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ORIGINAL ARTICLE

Relationships of pain in pancreatic cancer patients with pathological stage and expressions of NF-κB and COX-2

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Summary

Purpose: *To explore the relationships of pain in pancreatic cancer patients with pathological stage and expressions of nuclear factor-κB (NF-κB) and cyclooxygenase-2 (COX-2).*

Methods: *A total of 54 patients with pancreatic cancer were enrolled to evaluate the pain before treatment, detect the expressions of NF-κB and COX-2, an inflammatory mediator, in tumor tissues by the immunohistochemical method and analyze their relationships with the pain in these patients.*

Results: *The expressions of NF-κB and COX-2 varied obviously among pancreatic cancer patients with different degrees of pain, and as the pain was aggravated, the patients had raised expressions of NF-κB and COX-2 in tumor tissues ($p<0.05$). The degree of pain also differed evidently among the patients at different tumor node metastasis (TNM) stages, and the higher the pathological stage, the higher the degree of pain in patients ($p<0.05$). The pain score of patients was positively correlated with the expressions of NF-κB and COX-2 ($p<0.05$).*

Conclusions: *The degree of pain in pancreatic cancer is closely related to the pathological stage and expressions of NF-κB and COX-2, and the expressions of NF-κB and COX-2 are raised and the pain is aggravated as well in the patients at a higher pathological stage.*

Key words: *pancreatic cancer, cancer pain, NF-Kb, COX-2*

Introduction

Clinically, pancreatic cancer is a common malignancy mainly originated from pancreatic ductal epithelium and has relatively high incidence and mortality rates [1]. Pancreatic cancer is characterized by insidious onset, inconspicuous early clinical symptoms and no specific diagnostic markers, and it is often accompanied by metastatic lesions once definitely diagnosed, when the patients have missed the best chance for operations, thus leading to a rather poor prognosis [2,3]. Epidemiologic studies have suggested that the onset of pancreatic cancer is closely associated with pancreatitis [4]. As a regulatory protein that participates in the transcription and regulation of inflammatory mediator genes, nuclear factor-κB (NF-κB) can promote the expression of inflammatory factors, and these inflammatory factors and reactive oxygen free radicals can damage cells, thereby stimulating the growth and metastasis of cancer cells [5]. Cyclooxygenase (COX) is a class of rate-limiting enzymes that modulate the production steps of prostaglandin, of which COX-2 is one of the most common isozyme forms of inflammatory mediators [6]. Many studies have confirmed that COX-2 can accelerate tumor cell proliferation, suppress apoptosis and promote angiogenesis, playing an important role in the occurrence, development and distant metastasis of tumors [7]. Pain is a subjective feeling due to the complex synergy of physiological and psychological factors, and it is often

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accompanied by tissue damage in organisms [8]. Cancer pain is one of the most common symptoms in patients with malignant tumors. In the early stage of cancer, 1/4 of the patients feel pain, while over 1/2 of the middle- and advanced-stage patients suffer from different degrees of cancer pain, so that symptoms such as anxiety, depression and insomnia are triggered or exacerbated in the patients, seriously affecting their quality of life [9,10]. The present study analyzed the relationships of pain in pancreatic cancer patients with the pathological stage and expressions of NF-κB and COX-2, so as to provide bases for formulating a pain control program for pancreatic cancer patients and improving their quality of life.

Methods

General information

A total of 54 patients with pancreatic cancer admitted and treated at our hospital from December 2017 to December 2018 were selected as the subjects of this study based on the following criteria: Inclusion criteria: 1) Patients conforming to the diagnostic criteria for pancreatic cancer [10], 2) those with complete case information, and 3) those undergoing surgical resection for the first time without receiving radiotherapy and chemotherapy preoperatively. Exclusion criteria: 1) Patients complicated with malignant tumors in other tissues or organs, 2) those suffering from autoimmune dysfunction, or 3) pregnant and lactating patients. Among the subjects, there were 38 males and 16 females, aged 34-76 years (mean 55.48 ± 3.75). In terms of tumor node metastasis (TNM) stage, they comprised 9 cases at stage I, 13 cases at stage II, 21 cases at stage III and 11 cases at stage IV. This study met with the requirement of the Ethics Committee on Cancer of the First Affiliated Hospital, Jinzhou Medical University. Signed informed consents were obtained from all participants before the study entry.

Main instruments and reagents

1) Main instruments: Embedding machine and microtome (Leica, Wetzlar, Germany), light microscope (Olympus, Tokyo, Japan) and incubator (Chongqing Medical Instrument Factory, Chongqing, China), and 2) main reagents: Rabbit anti-human COX-2 monoclonal antibody (Abcam, Cambridge, MA, USA), rabbit anti-human NF-κB p65 polyclonal antibody (Lab Vision, Fremont, CA, USA), diaminobenzidine (DAB) color development kit (Fuzhou Maixin Biotech. Co., Ltd., Fuzhou, China) and horseradish peroxidase-labeled goat anti-rabbit secondary antibody (Beyotime Biotechnology, Shanghai, China).

Determination of the expressions of the total NF-κB and COX-2

The expressions of NF-κB and COX-2 were observed using immunohistochemistry. Fresh tissues surgically removed were firstly sent to the Department of Pathology, and tumor cell-rich tissues were selected to be embedded using the embedding machine and numbered. Then, the paraffin-embedded tissues were sliced into 4

Table 1. IRS of NF-κB and COX-2 expressions in patients with different grades of pain

Grade of pain	*n*	*NF-κB expression (point)*	*COX-2 expression (point)*
Grade I	12	1.49±0.34	1.69±0.38
Grade II	19	3.08±0.38	3.57±0.45
Grade III	13	4.17±0.45	4.69±0.42
Grade IV	10	5.65±0.48	5.74±0.56
F		21.256	22.634
p		<0.001	<0.001

Table 2. Relationship between TNM stage and pain in patients

TNM stage	*n*	*VAS score (point)*	*F*	*p*
Stage I	9	3.49±0.54	19.415	<0.001
Stage II	13	5.58±0.47		
Stage III	21	7.03±0.43		
Stage IV	11	8.89±0.46		

Table 3. Analysis of correlations of pain score with NF-κB and COX-2 expressions

Item	*Correlation coefficient of pain score (r)*	*p*
NF-κB	0.523	0.007
COX-2	0.569	0.012

μm-thick sections, baked in an incubator at 60°C overnight, deparaffinized in xylene and successively placed in 95%, 85%, 80% and 75% ethanol each for 10 min. After being soaked in distilled water for 5 min, the sections were incubated with 3% hydrogen peroxide solution at 20°C for 10 min to block endogenous peroxidase activity, washed with phosphate buffered saline (PBS) 3 times, and incubated again with 50 μL of the primary antibody (diluted at 1:100) at 4°C overnight and with secondary antibody at 20°C for 10 min. The resulting sections were developed using the reagent from the DAB kit, and after the color development was terminated through washing away the reagent using distilled water, they were counterstained with hematoxylin for 2 min and sealed in neutral resins.

TNM stage of pancreatic cancer [11]

T consists of Tis: carcinoma *in situ*, T1: tumor with the maximum diameter ≤ 2 cm, T2: tumor with 2 cm < the maximum diameter ≤ 4 cm, T3: tumor with maximum diameter >4 cm, and T4: tumor invasion in the celiac trunk, superior mesenteric artery and common hepatic artery; N consists of N0: no metastasis in lymph nodes, N1: regional metastasis in 1-3 lymph nodes, N2: regional metastasis in 4 lymph nodes; and M consists of M0: no distant metastasis and M1: distant metastasis. TNM stage comprises stage I: T1N0M0 and T2N0M0, stage II: T3N0M0 and T1-T3N1M0, stage III: Tis-T4N2M0 and T4N0-N2M0 and stage IV: Tis-T4 N0-N2 M1.

Expressions of NF-κB and COX-2 in pancreatic cancer tissues [12]

Each section was observed in five fields randomly selected (400×), and the percentage of positive cells (PP) which were stained brownish-yellow was calculated. Then, the percentage scores were obtained based on the following criteria: 1) 0 point: no positive cells, 2) 1 point: PP<5%, 3) 2 points: 5%<PP≤20%, and 4) 3 points: PP>20%, and the staining intensity (SI) was scored according to the following criteria: 1) 0 point: stainless, 2) 1 point: light yellow, 3) 2 points: brownish-yellow and 4) 3 points: tan. Finally, immunoreactivity score (IRS) was calculated using the formula: IRS=PP×SI, and IRS>4 and IRS≤4 were defined as high expression and low expression, respectively.

Evaluation of pain in patients using the visual analogue scale (VAS)

The VAS score ranges from 0 to 10 points (0 point denotes no pain, and 10 unbearable severe pain) [13]: 1) grade I: VAS score is 0-2 points, indicating that the patients have no pain or mild and imperceptible pain, 2) grade II: VAS score is 3-5 points, indicating that the patients have bearable pain, 3) grade III: VAS score is 6-8 points, indicating that the patients have obvious pain which affects their normal activities, and 4) grade IV: VAS score is no less than 8 points, indicating that the patients have unbearable pain [8].

Statistics

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used for processing data. Measurement data was expressed as mean±SD and t-test and one-way analysis of variance (ANOVA) were performed for inter-group and intragroup comparisons, respectively. Enumeration data were expressed as ratio and analyzed using x^2 test. Pearson's correlation coefficient was used for correlation analysis. P<0.05 suggested that the difference was statistically significant.

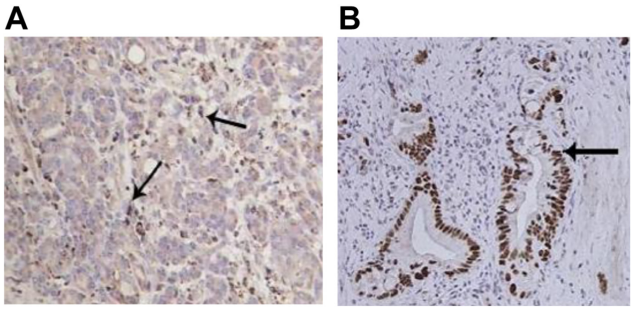

Figure 1. A: Positive expressions of NF-κB in the pancreatic cancer tissues in a 54-year-old male patient (400×). **B:** Positive expressions of COX-2 in the pancreatic cancer tissues in a 54-year-old male patient (400×). Arrows indicate COX-2 positive sites.

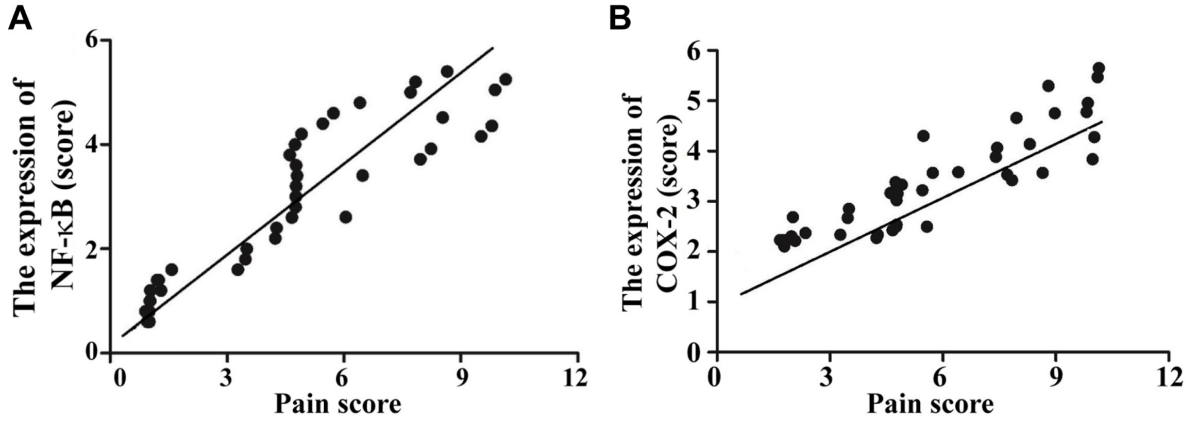

Figure 2. A: Correlations of pain score with NF-κB expressions. **B:** Correlations of pain score with COX-2 expressions. The Figure shows that the pains core of patients was positively correlated with the expressions of NF-kB and COX-2.

Results

Expressions of NF-κB and COX-2 in pancreatic cancer tissues and their relationship with different grades of pain in patients

NF-κB and COX-2 were mainly expressed in the cytoplasm of pancreatic cancer cells, and also in the nucleus, and showed a relatively uniform of brownish-yellow particles (Figure 1A,1B). Pancreatic cancer patients with different degrees of pain exhibited obviously different expressions of NF-κB and COX-2, and as the pain was aggravated, they were raised in tumor tissues ($p<0.05$) (Table 1).

Pain and pathological stage in pancreatic cancer patients

The patients at different TNM stages suffered from evidently different degrees of pain, and those at a higher pathological stage had aggravated pain ($p<0.05$) (Table 2).

Analysis of correlations of the pain in pancreatic cancer patients with the expressions of NF-κB and COX-2

The Pearson's correlation coefficient analysis results showed that the pain score of the patients was positively correlated with the expressions of NF-κB and COX-2 ($p<0.05$) (Table 3, Figure 2A, 2B).

Discussion

Pancreatic cancer, one of the common malignant tumors in the digestive system, represents 1-2% of all the malignancies and is highly prevalent in the population aged 50-60 years old, among whom males are more susceptible to the disease than females [14]. In recent years, the incidence rate of pancreatic cancer is increasing worldwide. Since the early-stage pancreatic cancer exhibits no typical clinical symptoms, it is relatively difficult to be diagnosed, and over 80% of the patients are not definitely diagnosed until the late stage, the majority of whom have metastasis. Additionally, it is difficult to achieve the desired efficacy through simple surgical treatments, and the disease normally needs to be controlled combined with radiotherapy and chemotherapy. However, these treatments are connected with very poor prognosis and an extremely low 5-year survival rate (lower than 5%) of the patients. Most of middle- and advanced-stage cancer patients are complicated with pain that has a serious influence on their quality of life. As the medical model is currently shifting from biomedicine to bio-psycho-social medicine, effective analgesia and improvement of the prognosis in patients in treating pancreatic cancer has become one of hot spots in gastrointestinal tumor research.

The mechanism of cancer pain is diversified, and tumors are able to change the microenvironment *in vivo*, thereby enabling the changed chemical components to activate the sensillum receptors that will also be activated due to the extrusion and pulling by tumors [15]. Pain is a symptom belonging to the physiological and mental activities and there are two types of pain: neuropathologic and nociceptive pain, both of which normally occur in patients with pancreatic cancer due to numerous influencing factors, such as surgical trauma, inflammatory stimulation, tumor growth, increased dose of therapeutic drugs and drug side effects [16]. The results of the present study revealed that the higher the pathological stage, the worse the pain in patients ($p<0.05$), probably because with a higher pathological stage, the tumor has an increasing volume and exerts larger extrusion and pulling effects on the patients and as pancreatic cancer progresses, metastasis occurs in several lymph nodes, resulting in nociceptive pain. In addition, the higher the pathological stage, the higher the malignancy of tumor in patients. As the microenvironment in their bodies is largely changed and chemotactic factors are increased, neuronal excitability is enhanced, thus inducing neuropathologic pain.

Inflammatory response serves as an important defense mechanism in the body, and it can defend against external harmful stimuli [17]. Related research suggested that about 15% of cancers start with chronic inflammatory response [18]. As a multi-directional transcriptional regulator, NF-κB can transcribe and modulate multiple inflammatory mediator genes. According to a relevant study, NF-κB is closely related to the occurrence and development of tumors, and its activity is correlated with the clinicopathological features of malignant tumors, such as infiltration depth and lymphatic metastasis, and the prognosis of patients [5]. The findings in this study showed that as the pain was worsened, the expression of NF-κB was raised in tumor tissues, and the pain score of patients was positively correlated with it ($p<0.05$). It is probably because NF-κB is activated in pancreatic cancer tissues, and with the up-regulation on inflammation and oncogene pathways, it induces epithelial-mesenchymal transformation to promote the infiltration and metastasis of this cancer through regulating the changes in the epithelium- and mesenchyme-derived markers. NF-κB can accelerate the secretion of numerous inflammatory factors in the initial stage of inflammatory

response and stimulate the growth and metastasis of tumors, thereby aggravating the pain in the patients.

The inflammatory mediator COX-2 can be overexpressed in breast, colon, lung, prostate and pancreatic cancer tissues [20-24]. COX-2 plays a role as a bridge between inflammatory response and pancreatic cancer, and the occurrence and development of pancreatic cancer is associated with its high expression [19]. This study found that as the pain was worsened, the expression of COX-2 was raised in the tumor tissues, and the pain score of patients was positively correlated with COX-2 expression ($p<0.05$). The cause may be that generated under the induction by various inflammatory factors and mitogens, COX-2 can accelerate the proliferation of pancreatic cancer cells, inhibit cell apoptosis and promote angiogenesis so as to continue deteriorating pancreatic cancer, thereby participating in chronic pain mechanism and raising the degree of cancer pain in patients.

Conclusions

In conclusion, the degree of pain in pancreatic cancer patients is closely related to pathological stage and expressions of NF-κB and COX-2, and as the pathological stage and expressions of NF-κB and COX-2 are increased, the pain is aggravated in the patients. The above findings can provide a novel idea and target for the clinical pain management in pancreatic cancer patients.

Conflict of interests

The authors declare no conflict of interests.

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