

Expression of p27^{kip1}, Rb protein and proliferating cell nuclear antigen and its relationship with clinicopathology in human pancreatic cancer

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OBJECTIVE: To investigate the effect of inhibiting factor of cell cycle regulation p27^{kip1}, retinoblastinoma protein (Rb protein), and proliferating cell nuclear antigen (PCNA) on the genesis and progression of human pancreatic cancer.

METHODS: The expression of p27^{kip1}, Rb protein and PCNA in the tumor tissue and adjacent tissue of 32 patients with pancreatic cancer was detected by SP immunohistochemical technique.

RESULTS: The p27^{kip1} protein positive-expression rate in the tumor tissue of pancreatic cancer was 56.25%, which was lower than that in the adjacent pancreatic tissue ($P < 0.05$). p27^{kip1} protein positive-expression was correlated significantly with tumor cell differentiation and lymph node metastasis ($P < 0.05$). The Rb gene protein positive-expression rate in the tumor tissue was 50%, which was also lower than that in the adjacent pancreatic tissue ($P < 0.05$). The PCNA positive-expression rate was 71.87%, which was higher than that in the adjacent pancreatic tissue ($P < 0.05$). PCNA positive-expression was also correlated significantly with tumor cell differentiation and lymph node metastasis ($P < 0.05$).

CONCLUSION: The decreased expression of p27^{kip1}, Rb protein and over-expression of PCNA may play an important role in the genesis and progression of pancreatic cancer.

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Key words: p27^{kip1}; retinoblastinoma protein; proliferating cell nuclear antigen; pancreatic cancer; immunohistochemistry

Introduction

Abnormality of mammalian cell cycle regulation is an important cause of cell over-proliferation and oncogenesis.^[1] Orderly progression of the cell

cycle is controlled by a family of cyclins and cyclin-dependent kinases (CDKs), which are restrictively counterbalanced by CDK inhibitors (CDK-Is).^[2] Two distinct families of CDKIs, the INK4 and CIP/KIP families which regulate the activity of the cyclin-CDK complexes, have been described.^[3] The CIP/KIP family, including p21, p27 and p57 proteins, harbors homologous CDK binding domains or function of cyclin-CDK complexes and makes cell cycle to arrest in G₁ phase. Retinoblastinoma protein (Rb protein) is one of tumor suppressor protein and affects the progression of G₁ phase of cell cycle. The expression of proliferating

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cell nuclear antigen (PCNA) is obviously associated with cell proliferation. But the relationship between p27^{kip1} protein and pancreatic cancer is less reported in China. In this study, the expression of p27^{kip1}, Rb and PCNA protein in the tissue of pancreatic cancer was detected by immunohistochemical technique in an attempt to investigate the effect of inhibiting proteins of cell cycle regulation of p27^{kip1}, Rb protein and PCNA on the genesis and progression of human pancreatic cancer.

Methods

Patients and tumor samples

Specimens were collected from 32 patients with primary pancreatic cancer after pancreatic resection at the Hepatobiliary Department, General Hospital of Shenyang Military Command and the First Clinical College, Chinese Medical University, Shenyang, China. Of the patients, 20 (62.5%) were male and 12 (37.5%) female. Their mean age was 59.5 years (range, 26–72 years). Nineteen patients (59.38%) had well-differentiated pancreatic cancer, and 13 (40.63%), moderate or low-differentiated pancreatic cancer. Twelve patients (37.5%) showed lymph node metastasis. All the patients were confirmed pathologically. Tumor tissues or adjacent tissues from the 32 patients were fixed in 10% buffered formalin, processed routinely, and embedded in paraffin. They were stained with hematoxylin and eosin and sectioned. Representative blocks were chosen for further studies.

Immunohistochemical study

Four micrometer-thick sections were placed on poly-L-lysine-coated slide for immunohistochemical study. The expression of p27^{kip1}, Rb protein and PCNA were assessed by SP method of immunohistochemical examination using an anti-human p27^{kip1} monoclonal antibody (DCS-72.F6), anti-human Rb protein monoclonal antibody (1F8), anti-human PCNA monoclonal antibody (PC10) and UltraSensitive™ S-P Kit (Kit-9720). The deparaffinized sections were boiled in the EDTA buffer at high temperature and high pressure for antigen retrieval and incubated with each antibody at 4

°C overnight. Immunohistochemical staining for these proteins was then performed according to the UltraSensitive™ S-P Kit manual. All the reagents were purchased from Maixin-Bio Co., Fuzhou, China. The cell with brown-yellow color granules in the nuclei or cytoplasm was taken as positive cell. Four hundred cells on each slide were counted. The slides were distinguished as negative (-), positive (+), stronger positive (++) and strongest positive (+++) when the count of positive cells was less than 10%, ranged from 10% to 25%, ranged from 25% to 50%, and more than 50% respectively for p27^{kip1} and Rb proteins. Contrarily, the slides were distinguished as negative (-), positive (+) when the count of positive cells was less than 50% and exceeded 50% for PCNA respectively.

Statistical analysis

The Chi-square test and Fisher's exact test of SAS system statistical software (Release 6.12) were adopted. $P < 0.05$ was considered significant.

Results

Expression of p27^{kip1} protein and its relationship with clinicopathology in human pancreatic cancer

p27^{kip1} protein was located in the nuclei or cytoplasm of normal pancreatic cell and positive pancreatic cancer cell with brown-yellow color granules. p27^{kip1} protein positive-expression rate in the tumor tissue of pancreatic cancer was 56.25%, which was lower than that in the adjacent pancreatic tissue ($\chi^2 = 6.163$, $P < 0.05$, Table 1). p27^{kip1} protein positive-expression rate in the moderate or low-differentiated group of pancreatic cancer was 30.76%, which was lower than that in the well-differentiated group ($\chi^2 = 5.776$, $P < 0.05$, Table 1). p27^{kip1} protein positive-expression rate in the lymph node metastasis group of pancreatic cancer was 33.33%, which was lower than that in the non-lymph node metastasis group ($\chi^2 = 4.097$, $P < 0.05$, Table 1).

Table 1. Expression of p27^{kip1} protein in tissue of pancreatic cancer

Characteristics	n	Negative	Positive			Positive-expression rate (%)	P <
			+	++	+++		
Tissue type							
Tumor tissue	32	14	8	8	2	56.25	0.05
Adjacent tissue	32	5	6	16	5	84.37	
Degree							
Well-differentiated	19	5	4	7	3	73.68	0.05
Moderate or low-differentiated	13	9	2	2	0	30.76	
Lymph node metastasis							
Yes	12	8	4	0	0	33.33	0.05
No	20	6	4	8	2	70.00	

Table 2. Expression of Rb protein in tissue of pancreatic cancer

Characteristics	n	Negative	Positive			Positive-expression rate (%)	P
			+	++	+++		
Tissue type							
Tumor tissue	32	16	10	2	4	50.00	<0.05
Adjacent tissue	32	7	12	8	5	78.13	
Degree							
Well-differentiated	19	9	4	2	4	52.63	>0.05
Moderate or low-differentiated	13	7	6	0	0	46.15	
Lymph node metastasis							
Yes	12	8	3	1	0	33.33	>0.05
No	20	8	7	1	4	60.00	

Expression of Rb protein and its relationship with clinicopathology in human pancreatic cancer

Rb protein was located in the nuclei or cytoplasm of normal pancreatic cell and positive pancreatic cancer cell with brown-yellow color granules. Rb protein positive-expression rate in the tumor tissue of pancreatic cancer was 50.00%, which was lower than that in the adjacent pancreatic tissue ($\chi^2 = 5.497$, $P < 0.05$, Table 2). Rb protein positive-expression rate in the moderate or low-differentiated group of pancreatic cancer was 46.15%, which was lower than that in the well-differentiated group ($P > 0.05$, Table 2). Rb protein positive-expression rate in the lymph node metastasis group of pancreatic cancer was 33.33%, which was lower than that in the non-lymph node metastasis group ($P > 0.05$, Table 2).

Expression of PCNA protein and its relationship with clinicopathology in human pancreatic cancer

PCNA protein was located in the nuclei or cytoplasm of normal pancreatic cell and positive pancreatic cancer cell with brown-yellow color granules. PCNA protein positive-expression rate in the tumor tissue of pancreatic cancer was 71.87%, which was higher than that in the adjacent pancreatic tissue ($\chi^2 = 5.189$, $P < 0.05$, Table 3). PCNA protein positive-expression rate in the moderate or low-differentiated group of pancreatic cancer was 92.30%, which was higher than that in the well-differentiated group ($\chi^2 = 4.522$, $P < 0.05$, Table 3). PCNA protein positive-expression rate in the lymph node metastasis group of pancreatic cancer was 100%, which was higher than that in the non-lymph node metastasis group ($\chi^2 = 7.513$, $P < 0.05$, Table 3).

Table 3. Expression of PCNA protein in the tissue of pancreatic cancer

Characteristics	n	Negative	Positive	Positive-expression rate (%)	P <
Tissue type					
Tumor tissue	32	9	23	71.87	0.05
Adjacent tissue	32	18	14	43.75	
Degree					
Well-differentiated	19	8	11	57.89	0.05
Moderate or low-differentiated	13	1	12	92.30	
Lymph node metastasis					
Yes	12	0	12	100.00	0.05
No	20	9	11	55.00	

Table 4. Relationship of p27^{k_{ip}1} and Rb protein in pancreatic cancer

p27 ^{k_{ip}1}	n	Rb				Positive-expression rate (%)
		-	+	++	+++	
-	32	16	10	2	4	42.87
+	14	8	4	2	0	
++	8	3	3	0	2	55.56
+++	8	3	3	0	2	
+++	2	2	0	0	0	

Relationship of expression between p27^{k_{ip}1} and Rb protein in pancreatic cancer

Rb protein positive-expression rate in the tumor tissue of p27^{k_{ip}1} protein positive-expression group was 55.56%, and in the tumor tissue of p27^{k_{ip}1} protein negative-expression group, 42.87%. There was no significant correlation between the two groups ($r=0.0789$, $P>0.05$, Table 4).

Discussion

Cell cycle regulation is a hot topic on oncological research at present. Recent studies showed that G₁ phase regulation is a complex process in which multiple cell factors take part in and abnormal cell cycle regulation is significantly correlated with the genesis and progression of tumors.^[4-7] The p27^{k_{ip}1} gene is located in chromosome 12p13, and p27^{k_{ip}1} protein as a cell cycle inhibitor with a molecular weight of 27kD is included in the CIP/KIP family and is similar to p21, p57 proteins in function. Polyak et al^[8] suggested that the p27^{k_{ip}1} gene plays

an important role in the genesis and progression of tumor, but mutations and loss of the p27^{k_{ip}1} gene are rarely detected, and that the tumor suppressor mechanism of p27^{k_{ip}1} protein may be integrated with cyclin-CDK complexes, making cell cycle to arrest in the G₁ phase. In this study, the p27^{k_{ip}1} protein positive-expression rate in the tumor tissue of pancreatic cancer was significantly lower than that in the adjacent tissue. The worse the expression of p27^{k_{ip}1} protein decreased, the stronger the malignant degree and lymph node metastasis increased. Our results suggested that reduced expression of p27^{k_{ip}1} is correlated with the genesis and progression of pancreatic cancer. This is consistent with Thomas GV's conclusion about the relationship of the reduced expression of p27^{k_{ip}1} protein and metastasis of colorectal adenocarcinoma.^[9] The Rb gene as the first isolated and detected tumor suppressor gene is an important factor in the regulating system of G₁ phase. Inactivity of Rb protein is associated with liver carcinoma and small cell lung carcinoma apart from retinoblastoma. In this study, the Rb protein positive-expression rate in the tumor tissue was significantly lower than that in the adjacent tissue, and the worse the expression of Rb protein decreased, the stronger the malignant degree and lymph node metastasis increased. However, there was no significant difference between the two groups because of insufficient cases of pancreatic cancer. PCNA or δ -assistant factor of DNA synthetase participates in DNA biological synthesis and regulates cell cycle and cell proliferation by tetramer with cyclin, CDK and

p21. In our study, the PCNA protein positive-expression rate in the tumor tissue was higher than that in the adjacent tissue, and the PCNA protein positive-expression rate in the moderate or low-differentiated group was higher than that in the well-differentiated group. The PCNA protein positive-expression rate in the lymph node metastasis group was higher than that in the non-lymph node metastasis group. These findings suggested that the over-expression of PCNA is associated with the genesis and progression of pancreatic cancer and that the malignancy of pancreatic cancer determined by the expression of PCNA is of practical value. We found that cell proliferative activity is high for the negative or reduced expression of p27^{kip1}, Rb protein, or p27^{kip1}, and Rb protein plays a suppressor role in cell proliferation. In our study, no significant correlation was found in Rb protein positive-expression rate between the p27^{kip1} positive-expression group and the p27^{kip1} negative-expression group, indicating that there may be a different pathway of cell cycle regulation in tumor suppression between p27^{kip1} and Rb protein.

In summary, p27^{kip1}, Rb and PCNA expression at protein level suggests that reduced expression or loss of p27^{kip1}, Rb protein expression and over-expression of PCNA protein might contribute to the genesis or progression of pancreatic cancer. p27^{kip1}, Rb protein and PCNA might play a role in regulating different pathway of cell cycle.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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