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Original Article

Increased expression of tumor necrosis factor receptor-associated factor 6 in pancreatic cancer and its clinical significance

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Abstract: Objective: Tumor necrosis factor receptor-associated factor 6 (TRAF6) has been proved to play an important role in various malignancies. However, the role of TRAF6 in pancreatic adenocarcinoma (ADC) has not been clearly defined. The objective of this study was to assess the aberrant expression of TRAF6 and its clinicopathological features in pancreatic ADC. Methods: Immunohistochemistry was used to detect the expression of TRAF6 in 153 pancreatic ADC tissues. We also analyzed the association of TRAF6 expression with clinical pathological characteristics and the survival of pancreatic ADC patients. Furthermore, genomic alterations of TRAF6 were analyzed from The Cancer Genome Atlas (TCGA) via cBioPortal. Results: The expression level of TRAF6 was higher in pancreatic ADC tissues (54.2%) than that in normal pancreas (11.1%, P=0.001) and para-tumor pancreatic tissues (16.7%, P<0.001). The area under curve (AUC) of TRAF6 was 0.694 to diagnose pancreatic ADC. Positive correlations were also noted between TRAF6 expression and N stage (r=0.285, P<0.001), M stage (r=0.19, P=0.019) and TNM stage (r=0.334, P<0.001). Furthermore, cBioPortal provided OncoPrints of TRAF6 in pancreatic ADC (TCGA, provisional) and 8% of 185 cases showed different genetic alteration including amplification, mRNA upregulation, mRNA downregulation, and missense mutation. Conclusion: TRAF6 is up-regulated in pancreatic ADC tissues and could serve as a tumor promoter in the initiation and progression of pancreatic ADC.

Keywords: TNF receptor-associated factor 6, pancreatic adenocarcinoma, immunohistochemistry, TNM stage

Introduction

Pancreatic adenocarcinoma (ADC) is the main histologic type of pancreatic cancer, which has the following characteristics: the treatment is difficult and the mortality rate is high. Most pancreatic ADC patients are diagnosed at an advanced stage with serious clinical symptoms, and distant metastases will then lead to poor prognosis. Thus, early detection and diagnosis of pancreatic ADC remains a clinical challenge [1-7]. Nowadays, though the systemic recurrence rate of pancreatic ADC is higher, the only potentially curative treatment of pancreatic ADC is still surgical resection, which can cover only small portion of the pancreatic ADC patients. Presently, people have been working on the molecule associated with pancreatic ADC and attempted to discover new target molecules for clinical diagnosis and treatment for pancreatic ADC [4, 5, 8-13]. However, the mechanism underlying pancreatic ADC initiation and progression has not been well elucidated.

Tumor necrosis factor receptor-associated factors (TRAFs) are an adapter protein in the cell, which act as a bridging protein to link cell surface receptors and the downstream signaling cascades, and eventually the process will lead to the release of proinflammatory cytokines

Table 1. Expression of TRAF6 protein in non-cancerous pancreas and pancreatic adenocarcinoma (ADC) tissues

Tissues	n	TRAF6 positive (n, %)	*P*-value
Normal pancreas	18	2/18 (11.1%)	0.001[A]
Para-tumor	66	11/66 (16.7%)	0.566[B]
Pancreatic ADC	153	83/153 (54.2%)	<0.001[C]

A: Normal pancreas vs Pancreatic ADC, B: Normal pancreas vs Para-tumor, C: Para-tumor vs Pancreatic ADC.

[14]. As the especial family member in the TRAFs, TRAF6 can not only activate signaling pathways but are also extensively involved in the inflammatory response and immune response. In the other hand, TRAF6 can be a signal transducer in the pathway of NF-κB by activating IKK in response to proinflammatory cytokines [15]. More importantly, studieshave recently demonstrated that TRAF6 may have a close relationship with some neoplasms [16-25].

However, only one study has reported the clinical value of TRAF6 in pancreatic cancer. Rong Y, et al [18] revealed that the expression level of TRAF6 mRNA and protein was both increased in pancreatic cancer samples than that in the paired normal pancreatic tissues. However, the sample size was small (n=53) and the relationship between TRAF6 and disease progression has not been investigated. Thus, in the present study, both immunohistochemistry (n=237) and bioinformatics based on The Cancer Genome Atlas (TCGA, n=185) were performed to explore the clinicopathological implication of TRAF6 in pancreatic ADC.

Materials and methods

Study design

The study included a total of 237 specimens of pancreatic tissues. All of pancreatic specimens were randomly selected from the Department of Pathology of the First Affiliated Hospital of Guangxi Medical University, Department of Pathology of the First Affiliated Hospital of Guangxi University of Science and Technology, or purchased from Fanpu Biotech, Inc (PAC481 and PAC961, Guilin, China). In this study, all of patients did not receive any radiotherapy or chemotherapy before surgery. The patients all agreed that these specimens could be applied for research work. All the pancreatic specimens in the current study were fixed by 10% formalin and embedded with paraffin according to the standard procedure in pathology department. Specimens of pancreatic tissues were clinically classified into ADC (n=153), para-tumor pancreatic tissues (n=66) and non-tumor normal pancreatic tissues (n=18, **Table 1**).

Immunohistochemical staining to detect TRAF6

All of specimens were performed according to the following process: treated with high pressure hotfix, retied by hematoxylin, differentiated by hydrochloric acid-alcohol, dehydrated by anhydrous ethanol, and sealed by neutral gum. Next primary and second antibodies were incubated and lastly 3.3'-Diaminobenzidine spectrophotometry (DAB) chromogenic was used as the color reagent. Immunostaining was performed with TRAF6 mouse monoclonal antibody from Santa Cruz Biotechnology Inc. (D-10, sc-8409, dilution: 1:300, Heidelberg, Germany). All steps were strictly carried out with the manufacturer's protocols as previously reported [20].

Evaluation of immunostaining

The immunohistochemical staining of pancreatic specimens were evaluated by three authors (Lin Shi, Dan-ming Wei and Gang Chen) without the information about patient's clinical manifestations. The average percentage of positive cells were used as criteria to score the TRAF6 expression semi quantitatively: no staining was marked as "0", <30% as "1", 30-70% as "2", and >70% as "3". The staining intensity was also recorded as following: no color was recorded as "0"; weaklystained with yellow as "1", moderately stained with yellow-brown as "2" and strongly stained with dark brown as "3"). According to the sum of the scores: 0~2 were divided into negative group(-), 3~6 were divided into positive group(+) [20].

OncoPrints of TRAF6 in pancreatic ADC based on TCGA data via cBioPortal

OncoPrints was produced to present the genomic alterations directly from cBioPortal in pancreatic ADC (TCGA, provisional) containing 185 patients [26] (www.cbioportal.org).

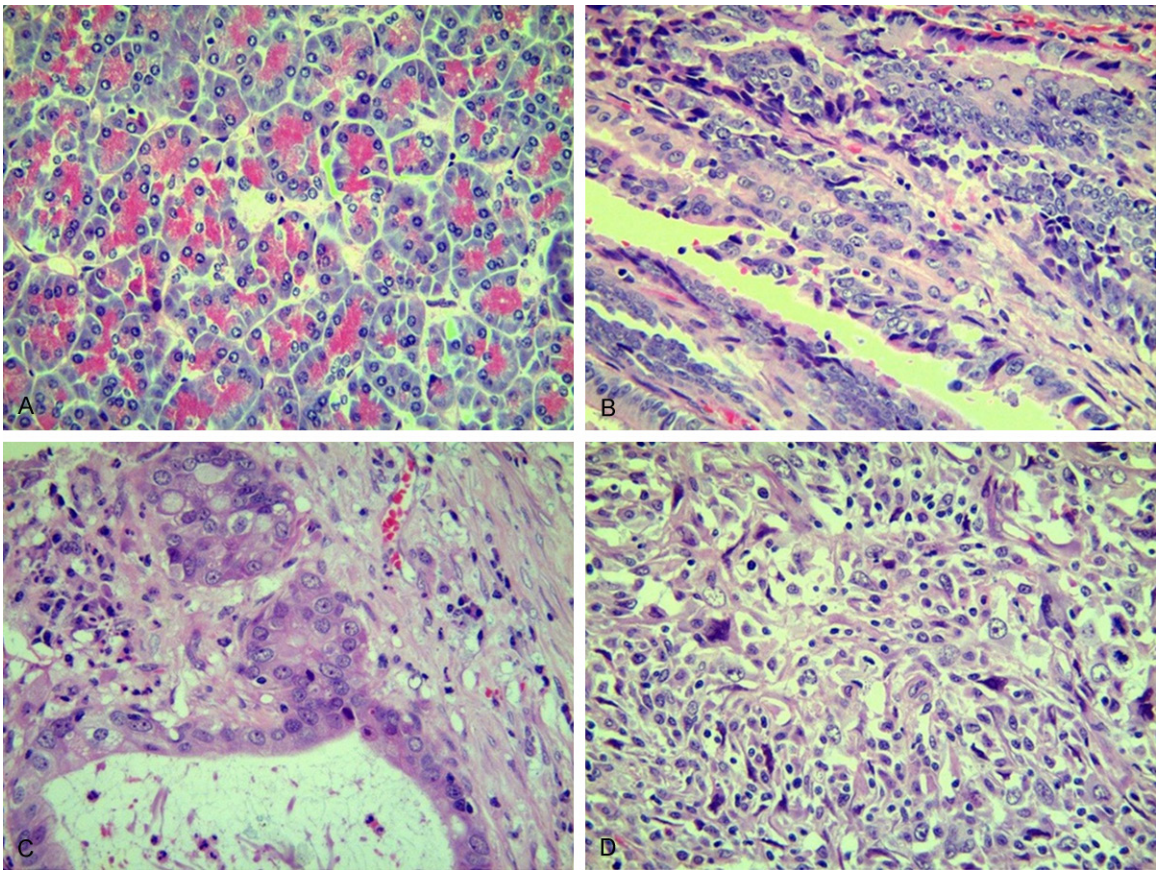

Figure 1. Hematoxylin-Eosin staining of pancreatic adenocarcinoma (ADC) tissues. Non-cancer pancreatic tissue (A). Pancreatic ADC tissue TNM I, Grade I (B), TNM II, Grade I-II (C) and TNM III, Grade III (D), 400×.

Statistical analysis

All of the data was analyzed statistically by SPSS22.0 software. Mann-Whitney U tests were used to compare the difference of TRAF6 expression with two categories for different parameters. Kruskal-Wallis H was selected for the comparison of the difference of TRAF6 expression among more than two categories for some parameters, for instance, four T stages and four TNM stages. Receiver Operating Characteristic Curve (ROC) was drawn with SPSS22.0 as well. Spearman's correlation test was performed to assess the correlation between TRAF6 expression and various clinicopathological features. When the value of the *P* with two-tailed was less than 0.05, the tests had statistical significance.

Results

HE and immunohistochemical staining

HE staining of the pancreatic tissues including ADC and non-cancerous pancreatic tissues was shown in **Figure 1**. Immunohistochemically, TRAF6 positive signaling was observable in the cytoplasm of pancreatic ADCcells and some areas of non-cancerous pancreatic tissues (**Figure 2**). The positive ratio of TRAF6 protein was 54.2% (83/153) in pancreatic ADC tissues, significantly higher than that in normal pancreas (11.1%, 2/18, P=0.001) and papa-tumor pancreatic tissues (16.7%, 11/66, P<0.001, **Table 1**; **Figure 2**). Further, the area under curve (AUC) of TRAF6 was 0.694 (95% confidence interval: 0.626-0.762, P<0.001) to diagnose pancreatic ADC. From normal pancreas, para-tumor pancreatic tissue to pancreatic ADC, the TRAF6 expression also rose from 11.1%, 16.7% to 54.2%, showing an increasing trend (r=0.367, P<0.001).

The relationship between TRAF6 expression and clinical parameters in pancreatic ADC

As opposed to lymphatic metastasis group (46/65, 70.8%), the patients without lymph node metastasis (37/88, 42%) showed a signifi-

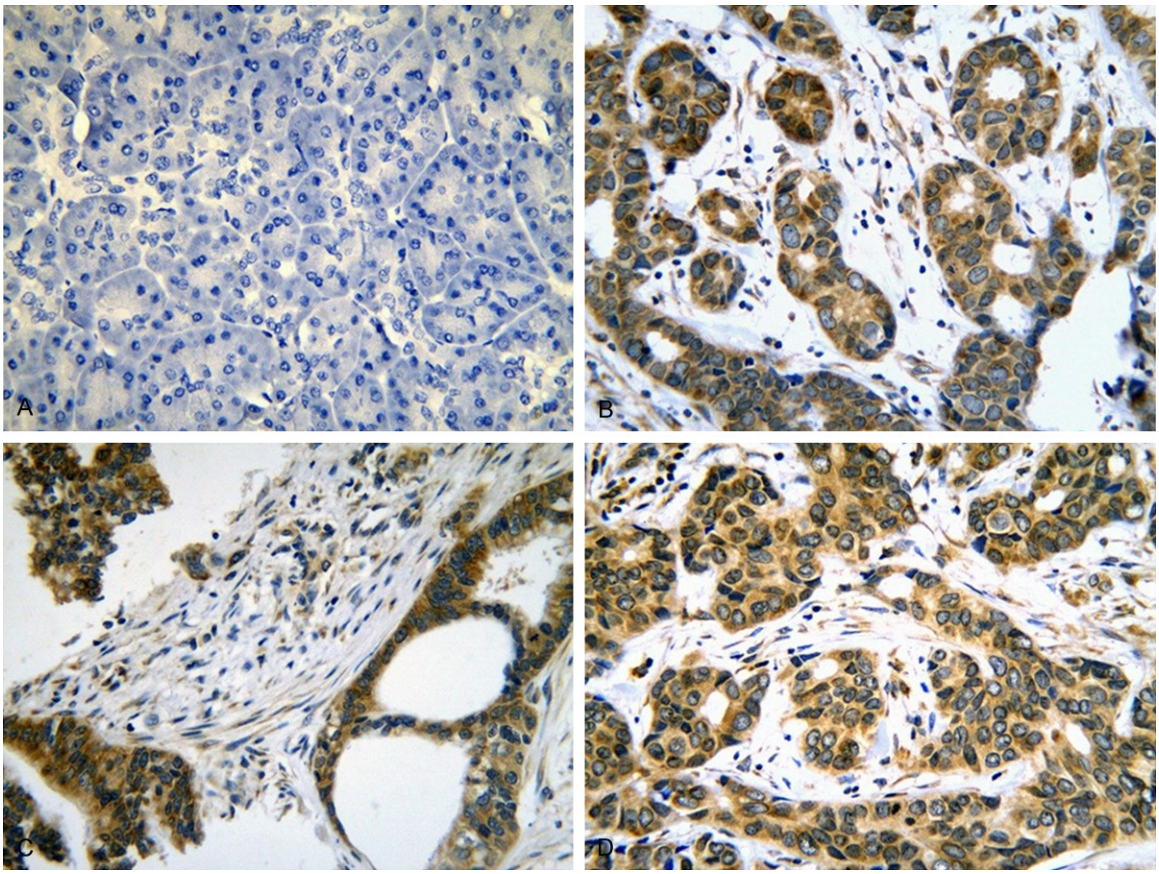

Figure 2. Immunohistochemical Staining of TRAF6 protein in various pancreatic tissues. Non-cancer pancreatic tissue (A). Pancreatic adenocarcinoma (ADC) tissue TNM I, Grade I (B), TNM II, Grade I-II (C) and TNM III, Grade III (D), 400×.

cantly lower TRAF6 expression level (P<0.001). With regard to tumor distant metastasis, higher level TRAF6 expression was found in metastasis group (9/10, 90%) compared to that in non-metastasis group (74/143, 51.7%, P=0.019). Furthermore, with TNM stage increased, the positive ratio of TRAF6 was growing from 38%, 53.3%, 68.4% to 90% for each stage (**Table 2**). Positive correlations were also found between TRAF6 expression and N stage (r=0.285, P<0.001), M stage (r=0.19, P=0.019) and TNM stage (r=0.334, P<0.001). No significant correlation was notedbetween TRAF6 expression and other characteristics (data not shown).

Survival analysis

The survival data was collected from 49 patients and the mean follow-up time was 15.913±1.754 months (range: 0.5-36.0 months). Thirty-five patients died during the follow-up period. The positive expression rate of TRAF6 among 49 patients followed-up was 59.2% (29/49). Kaplan-Meier was performed to evaluate the relationship between TRAF6 expression and survival of pancreatic ADC patient. However, no statistics significance was found between TRAF6 negative group (15±2.683 months) and TRAF6 positive group (16.221±2.214, P=0.754, **Figure 3**).

OncoPrints of TRAF6 of pancreatic ADC based on TCGA

OncoPrints of TRAF6 in pancreatic ADC (TCGA, provisional) was assessed by cBioPortal (www.cbioportal.org). Altogether 185 cases in TCGA were available for the OncoPrints. Genetic alteration of TRAF6 in 14 cases (8%) was noted including amplification, mRNA upregulation, mRNA downregulation, and missense mutation (**Figure 4**).

Discussion

In the current study, we investigated the clinicopathological role of the TRAF6 expression in

Table 2. Relationship between TRAF6 expression and clinical parameters in pancreatic adenocarcinoma patients

Parameters		n	TRAF6 positive (n, %)	*P*-value
T	T1a	2	1/2 (50%)	0.948
	T1b	43	22/43 (51.2%)	
	T2	77	42/77 (54.5%)	
	T3	31	18/31 (58.1%)	
N	N0	88	37/88 (42%)	<0.001
	N1	65	46/65 (70.8%)	
M	M0	143	74/143 (51.7%)	0.019
	M1	10	9/10 (90%)	
TNM	I	71	27/71 (38%)	0.005*
	II	15	8/15 (53.3%)	
	III	57	39/57 (68.4%)	
	IV	10	9/10 (90%)	

*I vs II: P=0.276, I vs III: P=0.001, I vs IV: P=0.002, II vs III: P=0.278, II vs IV: 0.129, III vs IV: P=0.166.

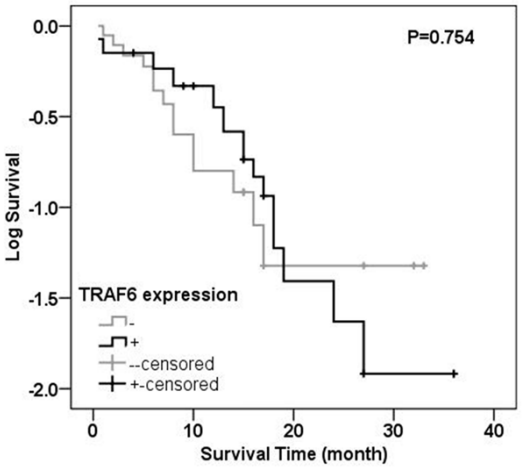

Figure 3. Correlation of TRAF6 expression with survival of pancreatic adenocarcinomas. Kaplan-Meier test was performed.

pancreatic ADC, and found that TRAF6 might be related to the initiation and progression of pancreatic ADC.

TRAF6 is a potential factor in the development of various cancers as well as in immune and inflammation. The expression of TRAF6 has been reported to be overexpressed in both esophageal squamous cell carcinoma (ESCC) clinical samples and ESCC cell lines [16, 17]. TRAF6 was also overexpressed in gastric cancer [23], colon cancer [19, 25] and primary as well as metastatic melanoma tumors and melanoma cell lines [24]. Previously, we found that the expression of TRAF6 protein in both non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) was markedly increased than that in non-tumorous lung tissues. Furthermore, the AUC of ROC for TRAF6 was 0.663, which indicated a moderate diagnostic value for lung cancer [20]. Taken together, TRAF6 may play an oncogenic role in several cancers. However, when concerning the clinical role of TRAF6 in pancreatic ADC, only one paper performed by Rong Y, et al [18] was available. Rong Y, et al [18] detected both of the mRNA and protein level of TRAF6 in 53 cases of pancreatic cancer samples and paired normal pancreatic tissues with real-time PCR and immunohistochemistry assay, respectively. They found that the expression level of TRAF6 was upregulated in pancreatic cancer samples. They further performed western blot analysis on six randomly selected pancreatic cancer tissues and paired normal tissues, as well as several pancreatic cancer cell lines (MIAPaca2, BXPC3 and Suit2) and achieved concordant overexpression of TRAF6 in pancreatic cancer tissues and cells. In the current study, we confirmed the upregulation of TRAF6 protein level with a larger size of clinical samples with 153 cases of pancreatic ADC and the AUC was 0.694 with a moderate diagnostic effect. We also attempted to analyze the TRAF6 mRNA level based on TCGA data, however, since only four samples were available for the non-cancerous controls, we could not compare the difference of TRAF6 mRNA between 186 pancreatic ADCs and only four normal pancreases, which did not meet the requirements of the statistics. However, cBioPortal provided OncoPrints of TRAF6 in pancreatic ADC (TCGA, provisional) and 8% of 185 cases showed different genetic alteration including amplification, mRNA upregulation, mRNA downregulation, and missense mutation. The association between the genetic alteration of TRAF6 and pancreatic ADC remains to be investigated. Even though, our immunohistochemical verification with previous report of Rong Y, et al [18] strongly indicated that TRAF6 might play an essential part in the tumorigenesis of pancreatic ADC.

Besides the tumorigenic role of TRAF6, several studies have revealed that TRAF6 is closely related to the progression of malignancies,

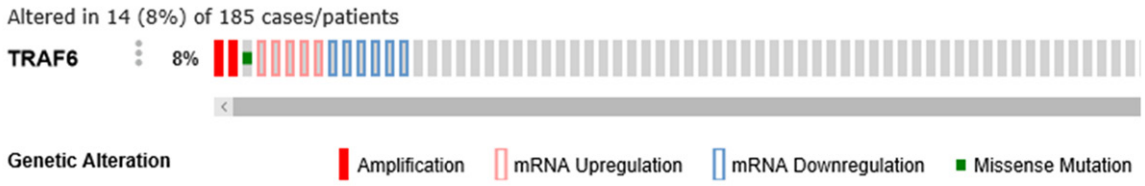

Figure 4. OncoPrints of TRAF6 of pancreatic adenocarcinomas (ADC) based on TCGA. OncoPrints of TRAF6 in pancreatic ADC (TCGA, provisional) was assessed by cBioPortal (www.cbioportal.org). Altogether 185 cases were available for the OncoPrints. Genetic alteration of TRAF6 included amplification, mRNA upregulation, mRNA downregulation, and missense mutation.

including lung cancer [20], gastric cancer [23] and colon cancer [19, 25]. To our knowledge, we are the first to explore the relationship between TRAF6 expression and progression of pancreatic ADC. We foundaccordant significant upregulation of TRAF6 in the more advanced periods of pancreatic ADC, including when patients had lymph node metastasis, distant metastasis and in TNM stage III or IV. Therefore, our finding supports that TRAF6 could also lead to tumorprogression of pancreatic ADC. However, the follow-up data showed TRAF6 aberrant expression was not related to the patient survival based on only a small number of patients. Thus, larger sample size and longer following duration are considered necessary to study the influence of TRAF6 on patient survival of pancreatic ADC.

Considering the mechanism of TRAF6 in pancreatic ADC, Rong Y, et al [18] showed that over-expression of TRAF6 in pancreatic cancer-MIAPaca2 and BXPC3 cells could promote cell proliferation and migration. On the contrary, knock-down of TRAF6 could weaken the tumorigenicity of MIAPaca2 and Suit2 cells both *in vitro* and *in vivo*. Mechanistically, TRAF6 could regulate the expression of a series of multiple genes (c-Myc, CyclinD1, Snail, active Caspase 3, Bax, E-cadherin and cleaved PARP) involved in cell growth, apoptosis and migration. Chiu HW et al [22] also reported that down-regulation of TRAF6 could cause a large increase in apoptosis and autophagy in human pancreatic cancer cells. Hence, the above reports suggested several vital roles of TRAF6 in the carcinogenesis and progression of pancreatic ADC.

It is well-known that early diagnosis with body fluid can assist toreduce the mortality of pancreatic ADC effectively. Serum TRAF6 expression has been detected in breast cancer (BC) [21]. Median serum TRAF6 expression was 0.90 ng/ml in 13 triple-negative breast cancer (TNBC) patients, significantly higher than 0.63 ng/ml in 35 hormone receptor-(HR-) positive BC patients. Also, TRAF6 expression was obviously increased in the TNBC patients than that in the obese control group. Additionally, median serum TRAF6 expression levels rose remarkably in HR-negative patients than that in HR-positive patients. Thus, the study in BC proved that serum TRAF6 expression increased significantly in TNBC and HR-negative patients with non-metastatic BC, than that in HR- and human epidermal growth factor receptor 2 (HER2)-positive cases or the obese healthy controls, which suggested that increased TRAF6 expression may act as a poor prognostic biomarker in non-metastatic BC. Interestingly, Słotwiński R [27] also tested the TRAF6 level in peripheral blood leukocytes of 55 patientswith pancreatic ADC using real-time polymerase chain reaction (RT-PCR) and found that the level of expression of TRAF6 gene was significantly elevated. Over-expression of TRAF6 gene may contribute to chronic inflammation and tumor progression by up-regulation of the innate antibacterial response. However, the clinical role of serum TRAF6 still needs to be confirmed with new designed study with larger sample size in pancreatic ADC.

In conclusion, the current study indicates that the upregulated expression of TRAF6 may play a vital part in the development and progression of pancreatic ADC. Moreover, TRAF6 may become a new biomarker for diagnosis and treatment of pancreatic cancer. Nevertheless, further study should be conducted to address the molecular mechanism of TRAF6 underlying the carcinogenesis of pancreatic ADC, as well as the clinical potential of serum TRAF6 detection.

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Disclosure of conflict of interest

None.

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References

[1] Wolfgang CL, Herman JM, Laheru DA, Klein AP, Erdek MA, Fishman EK, Hruban RH. Recent progress in pancreatic cancer. CA Cancer J Clin 2013; 63: 318-348.

[2] Liu JH, Chen G, Dang YW, Li CJ, Luo DZ. Expression and prognostic significance of lncRNA MALAT1 in pancreatic cancer tissues. Asian Pac J Cancer Prev 2014; 15: 2971-2977.

[3] Cao Z, Tian R, Zhang T, Zhao Y. Localized Autoimmune Pancreatitis: Report of a Case Clinically Mimicking Pancreatic Cancer and a Literature Review. Medicine (Baltimore) 2015; 94: e1656.

[4] Cotterchio M, Lowcock E, Bider-Canfield Z, Lemire M, Greenwood C, Gallinger S, Hudson T. Association between Variants in Atopy-Related Immunologic Candidate Genes and Pancreatic Cancer Risk. PLoS One 2015; 10: e0125273.

[5] Goral V. Pancreatic Cancer: Pathogenesis and Diagnosis. Asian Pac J Cancer Prev 2015; 16: 5619-5624.

[6] Krska Z, Svab J, Hoskovec D, Ulrych J. Pancreatic Cancer Diagnostics and Treatment--Current State. Prague Med Rep 2015; 116: 253-267.

[7] Gharibi A, Adamian Y, Kelber JA. Cellular and molecular aspects of pancreatic cancer. Acta Histochem 2016; 118: 305-16.

[8] Chen C, Wu CQ, Chen TW, Tang MY, Zhang XM. Molecular Imaging with MRI: Potential Application in Pancreatic Cancer. Biomed ResInt 2015; 2015: 624074.

[9] Hu H, Jiao F, Han T, Wang LW. Functional significance of macrophages in pancreatic cancer biology. Tumour Biol 2015; 36: 9119-9126.

[10] Ogrendik M. Oral bacteria in pancreatic cancer: mutagenesis of the p53 tumour suppressor gene. Int J Clin Exp Pathol 2015; 8: 11835-11836.

[11] Tang B, Li Y, Qi G, Yuan S, Wang Z, Yu S, Li B, He S. Clinicopathological Significance of CDKN2A Promoter Hypermethylation Frequency with Pancreatic Cancer. Sci Rep 2015; 5: 13563.

[12] Tu C, Zheng F, Wang JY, Li YY, Qian KQ. An Updated Meta-analysis and System Review:is Gemcitabine+Fluoropyrimidine in Combination a Better Therapy Versus Gemcitabine Alone for Advanced and Unresectable Pancreatic Cancer? Asian Pac J Cancer Prev 2015; 16: 5681-5686.

[13] Zhang Y, Yang J, Li H, Wu Y, Zhang H, Chen W. Tumor markers CA19-9, CA242 and CEA in the diagnosis of pancreatic cancer: a meta-analysis. Int J Clin Exp Med 2015; 8: 11683-11691.

[14] Crunkhorn S. Inflammatory disorders: targeting TRAFs tames inflammation. Nat Rev Drug Discov 2013; 12: 423.

[15] Khan KA, Abbas W, Varin A, Kumar A, Di Martino V, Dichamp I, Herbein G. HIV-1 Nef interacts with HCV Core, recruits TRAF2, TRAF5 and TRAF6, and stimulates HIV-1 replication in macrophages. J Innate Immun 2013; 5: 639-656.

[16] Yao F, Han Q, Zhong C, Zhao H. TRAF6 promoted the tumorigenicity of esophageal squamous cell carcinoma. Tumour Biol 2013; 34: 3201-3207.

[17] Han Q, Yao F, Zhong C, Zhao H. TRAF6 promoted the metastasis of esophageal squamous cell carcinoma. Tumour Biol 2014; 35: 715-721.

[18] Rong Y, Wang D, Wu W, Jin D, Kuang T, Ni X, Zhang L, Lou W. TRAF6 is over-expressed in pancreatic cancer and promotes the tumorigenicity of pancreatic cancer cells. Med Oncol 2014; 31: 260.

[19] Sun H, Li X, Fan L, Wu G, Li M, Fang J. TRAF6 is upregulated in colon cancer and promotes proliferation of colon cancer cells. Int J Biochem Cell Biol 2014; 53: 195-201.
[20] Zhang XL, Dang YW, Li P, Rong MH, Hou XX, Luo DZ, Chen G. Expression of tumor necrosis factor receptor-associated factor 6 in lung cancer tissues. Asian Pac J Cancer Prev 2014; 15: 10591-10596.
[21] Bilir C, Engin H, Can M, Likhan S, Demirtas D, Kuzu F, Bayraktaroglu T. Increased serum tumor necrosis factor receptor-associated factor-6 expression in patients with non-metastatic triple-negative breast cancer. Oncol Lett 2015; 9: 2819-2824.
[22] Chiu HW, Lin SW, Lin LC, Hsu YH, Lin YF, Ho SY, Wu YH, Wang YJ. Synergistic antitumor effects of radiation and proteasome inhibitor treatment in pancreatic cancer through the induction of autophagy and the downregulation of TRAF6. Cancer Lett 2015; 365: 229-239.
[23] Han F, Zhang L, Qiu W, Yi X. TRAF6 promotes the invasion and metastasis and predicts a poor prognosis in gastric cancer. Pathol Res Pract 2016; 212: 31-37.
[24] Luo Z, Zhang X, Zeng W, Su J, Yang K, Lu L, Lim CB, Tang W, Wu L, Zhao S, Jia X, Peng C, Chen X. TRAF6 regulates melanoma invasion and metastasis through ubiquitination of Basigin. Oncotarget 2016; 7: 7179-7192.
[25] Zhang T, Wang H, Han L. Expression and Clinical Significance of Tumor Necrosis Factor Receptor-Associated Factor 6 in Patients With Colon Cancer. Iran Red Crescent Med J 2016; 18: e23931.
[26] Wang OH, Azizian N, Guo M, Capello M, Deng D, Zang F, Fry J, Katz MH, Fleming JB, Lee JE, Wolff RA, Hanash S, Wang H, Maitra A. Prognostic and Functional Significance of MAP4K5 in Pancreatic Cancer. PLoS One 2016; 11: e0152300.
[27] Slotwinski R, Dabrowska A, Lech G, Slodkowski M, Slotwinska SM. Gene expression disorders of innate antibacterial signaling pathway in pancreatic cancer patients: implications for leukocyte dysfunction and tumor progression. Cent Eur J Immunol 2014; 39: 498-507.