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Original Article

Up-regulation of PFTK1 is significantly associated with metastasis and poor prognosis in pancreatic ductal adenocarcinoma

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Abstract: PFTK1, whose alias was Cyclin-Dependent Kinase 14 (CDK14), has been reported to be overexpressed promoting proliferation and migration in pancreatic cancer cell lines in vitro; however, both expression status and clinicopathological significance in pancreatic ductal adenocarcinoma (PDAC) haven't been evaluated till now. In our study, to explore the clinicopathological significance of PFTK1 expression, PDAC tissue microarray was used to immunohistochemically assess the relationship between expression and various clinicopathological parameters. It was found that PFTK1 was significantly up-regulated in PDAC tissues compared with paired normal control. In addition, up-regulation of PFTK1 was also remarkably associated with metastasis and TNM stage ($P<0.05$). Kaplan-Meier survival analysis showed that overall prognosis of patients with positive expression for PFTK1 was inferior to those with negative expression ($P<0.05$). Moreover, PFTK1 expression as well as lymph node metastasis, differentiation degree, M classification can be used as an independent prognostic predictor for patients with PDAC after multivariate analysis. In our study, we for the first time found that Up-regulation of PFTK1 is significantly associated with metastasis and poor prognosis in PDAC, suggesting that PFTK1 was able to be used as an independent prognostic biomarker for patients diagnosed with PDAC.

Keywords: Pancreatic ductal adenocarcinoma (PDAC), PFTK1, metastasis, prognosis

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a malignancy with a lowest prognosis, with the majority of patients diagnosed with advanced disease that often happened to metastasize from lymph nodes to distant organs [1]. Therefore, there is a great need to understand the biological mechanisms that contribute to pancreatic cancer development and progression so as to develop effective therapies.

PFTK1, aliased for CDK14 or PFTAIRE1, was first identified from Hela cell cDNA library [2]. Later, PFTK1 was found to be involved in the metastasis of malignancies [3], to be exact, mainly in hepatocarcinoma [3-5] and rarely in oesophageal squamous cell carcinoma [6], breast carcinoma [7], Clear Cell Renal Cell Carcinoma [8] and glioma [9]. Actually, the majority of biomedical reports available regarding PFTK1 in the setting of cancers focused on the migration aspect of cancer cells [3, 4, 7, 9], indicating that PFTK1 can promote the migration of cancer cells. While, Just only one report found that up-regulation of PFTK1 was significantly associated with chemoresistance in oesophageal squamous cell carcinoma [6]. Another basic or biological literatures concerning PFTK1 were mainly about the findings of interactive protein of PFTK1, including TAGLN2 [4], Cyclin Y [5, 10], 14-3-3 proteins [11] and KIAA0202 [12]. However, there has been no report regarding PFTK1 in PADC. Therefore, both the expression status and clinicopathological significance of PFTK1 in PDAC are unknown and hasn't been unraveled, which deserves to be further explored.

In the present study, to understand the clinicopathological significance of PFTK1 in PDAC, immunohistochemistry was performed with

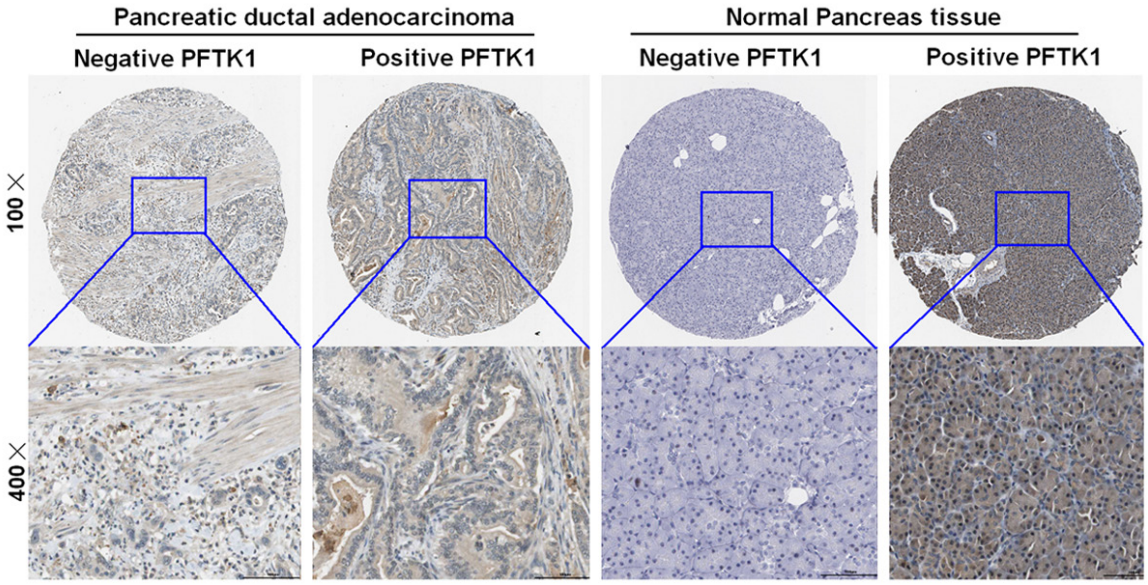

Figure 1. PFTK1 was significantly up-regulated in pancreatic cancer in comparison with paired normal control tissue. Immunostaining of PFTK1 in pancreatic cancer tissues and normal pancreatic tissue with tissue microarray by PFTK1 antibody (1:100). Representative photographs with different expression status of PFTK1, ranging from negative expression and positive expression in pancreatic cancer tissues as well as in normal pancreatic tissue, were taken at different magnification in pancreatic cancer tissues and normal pancreatic tissue, respectively.

PDAC tissue microarray. Ensuing clinicopathological significance was statistically analyzed. To explore the relationship between overall prognosis and expression of PFTK1, Kaplan-Meier survival analysis was also conducted. It was found that Up-regulation of PFTK1 is significantly associated with metastasis and poor prognosis in PDAC, suggesting that PFTK1 could be used as a prognostic biomarker for patients diagnosed with PDAC.

Materials and methods

Clinical samples

The present study was approved by the Medical Ethnic Committee of The First Affiliated Hospital of Wenzhou Medical University. 81 cases of PDAC tissues and 79 cases of normal control tissues were collected in the department of Hepatobiliary Surgery. The signed informed consents of all patients involved were obtained before surgery. And all the patients underwent no any chemo-radio therapy prior to surgical resection. The Haematoxylin-eosin (HE) staining of PDAC and paired normal control tissues were diagnosed and confirmed blindly by two separate clinical gastrointestinal pathologists. Only those slide sections on which the tumor cells accounting for more than 50% were included, otherwise was omitted. All the clinicopathological parameters include overall prognosis, demographic information (gender, age) and TNM staging and clinical grading.

Immunohistochemistry (IHC)

HE stained slides and unstained slides for immunohistochemical analysis were prepared from formalin-fixed, paraffin-embedded blocks of PDAC tissues. Immunohistochemical staining was carried out using heat-induced epitope retrieval, an avidin-biotin complex method. The rabbit anti-PFTK1 antibody (TA 308191, Origene Technologies, Inc, USA) was diluted with 1:100. The sections were evaluated by light microscopic examination, and cellular localization of the protein and immunostaining level in each section was assessed by two pathologists. The staining patterns were scored generally as negative and positive expression according to the immunostaining intensity.

Statistical analysis

For correlations between PFTK1 immunostaining scores and clinicopathological variables, Fisher's exact or Chi-square tests were used. In

Table 1. Clinicopathological significance of PFTK1 expression in pancreatic cancer tissues

Clinicopathological parameters	Total	PFTK1 expression		χ^2	*p* value
		Positive	Negative		
PDAC	81	46	35	32.044	0.000
Normal control	79	11	68		
Gender					
Male	41	26	15	1.485	0.266
Female	40	20	20		
Age					
≤55	36	19	17	0.425	0.652
>55	45	27	18		
T classification					
T1-2	28	11	17	5.343	0.033
T3-4	53	35	18		
Clinical stage					
Stage I-II	32	13	19	5.633	0.023
Stage III-IV	49	33	16		
Lymph node metastases					
No	38	14	24	12.662	0.001
N1-2	43	32	11		
Differentiation degree					
Well-moderate	31	10	21	7.491	0.009
Low	50	36	14		
M classification					
M0	33	13	20	6.868	0.012
M1-2	48	33	15		

addition, Kaplan-Meier curve was performed to analyze the prognosis. All the statistical analysis was carried out with SPSS 17.0, and statistical figures were made using the Graphpad Prism 5.0 version. All results were considered significantly for P<0.05.

Results

PFTK1 was significantly up-regulated in PDAC tissues compared with paired normal control tissues

To explore the expression status of PFTK1 in PDAC, IHC was carried out with tissue microarray. It was shown that PFTK1 was positively and heterogeneously expressed both in PDAC and paired normal control tissues, with it being no and weak positive staining in PDAC or paired normal control tissues (**Figure 1**). On the whole, PFTK1 was remarkably overexpressed in PDAC compared with paired normal control (**Table 1**). histopathologically, the subcellular localization of PFTK1 was mainly Cytoplasmic, membranous and nuclear. That is, there are positive immunostaining both in cytoplasm, membrane and nucleus compartment of PDAC cells.

PFTK1 was significantly associated with metastasis and inferior overall prognosis

Having found that PFTK1 was significantly up-regulated in PDAC tissues in comparison with paired normal control tissues, we hypothesized that PFTK1 might be oncogene whose expression could be positively correlated with malignant behaviors of PDAC. To test the hypothesis, clinicopathological significance between expression of PFTK1 and clinicopathological parameters was statistically analyzed using Chi-square statistical method. It was shown that, of all the clinicopathological parameters, significant association was found between expression of PFTK1 and lymph node metastases (P=0.001), M classification (P=0.012), T classification (P=0.033), Clinical stage (P=0.023) and differentiation (P=0.009). No other significant difference was observed between expression of PFTK1 and age (P=0.652) and gender (P=0.266) (**Table 1**). Besides, Kaplan-Meier survival analysis showed that the overall prognosis of patients with positive staining of PFTK1 was significantly inferior to that of patients with negative staining of PFTK1 (**Figure 2**). However, in terms of progression free survival, the PFTK1-positive patients tended to have a poorer progression free survival than the PFTK1-negative patients, although the statistical significance between the two groups was not significant (**Figure 2**).

Univariate and multivariate analysis for prognosis

To evaluate the effect of PFTK1 expression and clinicopathologic characteristics (including age, gender, clinical stage, T classification, lymph node metastasis and M classification) on prog-

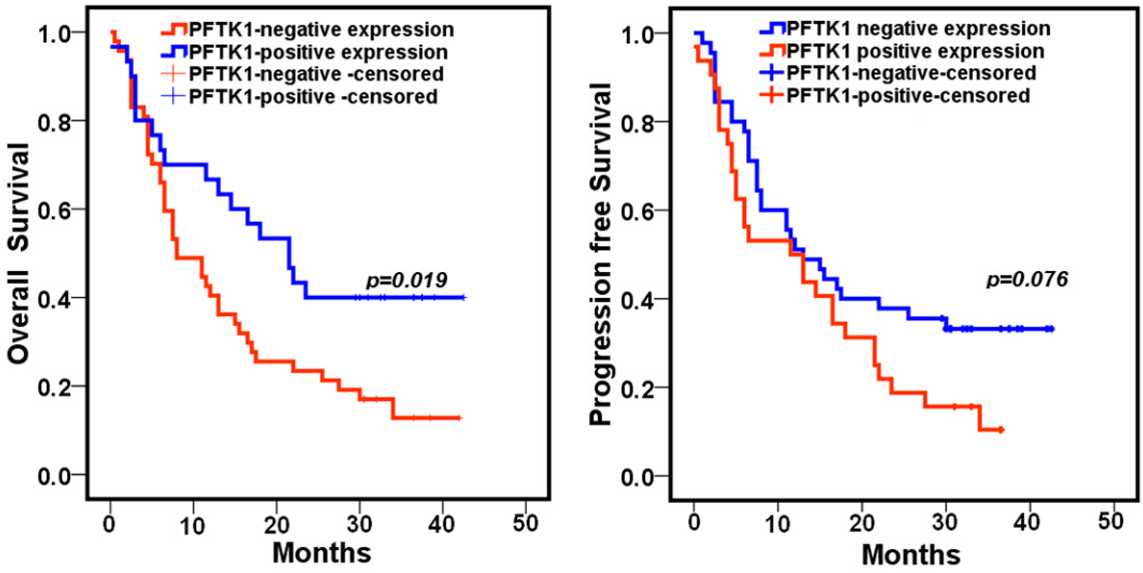

Figure 2. High expression of PFTK1 was significantly associated with poor prognosis. Kaplan-Meier survival analysis of overall prognosis and progression free survival were performed between patients with positive PFTK1 expression (46 cases) and patients with negative PFTK1 (35 cases). Log-rank test was used to statistically analyze the difference.

Table 2. Univariate and multivariate Cox-regression analysis of the prognostic parameters in patients with PDAC

	Univariate analysis		Multivariate analysis		
	P value	Regression coefficient (SE)	*P* value	Relative risk	95.0% CI
PFTK1 expression (negative vs. positive)	0.023	-0.656 (0.289)	0.045	1.852	1.013-3.387
Gender	0.065	0.672 (0.365)			
Age (years)	0.567	0.167 (0.291)			
Clinical stage	0.017	0.676 (0.283)	0.107	0.620	0.347-1.108
Lymph node metastasis	0.004	0.409 (0.140)	0.016	0.645	0.452-0.921
Differentiation degree	0.008	-0.472 (0.179)	0.010	1.450	1.091-1.927
M classification	0.006	0.404 (0.226)	0.013	0.605	0.406-0.900
T classification	0.073	0.103 (0.183)	0.56	1.147	0.723-1.820

Abbreviations: CI stands for confidence interval; PDAC, pancreatic adenocarcinoma; SE represents standard error.

nosis, we've carried out both univariate and multivariate survival analyses. Univariate Cox regression analysis showed PFTK1 expression (P=0.023), clinical stage (P=0.017), lymph node metastasis (P=0.004), differentiation degree (P=0.008) and M classification (P=0.006) were prognostic factors for PDAC. By using multivariate analysis, we further examined prognostic parameters of PDAC that were significant in univariate analysis. It can be seen that PFTK1 expression (P=0.045), lymph node metastasis (P=0.016), differentiation degree (P=0.010) and M classification (P=0.013) were independent prognostic factors influencing the 5-year overall survival, suggesting that PFTK1 expression can be used as an independent prognostic predictor for patients with PDAC (**Table 2**). Furthermore, lymph node metastasis, differentiation degree, M classification were all capable of being used as an independent prognostic factors in PDAC after multivariate analysis.

Discussion

In the present study, we for the first time found that Up-regulation of PFTK1 is significantly associated with metastasis and poor prognosis

in PDAC, suggesting that PFTK1 could be used as a prognostic biomarker for patients diagnosed with PDAC.

Original report regarding PFTK1 in the setting of cancer came from hepatocarcinoma [3] where the authors found that overexpression of PFTK1 may confer the motile phenotype in malignant hepatocytes that accounts for the association of up-regulation of PFTK1 in metastatic HCC. Then, the relevant investigation with regard to PFTK1 was extended in other different types of cancer, including gastric cancer [13], ovarian cancer [14], glioma [9], breast cancer [7], hepatocarcinoma [5], esophageal squamous cell carcinoma [6] and pancreatic cancer [15]. Mechanistic studies from cell culture and animal models have revealed the critical roles mediated by PFTK1 in the malignant behavior of cancers, however, there has been rather limited study regarding the clinicopathological significance of PFTK1 expression in the setting of PDAC other than a recent similar investigation mainly performed in vitro in pancreatic cancer cell lines [15]. Numerous reports available concerning PFTK1 focused on different cancers consistently discovered that PFTK1 alone [7, 9] or in combination with other interactive protein [4] was able to not only promote cell migration but also cell proliferation. PI3K/AKT signaling pathway [9], non-canonical Wnt signaling pathway [5] and PFTK1-DVL2-β-catenin axis [7] was found to be able to be activated by PFTK1. In terms of molecular mechanism by which PFTK1 plays in the setting of PDAC, it remains unknown that deserves to be further investigated. In our study, we've just focused on the clinicopathological role of PFTK1 on PDAC clinical tissue level, regardless of its function or potential working mechanism in vitro cell lines. We for the first time found that overexpression of PFTK1 was significantly associated with metastases, TNM stage and poor overall prognosis in PDAC, which was partially in agreement with Miyagaki H., et al's findings that up-regulation of PFTK1 was just markedly associated with inferior prognosis in patients with oesophageal squamous cell carcinoma, but did not correlate with any clinicopathological parameters including metastasis [6]. However, our results on clinical tissue level was entirely supported by Leung WK and colleagues' findings in hepatocellular carcinoma (HCC) that PFTK1 in primary HCC tumors significantly correlated with advance tumor grading and presence of microvascular invasion [4]. The possible reasons that lead to variation of final conclusions of ours and previous peer investigators may be in that the primary antibody used against PFTK1 was not the same and distinctive difference of clinical sample tissues involved. However, despite there existed nuances between our study and previous peer investigators' in the case of role mediated by PFTK1 on clinical tissue level, PFTK1 was uniformly up-regulated in cancer tissues in comparison with normal control, which is suggestive of its oncogenic roles in different types of cancers.

Despite our study for the first time provided evidences concerning PFTK1 on clinical tissue level in PDAC, there were still several limitations that we can't evade that should be acknowledged. Firstly, the clinical sample size is limited [16, 17], therefore may lead to the potentially biased or insufficient conclusions; secondly, considering that the specificity of primary antibodies used could lead to the irreproducibility of biomedical research [18, 19], all the primary antibodies should have been evaluated and tested prior to being used. However, we failed to test before it being used; thirdly, in terms of PFTK1 function in PDAC, our study lacks of experimental design in PDAC cell lines in vitro. In addition, in our series, immunophenotype was examined using tissue microarray [20], which may not reflect the heterogeneity of protein expression within individual tumors.

In conclusion, in the present study, we for the first time found that Up-regulation of PFTK1 is significantly associated with metastasis and poor prognosis in PDAC, suggesting that PFTK1 was able to be used as an independent prognostic biomarker for patients diagnosed with PDAC.

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Disclosure of conflict of interest

None.

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