, Shapero MH, Morkowski S, Laus R: Trp-p8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. Cancer Res, 2001; 61: 3760–69
19. Liu T, Fang Z, Wang G et al: Anti-tumor activity of the TRPM8 inhibitor BCTC in prostate cancer DU145 cells. Oncol Lett, 2016; 11: 182–88
20. Klijn's group, Tereemtschiopulou A, Chouchou C: Dose-dependent cytotoxic effects of menthol on human malignant melanoma A-375 cells: Correlation with TRPM8 transcript expression. Asian Pac J Cancer Prev, 2014; 15: 1551–56
21. Li G, Wang X, Yang Z et al: Menthol induces cell death via the TRPM8 channel in the human bladder cancer cell line T24. Oncology, 2009; 77: 335–41
22. Okamoto Y, Ohkubo T, Iike T, Yamazaki J: Blockade of TRPM8 activity reduces the invasion potential of oral squamous carcinoma cell lines. Int J Oncol, 2012; 40: 1431–40
23. Zhao W, Xu H: High expression of TRPM8 predicts poor prognosis in patients with osteosarcoma. Oncol Lett, 2016; 12: 1373–79
24. Xiao N, Jiang LM, Ge B et al: Over-expression of TRPM8 is associated with poor prognosis in uterine cervical carcinoma of bladder. Tumour Biol, 2014; 35: 11499–504
25. Zang L, Barrett GI: TRPM8 in prostate cancer cells: A potential diagnostic and prognostic marker with a secretory function? Endocr Relat Cancer, 2006; 13: 27–38
26. Yee NS, Zhou W, Lee M: Transient receptor potential channel TRPM8 is over-expressed and required for cellular proliferation in pancreatic adenocarcinoma. Cancer Lett, 2010; 297: 49–55
27. Yee NS, Li Q, Kazi AA et al: aberrantly over-expressed TRPM8 channels in pancreatic adenocarcinoma: Correlation with tumor size/stage and requirement for cancer cells invasion. Cells, 2014; 3: 500–16
28. Wang Y, Yang Z, Meng Z et al: Knockdown of TRPM8 suppresses cancer malignancy and enhances epirubicin-induced apoptosis in human osteosarcoma cells. Int J Biol Sci, 2011; 10: 90–92
29. Liu J, Chen Y, Shuai S et al: TRPM8 promotes aggressiveness of breast cancer cells by regulating EMT via activating AKT/GSK-3beta pathway. Tumour Biol, 2014; 34: 8967–79
30. Zhang L, Barrett GI: Evidence that TRPM8 is an androgen-dependent Ca2+ channel required for the survival of prostate cancer cells. Cancer Res, 2004; 64: 8365–73
31. Mukerji G, Yiangou Y, Corcoran SL et al: Cool and menthol receptor TRPM8 in human urinary bladder disorders and clinical correlations. BMC Urol, 2006; 6: 6
32. Yee NS, Chen AS, Yee JD, Yee KK: TRPM7 and TRPM8 ion channels in pancreatic adenocarcinoma: Potential roles as cancer biomarkers and targets. Scientifica (Cairo), 2012; 2012: 415158
33. Du GJ, Li JH, Liu WL et al: The combination of TRPM8 and TRPA1 expression causes an invasive phenotype in lung cancer. Tumour Biol, 2014; 35: 1251–61
34. Yudin Y, Rohacs T: Regulation of TRPM8 channel activity. Mol Cell Endocrinol, 2012; 353: 68–74
35. Vervliet T, Clerix E, Seitaj B et al: Modulation of Ca(2+) signaling by anti-apoptotic B-cell lymphoma 2 proteins at the endoplasmic reticulum-mitochondrial interface. Front Oncol, 2017; 7: 75
36. Hwang MS, Schwall CT, Pazarentzos E et al: Mitochondrial Ca(2+) influx targets cardiolipin to disintegrate respiratory chain complex II for cell death induction. Cell Death Differ, 2014; 21: 1733–45
37. Rizzuto R, De Stefano D, Raffaello A, Mammucari C: Mitochondria as sensors and regulators of calcium signalling. Nat Rev Mol Cell Biol, 2012; 13: 566–78