Expression of Smad2 and TGF-β1 in Human Pancreatic Carcinoma Tissues and its Signifiance*

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ABSTRACT Objective: To investigate relationships of the expression of Smad2, TGF-β1 in the tissues of pancreatic adenocarcinoma and normal pancreas and the clinicopathological parameters of pancreatic adenocarcinomas. Methods: The expressions of the Smad2, TGF-β1 in formalin-fixed paraffin embedded samples of 50 patients and 10 normal pancreatic tissues were detected by pancreatic adenocarcinoma. The ages, gender, tumor size, location, degree of differentiation and stage were recorded. Results: The expression of Smad2, TGF-β1 was higher in pancreatic adenocarcinoma than that in the normal pancreatic parenchyma. There were significant differences between the two groups (P<0.05). In pancreatic adenocarcinoma, the expression of Smad2, TGF-β1 in different TNM staging were significantly different (P<0.05). Conclusions: The expression of Smad2, TGF-β1 in pancreatic adenocarcinoma was higher than that in the normal pancreatic parenchyma. The expression of Smad2, TGF-β1 in pancreatic cancer tissues was related to the tumor stage.

Key Words: Pancreatic carcinoma; Smad2; TGF-β1; Immunohistochemistry
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Introduction

Pancreatic cancer is a malignant tumor with an extremely poor prognosis. This tumor is highly aggressive and patients with this form of cancer have a short survival after diagnosis. Even when the tumor is localized, the mean survival time after radical resection varies from 10 to 20 months. The mechanisms of the aggressive growth and metastasis are not yet extensively understood [1-2]. The incidence of pancreatic cancer has increased 4-fold over the past 20 years in China. Numerous studies had shown that the expression levels of both Smad2 and TGF-β1 (Transforming growth factor-β1) in pancreatic carcinoma were tightly related with the proliferation and invasion of pancreatic carcinoma, available for the diagnosis and treatment of pancreatic carcinoma and serving as indicators of prognosis [3-6]. However, it remains under hot dispute whether the expression of TGF-β1 and Smad2 in pancreatic carcinoma is related with the pathological grading and staging, histological types. In this study, immunohistochemical pv-9000 method was used to detect the expressions of TGF-β1 and Smad2 in pancreatic carcinoma tissues and normal pancreatic tissues, so as to investigate their correlation with the clinical pathology of pancreatic carcinoma and the mutual relationship of Smad2 expression and TGF-β1 expression.

1 Material and Method

1.1 Material

Experiment group consisted of 50 paraffin-embedded specimens, which were resected from patients with confirmed pancreatic ductal carcinoma by postoperative pathology in Hepatobiliary Surgery Department of Associated Hospital to Medical College of Qingdao University in 2003-2008. 30 male cases and 20 female cases aged from 30 to 78 (58± 9). 45 cases of carcinoma were in heads of pancreas while 5 cases in bodies and tails of pancreas. All tumors were pathologically confirmed as ductal adenocarcinomas with good differentiation in 21 cases, moderate differentiation in 19 cases, and poor differentiation in 10 cases. According to UICC staging, 34 cases were in Ⅰ, Ⅱ stages, while 16 cases were in Ⅲ, Ⅳ stages. Meanwhile, 10 specimens were taken from normal pancreatic tissues.

1.2 Methods

1.2.1 Preparation of tissue microarray Paraffin-embedded specimens were used to make tissue microarray wax blocks. Serial sections in 3 ~ 4μm thickness of the wax blocks were stained with routine hematoxylin and eosin (H-E) staining.

1.2.2 Immunohistochemistry staining The immunohistochemical pv-9000 kit was used. Smad2 and TGF-β1 primary antibodies were purchased from Beijing Zhongshan Biotechnology Company, and diluted by 1:100 prior to use. The specification in pv-9000 kit were followed in the experiment.

1.2.3 Results determination Brown-yellow granules in the cytoplasm and / or the nucleus showed the positive expression of Smad2, and those in the cytoplasm indicate the positive expression of TGF-β1. There were 10 high power fields (× 400 times) randomly selected for each observation site. The results were determined by utilizing semi-quantitative classification. Standards were as follows: Staining Intensity: Transparent color is defined as 0;

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light yellow is 1; yellow-brown is 2; and brown is 3. Positive Percentage: No positive cell is defined as 0; percentage of positive cells lower than or equal to 25% is 1; 26-50% is 2; 51-75% is 3; and more than 75% is 4. Accumulated points are the sum of the above two scores. Grading criteria: 0 is (-); 2-3 is (±); 4-5 is (+); and 6-7 is (++). Both (+) and (++) are considered positive.

1.3 Statistics

The above analyses were all completed by spss13.0 software. Measurement data was expressed by $\bar{x}\pm s$, and count data by composition ratio. Contrast between the two groups was detected with X^2 test. Spearman is used to analyze the correlation of the two groups. $P<0.05$ was considered statistically significant.

2 Results

2.1 The expression of Smad2 and TGF-β1 in pancreatic carcinoma tissues and normal tissues

Smad2 had positive expressions in the 50 cases of the experiment group (82.0%), while only 2 cases showed positive expressions in the 10 cases of control group (20.0%). Smad2 expression in pancreatic carcinoma tissues was significantly higher than that in normal pancreatic tissues. ($P<0.05$, Table 1).

TGF-β1 in pancreatic cancer cells expressed mainly in the cytoplasm and most of them were yellow-brown or brown. Amount of peripheral interstitium and interstitial cells were colored, and there were no obvious colored nucleus was showed (Fig 1-B). There were 32 cases had positive expressions in the 50 cases of experiment group (64.0%), while 2 cases showed positive expression in the 10 cases of control group (20%) and most of them were light yellow. TGF-β1 expression in pancreatic cancer tissues was significantly higher than that in normal pancreatic tissues ($P<0.05$, Table 1).

Table 1 Expressions of Smad2 and TGF-β1 in pancreatic cancer and normal pancreatic tissue

	n	TGF-α				Positive rate (%)	TGF-β1				Positive rate (%)
		−	±	+	++		−	±	+	++	
pancreatic cancer※	50	0	9	12	29	82.0	0	18	7	25	64.0
normal pancrea	10	0	8	2	0	20.0	0	8	2	0	20.0

Note: ※: Compared with normal pancrea group, pancreatic cancer group was significantly higher ($P<0.05$)

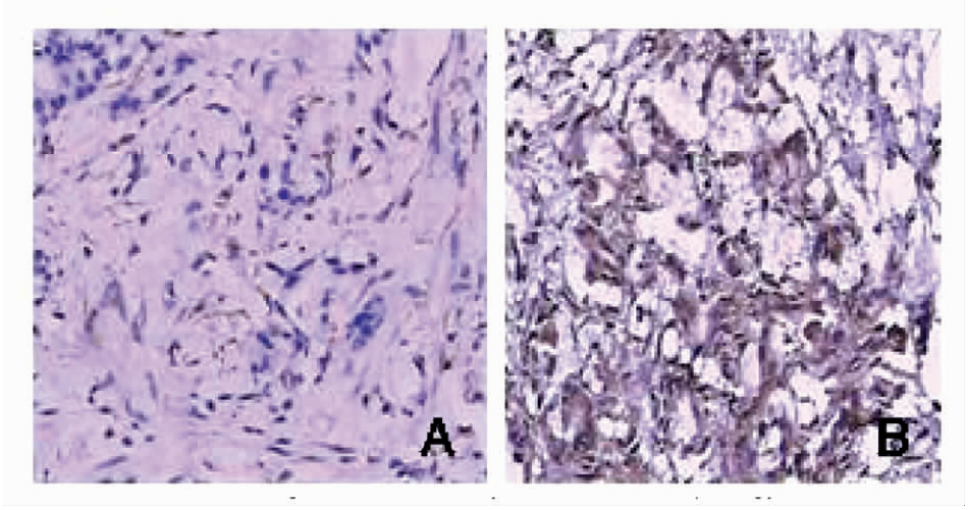

Fig. 1 A: positive expression of Smad2 in pancreatic carcinoma (SP× 200); B: positive expression of TGF-β1 in pancreatic carcinoma (SP× 200).

2.2 The expressions of Smad2 and TGF-β1 and their relationship with the clinicopathological parameters of pancreatic carcinoma

There was no significant difference for the expressions of Smad2 and TGF-β1 among patients of different age, tumor sizes, gender, location, degree of differentiation ($P>0.05$), but they showed obvious difference in different tumor stages ($P<0.05$) (Table 2).

2.3 The mutual relationship of Smad2 expression and TGF-β1 expression in pancreatic carcinoma

There was significant correlation between the expression of Smad2 and TGF-β1, which demonstrated that Smad2 and TGF-β1 worked together in the development and progress of pancreatic carcinoma.

3 Discussions

There were not many reports on the role of Smad2 in pancreatic cancer. Smad2 was able to translocate to the nucleus on their own, but was unable to activate reporter genes on their own, suggesting that the whole Smad-receptor complex were essential for transcriptional regulation[7-9].

Arnold NB found that Smad2 mRNA levels were significant-

Table 2 Correlation of Smad2 and TGF-β1 with clinicopathologic parameters of pancreatic carcinoma

		n	Smad2				TGF-β1			
			−	±	+	++	−	±	+	++
Age	＜60	29	0	13	7	9	0	12	7	10
	≥60	21	0	6	7	8	0	6	4	11
Tumor size	＞3cm	33	0	13	12	8	0	12	12	9
	≤3cm	17	0	5	6	5	0	5	6	6
Gender	Male	30	0	10	5	15	0	10	7	13
	Femal	20	0	8	4	8	0	8	3	9
Location	Head of pancreas	45	0	19	10	16	0	17	8	20
	Body & tail of pancreas	5	0	1	2	2	0	1	2	2
Degree of differentiation	Well & moderately	40	0	14	13	13	0	15	11	14
	Poorly	10	0	3	3	4	0	2	3	5
	Ⅰ, Ⅱ	34	0	11	5	18	0	12	7	15
	Ⅲ, Ⅳ	16	0	3	2	11	0	6	3	7

Note: ※ :The expression of Smad2 and TGF-β1 had significant difference in different tumor stages ($P<0.05$)

ly increased in pancreatic cancer samples in comparison with normal pancreatic tissues and suggest that this might lead to excessive activation of specfic components of TGF-β-signaling pathway[10]. Smad2 was a direct mediator of TGF-β1 signalling and there was no ample evidence to suggest that Smad2 had distinct role in TGF-β1 signalling. However, relatively few studies on the roles of Smad2 in TGF-β1 signalling had been performed in pancreatic cancer[11-12]. In this study, it was found that expression of Smad2 in pancreatic cancer tissues was significantly higher than that in the normal tissues. There was no significant correlation between the expression of Smad2 in pancreatic cancer and patient ages, genders, histological grades, tumor locations. However, the expression of Smad2 in pancreatic cancer tissues was related with the tumor stage, which indicated that Smad2 had correlation with the infiltration and metastasis of cancer cells.

TGF-β1 was important in regulating cell proliferation and differentiation, participating in embryonic development regulation, promoting formation of extracellular matrix (ECM), and reaction of the immune regulation [13-16]. Studies showed that in the early stage of tumor, TGF-β1 functions as a tumor suppressor. However, the tumor cells could escape from the inhibition of TGF-β1 with the growth of tumors, and were even stimulated by TGF-β1 to grow. Tumor cells and/ or interstitial cells might produce TGF-β1 as a promoting factor to stimulate angiogenesis, cell dissemination, immunosuppression and synthesis of extracellular matrix in the late stage of tumor[17-20]. In this study, the expression of TGF-β1 in pancreatic cancer tissues was significantly higher than that in normal pancreatic tissues, which indicated that the expression of TGF-β1 might be related with the occurrence and development of pancreatic cancer. The expression of TGF-β1 in pancreatic cancer tissues was related with the tumor stage, suggesting that the over-expression of TGF-β1 was tightly related to the progress and metastases of pancreatic cancer.

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Smad2 与 TGF-β1 基因在胰腺癌中的表达及临床意义*

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摘要 **目的**:通过检测 Smad2 与 TGF-β1 基因在胰腺癌中的表达,初步探讨它们与胰腺癌临床病理的关系。**方法**:采用免疫组织化学 pv-9000 法检测 50 例胰腺癌组织和 10 例正常胰腺组织中 Smad2 与 TGF-β1 的表达情况,并分析其与胰腺癌临床病理的相关性。**结果**:Smad2 与 TGF-β1 在胰腺癌组织中的表达水平显著高于正常胰腺组织,两组之间存在显著差异($P<0.05$)。在胰腺癌组织中,Smad2 与 TGF-β1 的表达在胰腺癌不同分期中存在显著差异($P<0.05$)。**结论**:Smad2 与 TGF-β1 在胰腺癌中高表达,二者联合可作为反映胰腺癌临床分期的生物学指标。

关键词:胰腺癌;Smad2;TGF-β1;免疫组织化学

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