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Original Article

Prognostic significance of CD4 and interleukin-22 expression in pancreatic cancer

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Abstract: Pancreatic cancer is one of the most aggressive cancers. Interleukin-22 (IL-22) is a member of IL-10 cytokine family and primarily produced by Th17 cells which were differentiated from CD4 T cells. CD4 T cells play a central role in regulating the immune response and resisting cancer cells. However, the function of CD4 T cells and Interleukin-22 (IL-22) in the microenvironment of pancreatic cancer remains largely unknown. In the present study, we investigated expression of the IL-22 in tumor cells, CD4 expression in microenvironment of pancreatic cancer and assessed their effects on pathological characteristics and prognosis of pancreatic cancer. To analyze prognostic factors of pancreatic cancer, Kaplan-Meier survival and Cox proportional-hazards regression were applied. Expression of CD4 in pancreatic cancer was associated with pTNM stage (P=0.005). Expression of IL-22 in pancreatic cancer was related not only to pTNM stage (P=0.011) but also to lymph node involvement (P=0.016). Univariate analysis demonstrated that the main prognostic factors of pancreatic cancer are pathological differentiation, expression of low CD4, expression of high IL-22 and the combination of low CD4 expression and high IL-22 expression in pancreatic cancer tissues. Moreover, multivariate analysis clearly showed that pathological differentiation, and the combination of low CD4 expression and high IL-22 expression in pancreatic cancer tissues were independent prognostic factors for overall survival in pancreatic cancer. The present study indicated that CD4 and IL-22 might be used as independent prognostic markers and molecular targets for pancreatic cancer.

Keywords: CD4 expression, interleukin-22 expression, immunohistochemistry, pancreatic cancer, prognosis

Introduction

Pancreatic cancer is one of the most aggressive cancers with almost equivalent incidence and mortality rates and an average 5-year survival rate remains at approximately 5% [1]. Tumor microenvironment, comprising a large population of immune cells, cancer cells, cancer stem cell, cancer-associated fibroblast and endothelial cell, has important roles in the biological behavior of cancer [2]. Moreover, immune cells are one of the most important components of the tumor microenvironment [3].

Accumulated evidence indicates that the abundant tumor infiltrating lymphocytes in the certain tumor microenvironment is related with the prognosis of cancer patients. CD4 T cells play a central role in regulating the immune response through sending signals to other types of immune cells [4]. CD4 T cells are very important in resisting cancer cells. Naïve CD4 T cells can be differentiated into four types including helper T cells (Th1, Th2, Th17) and regulatory T cells (Tregs). These cells play important and different roles respectively in anti-tumor immunity, tumor immune evasion, tumor immune microenvironment and immune homeostasis [5]. Th17 cells has been shown to play a key role in the pathogenesis of autoimmune disorders and infectious diseases [6, 7]. Moreover, some studies indicates that Th17 cells are present in some cancers such as ovarian cancer and prostate cancer [8, 9].

Interleukin-22 (IL-22) is a member of IL-10 cytokine family and primarily produced by Th17 cells which were differentiated from CD4 T cells. IL-22 is a cytokine involved not only in inflammatory and healing processes [10], but also in malignant disease progress [11]. Cyto-

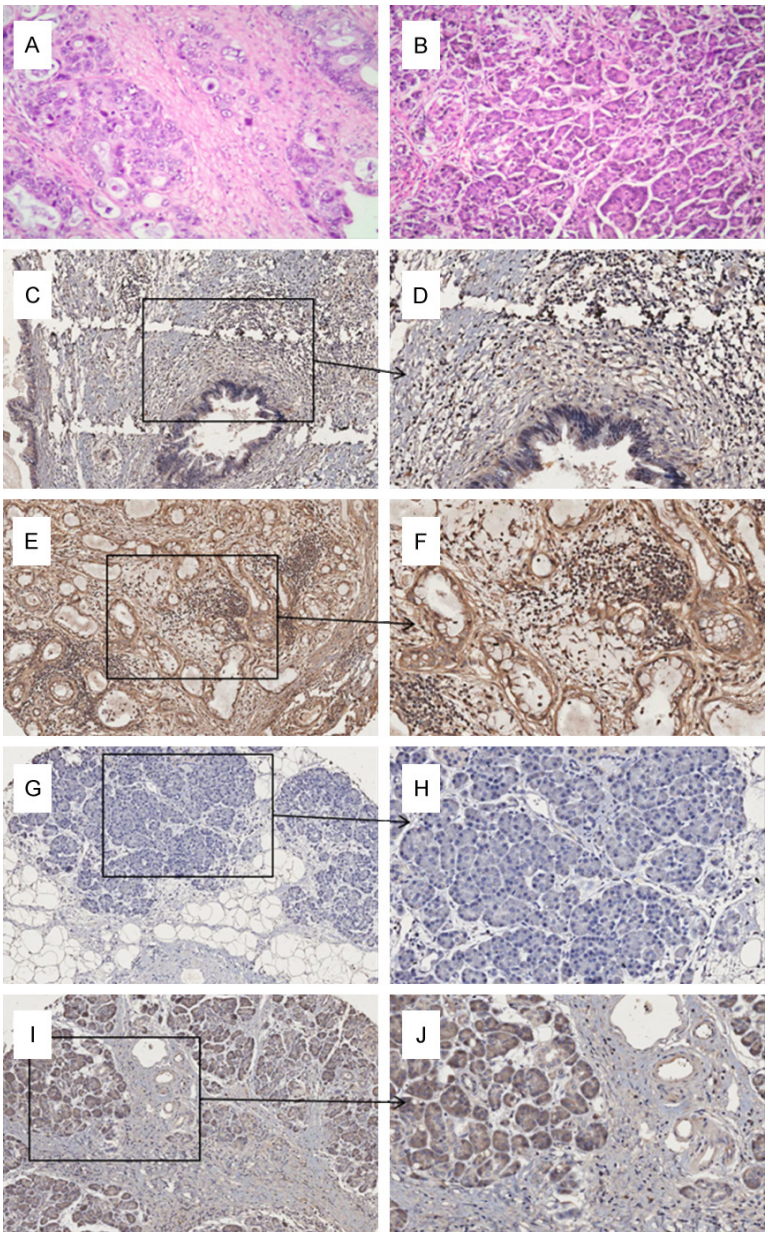

Figure 1. Expression of IL-22 in intratumoral and peritumoral tissues of pancreatic cancer. A: HE of intratumoral tissues of pancreatic cancer (×200). B: HE of peritumoral tissues of pancreatic cancer (×200). C and D: Low IL-22 expression in intratumoral tissues of pancreatic cancer (magnification: C ×100; D ×200). E and F: Low IL-22 expression in peritumoral tissues of pancreatic cancer (magnification: E ×100; F ×200). G and H: High IL-22 expression in intratumoral tissues of pancreatic cancer (magnification: G ×100; H ×200). I and J: High IL-22 expression in peritumoral tissues of pancreatic cancer (magnification: I ×100; J ×200).

kines produced by cancer cells play a critical role in the tumor microenvironment.

However, the function of CD4 T cells and IL-22 in the microenvironment of pancreatic cancer remains largely unknown. In this study, the expression of CD4 and IL22 in intratumoral tissues and peritumoral tissues was detected by immunohistochemistry method. Their effects on pathological characteristics and prognosis of pancreatic cancer were discussed.

Materials and methods

Patients

Pancreatic cancer tissues and corresponding adjacent cancer tissues were collected from 90 patients (66 males and 36 females) in the Inner Mongolia Medical College Affiliated Hospital. Pancreatic cancer samples were completely excised and confirmed by hematoxylin and eosin (H&E) staining by pathology. The adjacent cancer tissues were excised 3 cm from the pancreatic cancer tissues. Informed consent was obtained from all patients prior to surgery. The diagnosis and staging of pancreatic cancer was based on the 7th edition Staging Manual of American Joint Committee on Cancer. The pathological types of pancreatic cancer consist of duct adenocarcinoma, mucinous adenocarcinoma, mucinous cystadenocarcinoma and adenosquamous carcinoma. Complete follow-up history was available for at least 5 years. Overall survival was defined as the time from surgery to death. The study was approved by the Ethics Committee of the Inner Mongolia Medical College Affiliated Hospital.

Immunohistochemistry

Immunohistochemistry was performed on 4 μm thick paraffin-embedded sections. Briefly, the sections were deparaffinized and then hydrated. The Sections were washed with PBS. Then

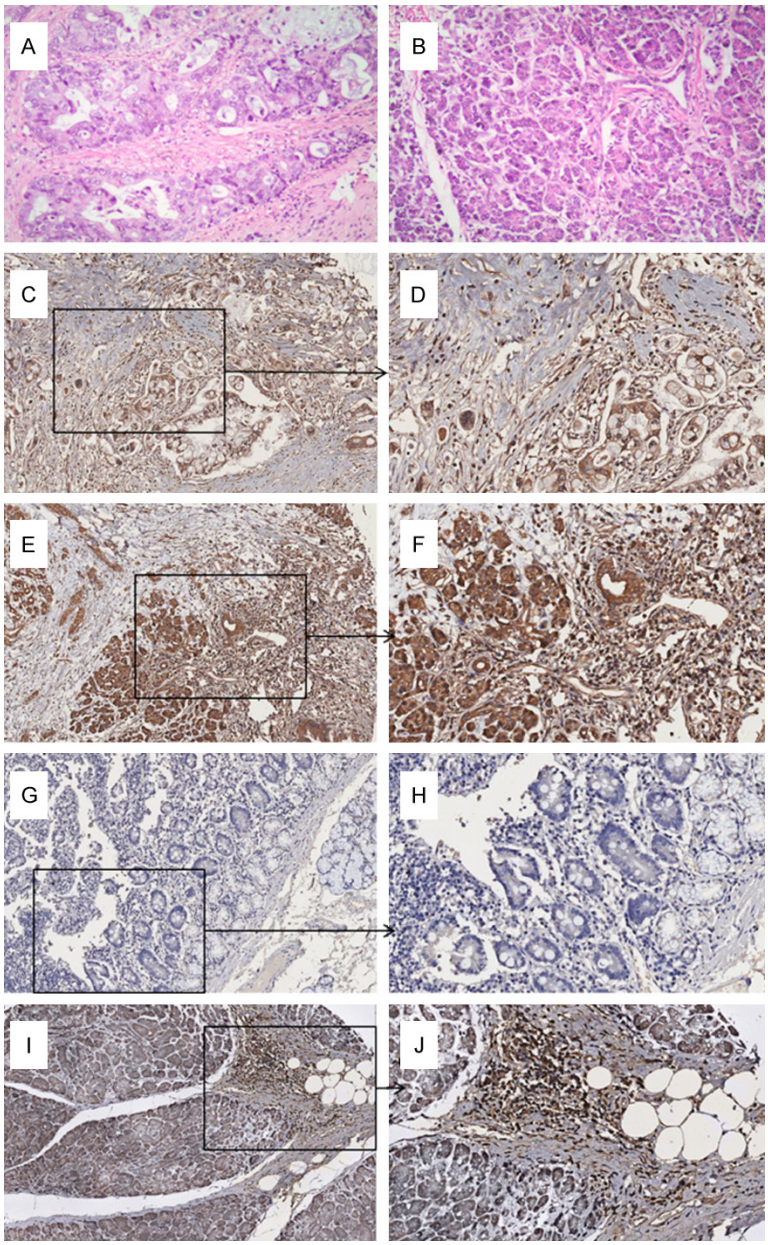

Figure 2. Expression of CD4 in intratumoral and peritumoral tissues of pancreatic cancer. A: HE of intratumoral tissues of pancreatic cancer (×200). B: HE of peritumoral tissues of pancreatic cancer (×200). C and D: Low CD4 expression in intratumoral tissues of pancreatic cancer (magnification: C ×100; D ×200). E and F: Low CD4 expression in peritumoral tissues of pancreatic cancer (magnification: E ×100; F ×200). G and H: High CD4 expression in intratumoral tissues of pancreatic cancer (magnification: G ×100; H ×200). I and J: High CD4 expression in peritumoral tissues of pancreatic cancer (magnification: I ×100; J ×200).

the sections were immersed in 0.01 mol/L citrate buffer solution (pH 6.0), and placed in microwave for 10 minutes. Peroxidase was quenched by 3% H_2O_2 in phosphate-buffered saline (PBS) for 15 minutes after washed with PBS, the sections were incubated at 4°C overnight with the primary antibody. After three washes with PBS, the sections were treated with the Envision detection system (EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse, Dako, Denmark). Finally, the sections were counterstained with hematoxylin (Hematoxylin, Sigma-Aldrich, Germany) to visualize the nuclei. Images were obtained with LEICA AMIL (LEICA, Germany).

Evaluation of staining

The CD4 and IL-22 staining of the tissue sections was located in cytoplasm. The IL-22 positive tumor cells and CD4 positive T cells were assessed. The CD4 and IL-22 immunostaining values were calculated with the percentage of immunostaining-positive cells. The total immunostaining values ranged from 5% to 95% with the gradient of 5%. We defined the value as low expression and high expression of CD4 and IL-22 with the median percentage value as cutoff (**Figures 1** and **2**).

Statistics

The Chi-square test was used to compare the data between different groups. Survival analyses were evaluated by Kaplan-Meier method and the COX regression model was used to analyze prognostic factors. *P* value less than 0.05 were considered as being statistically differences.

Results

Relationship between expression of CD4 and IL-22 and clinical factors of pancreatic cancer

The correlation between expression of CD4 and IL-22 and clinicopathological characteristics is summarized in **Table 1**. The results

Table 1. Association between expression of CD4 and IL-22 and clinicopathologic factors

Factors	IL-22 expression		*P* value	CD4 expression		*P* value
	Low	High		Low	High	
Gender			0.274			0.351
Men	26	31		30	27	
Women	19	14		14	19	
Age (years)			1.000			0.517
≤60	21	21		19	23	
>60	24	24		25	23	
Tumor size			0.288			0.691
≤4 cm	28	23		24	27	
>4 cm	17	22		20	19	
Pathological differentiation			0.095			0.233
High	33	26		27	32	
Low	9	16		15	10	
Lymph node involvement			0.016[a]			0.075
Negative	34	23		27	30	
Positive	11	22		17	16	
pTNM stage			0.011[b]			0.005[c]
I/II	26	14		13	27	
III/IV	19	31		31	19	

[a]Expression of IL-22 in pancreatic cancer was related lymph node involvement; [b]Expression of IL-22 in pancreatic cancer was related to pTNM stage; [c]Expression of CD4 in pancreatic cancer was associated with pTNM stage.

showed that CD4 expression in intratumoral tissue is lower than that of peritumoral tissue, but there is no statistically difference between two groups (P=0.201). On the contrary, it was showed that IL-22 expression in intratumoral tissue is higher than that of peritumoral tissue and there is statistically difference between two groups (P=0.031). Expression of CD4 in pancreatic cancer was associated with pTNM stage (P=0.005). Expression of IL-22 in pancreatic cancer was related not only to pTNM stage (P=0.011) but also to lymph node involvement (P=0.016). Expression of CD4 and IL-22 was not correlated with patient age, gender, tumor size and tumor differentiation (P>0.05).

Results of Cox regression analysis

Results of Cox regression analysis were shown in **Table 2**. Univariate analysis demonstrated that the main prognostic factors of pancreatic cancer are pathological differentiation, expression of low CD4, expression of high IL-22 and the combination of low CD4 expression and high IL-22 expression in pancreatic cancer tissues. Moreover, multivariate analysis clearly showed that pathological differentiation, and the combination of low CD4 expression and high IL-22 expression in pancreatic cancer tissues were independent prognostic factors for overall survival in pancreatic cancer. The age, sex and tumor size of pancreatic cancer patients were not correlated with the prognosis of pancreatic cancer.

Relationship between expression of CD4 and IL-22 and prognosis of pancreatic cancer

Kaplan-Meier survival curve also showed there was a significant correlation between expression of CD4 and IL-22 and overall survival (**Figure 3**). The median survival time in high IL-22 expression group (median 8 months; 95% CI 7.78-16.22 months) was significantly shorter than that in low IL-22 expression group (median 39 months; 95% CI 16.41-51.59 months). On the contrary, the median survival time in high CD4 expression group (median 30 months; 95% CI 7.78-16.22 months) was significantly longer than that in low CD4 expression group (median 10 months; 95% CI 7.78-16.22 months) (P< 0.05). The median survival time in the combination of high IL-22 and low CD4 expression group (median 10 months; 95% CI 7.78-16.22 months) was shorter than that in the combination of low IL-22 and high CD4 expression group (median 42 months; 95% CI 7.78-16.22 months) (P<0.05).

Discussion

Cellular immunity is an important part of the immune system, and plays a major role in killing cancer and preventing inflammation. T cells occupy a central position in the cellular immunity [12]. Traditionally, CD8+ T cytotoxic lymphocytes have been thought as the only component necessary for the elimination of cancerous tissue, while CD4 T cells were only thought as providers of additional stimuli [13]. However, CD8+ T cells lack the intrinsic ability to possess a extensive antitumor response

Table 2. Univariate and multivariate Cox regression analyses of factors associated with overall survival in pancreatic cancer

Factors	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender				
Men versus female	1.713 (0.974-3.013)	0.062		
Age (years)				
>60 versus ≤60	0.922 (0.553-1.538)	0.756		
Tumor size (cm)				
>4 versus ≤4	1.289 (0.763-2.175)	0.343		
Pathological differentiation				
Low versus high	0.535 (0.305-0.938)	0.029	0.256 (0.092-0.714)	0.009
Lymph node involvement				
Negative versus positive	1.741 (1.038-2.921)	0.861		
pTNM stage				
I/II versus III/IV	0.473 (0.275-0.814)	0.934		
IL-22 expression				
Low versus high	0.223 (0.125-0.396)	0.000		
CD4 expression				
Low versus high	1.932 (1.147-3.255)	0.013		
Combination of CD4 and IL-22 expression				
High IL-22 and low CD4 versus low IL-22 and high CD4	7.369 (3.109-17.467)	0.000	4.650 (1.670-12.945)	0.003

HR, hazard ratio; CI, confidence interval.

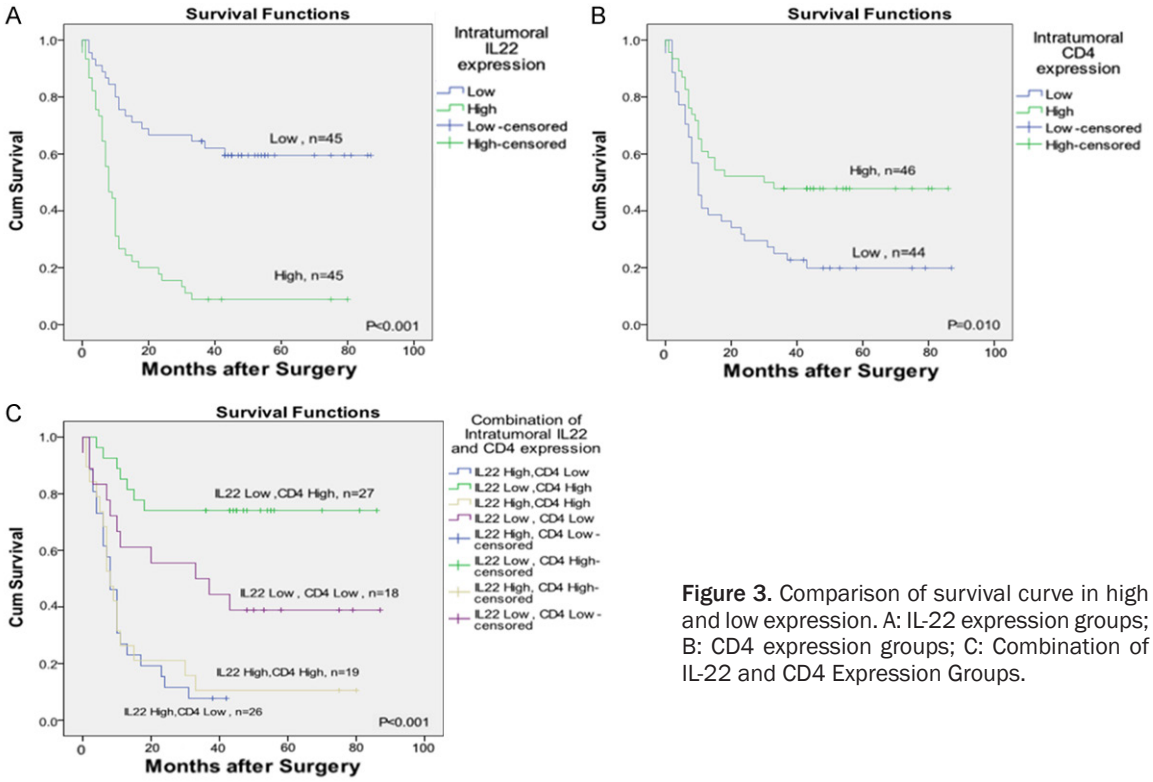

Figure 3. Comparison of survival curve in high and low expression. A: IL-22 expression groups; B: CD4 expression groups; C: Combination of IL-22 and CD4 Expression Groups.

that seems inherent in some CD4 T cell subsets [14]. In fact, the CD4 T cell is crucial to the development of CD8+ T cells function [15]. CD4 T cells are crucial for the activation and regula-

tion of the host defense against infections and for maintenance of adequate function of CD8+ T cytotoxic lymphocytes [16]. CD4 T cells are also important to the pathogenesis of many autoimmune diseases and eventually reject cancer through destroying a particular organ or tissue. Recent researches show that CD4 T cells can directly kill tumor cells. The role of CD4 T cells in cancer immunity remains controversial [17, 18].

Major targets of IL-22 mainly include the skin, kidney and epithelial cells from the digestive and respiratory systems, and the highest expression of IL-22 was found in the skin and pancreas [19]. Interestingly, although produced by immune cells, IL-22 does not affect the immune cells but regulates functions of certain tissue cells. IL-22 increases the innate immunity of tissue cells, protects tissues from damage, and enhances their regeneration [20]. Importantly, some researchers have reported that IL-22 can help to the pathogenesis of various malignant diseases. Gelebart P et al. reported that IL-22 signaling promotes cell growth in mantle cell lymphoma [11]. Zhang W et al. showed that IL-22 is highly expressed in primary tumor tissues and serum of patients with non-small cell lung carcinoma. The production of IL-22 contributes to survival of human lung cancer cell and resistance to chemotherapy [21]. Waidmann O et al. proved that Interleukin 22 serum levels are a negative prognostic indicator in patients with hepatocellular carcinoma [22].

The present study aimed to measure the expression of CD4 and IL-22 and assess the relationship of with clinicopathological characteristics and prognosis in pancreatic cancer. In our study, it was found that CD4 expression in intratumoral tissue is lower than that of peritumoral tissue. The results showed that survival time in high CD4 expression group is longer than that in the low group. The results suggested that $CD4^+$ T cells have a strong ability to directly or indirectly kill tumor cells and prolong the survival time of patients.

On the contrary, IL-22 expression in intratumoral tissue is significantly higher than that of peritumoral tissue and high expression of IL-22 were significantly associated not only with lymph node involvement but also pTNM stage. Survival time in high IL-22 expression group is shorter than that in the corresponding low expression group.

Kaplan-Meier analysis revealed that low CD4 expression and high IL-22 expression is associated with poor prognosis in pancreatic cancer. Results of Cox regression analysis demonstrated that pathological differentiation, lymph node involvement, pTNM staging, IL-22 expression in peritumoral tissues, low CD4 expression and high IL-22 expression are the main risk factors for prognosis of pancreatic cancer. Especially the combination of low CD4 expression and high IL-22 expression was significantly related with prognosis of pancreatic cancer. Furthermore, multivariate analysis showed that pathological differentiation and the combination of low CD4 expression and high IL-22 expression were independent predictor of survival in pancreatic cancer.

In conclusion, expression of CD4 and IL-22 in pancreatic cancer may provide a promising and useful markers for t monitoring occurrence, development and prognosis of pancreatic cancer. Detection of CD4 and IL-22 expression in pancreatic cancer tissues can help to understand the local immune response in tumor microenvironment, and provide a basis for postoperative antitumor immunity therapy.

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Disclosure of conflict of interest

None.

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