PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(32):5057-5060
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Expression of cell cycle regulator p57^{kip2}, cyclinE protein and proliferating cell nuclear antigen in human pancreatic cancer: An immunohistochemical study

Hui Yue, Hui-Yong Jiang

Hui Yue, Institute for Digestive Medicine, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China
Hui-Yong Jiang, Department of Pathology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China
Correspondence to: Dr. Hui Yue, Institute for Digestive Medicine, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China. yh12070430@vip.sina.com
Telephone: +86-20-61641545 Fax: +86-20-61363220
Received: 2004-11-02 Accepted: 2004-12-21

Abstract

AIM: To investigate the effects of p57^{kip2}, cyclinE protein and proliferating cell nuclear antigen (PCNA) on occurrence and progression of human pancreatic cancer.

METHODS: The expression of p57^{kip2}, cyclinE protein and PCNA in tumor tissues and adjacent tissues from 32 patients with pancreatic cancer was detected by SP immunohistochemical technique.

RESULTS: The positive expression rate of p57^{kip2} protein in tumor tissues was 46.9%, which was lower than that in adjacent pancreatic tissues ($\chi^2 = 5.317$, $P<0.05$). p57^{kip2} protein positive expression remarkably correlated with tumor cell differentiation ($P<0.05$), but not with lymph node metastasis ($P>0.05$). The positive expression rate of cyclinE protein in tumor tissues was 68.8%, which was higher than that in adjacent pancreatic tissues ($\chi^2 = 4.063$, $P<0.05$). CyclinE protein positive expression significantly correlated with tumor cell differentiation and lymph node metastasis ($P<0.05$). The positive expression rate of PCNA in the tumor tissues was 71.9%, which was higher than that in adjacent pancreatic tissues ($\chi^2 = 5.189$, $P<0.05$). PCNA positive expression remarkably correlated with tumor cell differentiation and lymph node metastasis ($P<0.05$).

CONCLUSION: The decreased expression of p57^{kip2} and/or overexpression of cyclinE protein and PCNA may contribute to the occurrence and progression of pancreatic cancer. p57^{kip2}, cyclinE protein, and PCNA play an important role in occurrence and progression of pancreatic cancer.

Key words: p57^{kip2}; CyclinE; PCNA; Human pancreatic cancer

Yue H, Jiang HY. Expression of cell cycle regulator p57^{kip2}, cyclinE protein and proliferating cell nuclear antigen in human pancreatic cancer: An immunohistochemical study. *World J Gastroenterol* 2005; 11(32): 5057-5060
http://www.wjgnet.com/1007-9327/11/5057.asp

INTRODUCTION

Abnormality in mammalian cell cycle regulation is an important cause of cell 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 (CDKIs)[2]. Two distinct families of CDKIs, INK4, and CIP/KIP families, which regulate the activity of cyclin-CDK complexes, have been described[3]. The CIP/KIP family, including p21, p27, and p57 proteins, harbors homologous CDK-binding domains or functions of cyclin-CDK complexes and causes the cell cycle to arrest in G_1 phase. CyclinE protein is a positive regulator of cell cycle, which promotes the transfer from G_1 to S phase. The expression of PCNA remarkably correlates with status of cell proliferation. There are few reports about the relationship between p57^{kip2} protein as negative factor of cell cycle regulation and pancreatic cancer. In this study, the expression of p57^{kip2}, cyclinE protein, and PCNA in pancreatic cancer tissues and adjacent tissues was detected by immunohistochemical technique to investigate the effects of p57^{kip2}, cyclinE protein, and PCNA on occurrence and progression of human pancreatic cancer.

MATERIALS AND METHODS

Patients and tumor samples

Thirty-two specimens of primary human pancreatic cancer were collected from pancreatic resection performed in the Department of General Surgery, General Hospital of Shenyang Military Command. There were 20 male and 12 female patients, with a mean age of 59.5 years (26-72 years). Nineteen patients had well-differentiated pancreatic cancer, 13 had moderately or poorly-differentiated pancreatic cancer, and 12 had lymph node metastasis. All patients were confirmed by clinicopathological diagnosis. These specimens were fixed in 10% buffered formalin and embedded in paraffin. All sections stained with hematoxylin and eosin were reviewed and kept for further studies.

Immunohistochemical study

Four-micrometer-thick sections from the tissues were cut for

immunohistochemical study. The expression of $p57^{kip2}$, cyclinE protein, and PCNA was assessed by SP immunohistochemical method using anti-human $p57^{kip2}$ mAb (57P06), anti-human cyclinE protein mAb (13A3), anti-human PCNA mAb (PC10), and the UltraSensitive™ SP kit (kit-9720). Immunohistochemical staining for these proteins was then performed according to the UltraSensitive™ SP kit manual. All reagents were supplied by Maixin-Bio Co., Fuzhou, China. The cells with brown-yellow granules in the nuclei or cytoplasm were taken as positive. Five hundred cells on each slide were counted. The slides were classified as negative (-), positive (+), strong positive (++), and strongest positive (+++) according to the count of positive cells for $p57^{kip2}$ and cyclinE proteins less than 10%, 10-25%, 25-50%, and more than 50%, respectively. The slides was distinguished as negative (-), and positive (+) when the count of positive cells were less than 50% and over 50% for PCNA respectively.

Statistical analysis

The χ^2 test and Fisher's exact test were used in the analysis by SAS system statistical software (release 6.12). $P<0.05$ was considered statistically significant.

RESULTS

Expression of $p57^{kip2}$ protein

The $p57^{kip2}$ protein was located in the nuclei or cytoplasm of normal pancreatic cells and positive pancreatic cancer cells with brown-yellow granules (Figure 1A).The positive expression rate of $p57^{kip2}$ protein in tumor tissues was 46.9%, which was lower than that in adjacent pancreatic tissues ($\chi^2 = 5.317$, $P<0.05$). The positive expression rate of $p57^{kip2}$ protein in the moderately or poorly differentiated tumor tissues was 23.1%, which was lower than that in well-differentiated tumor tissues ($\chi^2 = 4.979$, $P<0.05$). The positive expression rate of $p57^{kip2}$ protein in lymph node metastasis group was 25.0%, which was lower than that in non-lymph node metastasis group ($P>0.05$, Table 1).

Table 1 Expression of $p57^{kip2}$ protein in pancreatic cancer tissues

Characteristics	$p57^{kip2}$ protein expression				
	-	+	++	+++	Rate (%)
Tumor tissue	17	11	3	1	46.9[a]
Well-differentiated	7	9	2	1	63.2[c]
Moderately or poorly-differentiated	10	2	1	0	23.1[c]
Lymph node metastasis	9	2	1	0	25.0[e]
Non-lymph node metastasis	8	9	2	1	60.0[e]
Adjacent tissue	8	13	6	5	75.0[a]

[a]$P<0.05$, [c]$P<0.05$, [e]$P>0.05$ *vs* others.

Expression of cyclinE protein

CyclinE protein was located in the nuclei or cytoplasm of normal pancreatic cells and positive pancreatic cancer cells with brown-yellow granules (Figure 1B). The positive expression rate of cyclinE protein in tumor tissues was 68.8%, which was higher than that in adjacent pancreatic tissues ($\chi^2 = 4.063$, $P<0.05$). The positive expression rate of cyclinE protein in moderately or poorly differentiated tumor tissues was 84.6%, which was higher than that in well-differentiated tumor tissues ($\chi^2 = 5.128$, $P<0.05$). The cyclinE protein positive expression rate in lymph node metastasis group was 91.7%, which was higher than that in non-lymph node metastasis group ($\chi^2 = 4.693$, $P<0.05$, Table 2).

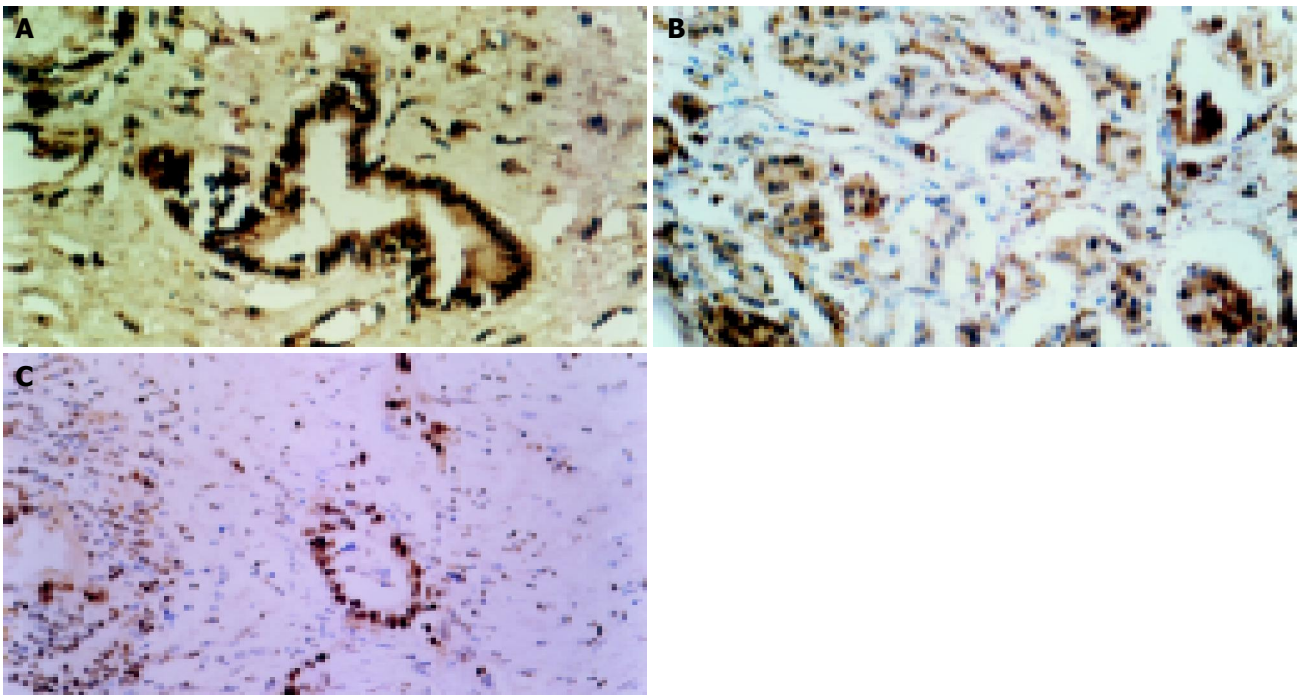

Figure 1 $P57^{kip2}$ protein in well-differentiated pancreatic adenocarcinoma (**A**), cyclin E protein in poorly-differentiated pancreatic adenocarcinoma (**B**), and PCNA protein in moderately-differentiated pancreatic adenocarcinoma (**C**).

Table 2 Expression of cyclinE protein in pancreatic cancer tissues

Characteristics	CyclinE protein expression				
	-	+	++	+++	Rate (%)
Tumor tissue	10	7	9	6	68.8[a]
Well-differentiated	9	5	4	1	52.6[c]
Moderately or poorly-differentiated	1	1	6	5	84.6[c]
Lymph node metastasis	1	1	5	5	91.7[e]
Non-lymph node metastasis	9	6	4	1	55.0[e]
Adjacent tissue	18	6	8	0	43.8[a]

[a]P<0.05, [c]P<0.05, [e]P<0.05 *vs* others.

Expression of PCNA

PCNA was located in the nuclei of normal pancreatic cells and positive pancreatic cancer cells with brown-yellow granules (Figure 1C). The positive expression rate of PCNA in tumor tissues was 71.9%, which was higher than that in adjacent pancreatic tissues (χ^2 = 5.189, P<0.05). The positive expression rate of PCNA in moderately or poorly differentiated tumor tissues was 92.3%, which was higher than that in well-differentiated tumor tissues (χ^2 = 4.522, P<0.05). The positive expression rate of PCNA in lymph node metastasis group was 100%, which was higher than that in non-lymph node metastasis group (χ^2 = 7.513, P<0.05, Table 3).

Table 3 Expression of PCNA in pancreatic cancer tissues

Characteristics	PCNA protein expression		
	-	+	Rate (%)
Tumor tissue	9	23	71.9[a]
Well-differentiated	8	11	57.9[c]
Moderately or poorly-differentiated	1	12	92.3[c]
Lymph node metastasis	0	12	100[e]
Non-lymph node metastasis	9	11	55.0[e]
Adjacent tissue	18	14	43.8[a]

[a]P<0.05, [c]P<0.05, [e]P<0.05 *vs* others.

Relationship between expression of p57^{kip2} and cyclinE proteins

The cyclinE protein positive expression rate in tumor tissues of the p57^{kip2} protein positive expression group and the p57^{kip2} protein negative expression was 60.0% and 76.5% respectively. There was no significant correlation between the two groups (r = -0.11211, P>0.05, Table 4).

Table 4 Relationship between expression of p57^{kip2} and cyclinE proteins

p57^{kip2}	CyclinE protein expression				
	-	+	++	+++	Rate (%)
–	4	4	6	3	76.5
+	4	3	2	2	
++	2	0	0	1	60.0
+++	0	0	1	0	

DISCUSSION

Studies in recent years have shown that G_1 phase regulation is a complex procedure[4-7]. p57^{kip2} gene is located in chromosome 11p15.5, and p57^{kip2} protein is a cell cycle inhibitor with molecular weight of 57 ku, which is included in the CIP/KIP family and similar to p21 and p27 proteins in functions[8,9]. Lee *et al*.[10], suggested that the tumor suppressor mechanism of p57^{kip2} protein may be integrated with cyclin-CDK complexes and makes cell cycle to arrest in the G_1 phase. Kondo *et al*.[11], considered that paternal alleles of p57^{kip2} are imprinted, maternal alleles of p57^{kip2} are expressed in the normal status, loss of imprinting and imprinting mistakes of p57^{kip2} lead to a decrease at level of gene expression in tumors. Matsumoto *et al*.[12], reported that p57^{kip2} protein positive expression rate is 43.3±3.2% in patients with esophageal squamous cell carcinoma. From then on, studies about p57^{kip2} protein expression in human colorectal carcinoma[13], hepatocellular carcinoma[14,15], prostate tumor[16], neoplastic thyroid tissues[17], epithelial ovarian tumor[18], extrahepatic bile duct carcinoma and intrahepatic cholangiocellular carcinoma have been reported[19,20], but few reports on the relationship between p57^{kip2} protein expression and pancreatic cancer are available[21]. In this study, we found that the positive expression rate of p57^{kip2} protein in pancreatic cancer tissues was significantly lower than that in adjacent pancreatic tissues. The worse the cancer cell differentiation, the lower was the p57^{kip2} protein expression, and there was no correlation between the reduced expression of p57^{kip2} protein and lymph node metastasis. The results suggest that reduced expression of p57^{kip2} protein correlates with the occurrence and malignant degree of pancreatic cancer. CyclinE protein is a positive regulating factor in the cell cycle and promotes the genesis and progression of tumors[22-24]. Our results suggest that overexpression of cyclinE is associated with the genesis and malignant degree, as well as lymph node metastasis of pancreatic cancer. PCNA is a δ-assistant factor of DNA synthetase, takes part in DNA biological synthesis and regulates cell cycle and cell proliferation by tetramer with cyclin, CDK and p21. Overexpression of PCNA is associated with a variety of tumors of digestive system including human colorectal cancer[25], gastric cancer[26], hepatocellular carcinoma[27], pancreas tumor[28,29]. The results in the present study suggest that overexpression of PCNA is associated with the occurrence and progression of pancreatic cancer, and malignant proliferation status of pancreatic cancer determined by expression of PCNA is of practical value. Our results suggest that cell proliferative activity is high for the negative or reduced expression of p57^{kip2} protein. Furthermore, p57^{kip2} protein plays a role in suppressing cell proliferation. Our findings are in accordance with the results of previous studies[5].

In summary, decreased expression of p57^{kip2} and/or overexpression of cyclinE protein and PCNA might contribute to the occurrence and progression of pancreatic cancer. The p57^{kip2}, cyclinE protein, and PCNA might play an important role in occurrence and progression of pancreatic cancer.

REFERENCES

1 **Clurman BE,** Roberts JM. Cell cycle and cancer. *J Natl Cancer*

Inst 1995; **87**: 1499-1501
2 **Grana X,** Reddy EP. Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinase(CDKs), growth suppressor and cyclin dependent kinase inhibitors(CDKIs). *Oncogene* 1995; **11**: 211-219
3 **Sherr CJ.** G_1 phase progression :cycling on cue. *Cell* 1994; **79**: 551-555
4 **Sherr CJ.** Cancer cell cycles. *Science* 1996; **274**: 1672-1677
5 **Kamb A.** Cell-cycle regulators and cancer. *Trends Genet* 1995; **11**: 136-140
6 **Hunter T,** Pines J. Cyclins and cancer II: CyclinD and CDK inhibitors come of age. *Cell* 1994; **79**: 573-582
7 **Kamb A,** Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian SV, Stockent E, Day RS, Johon BE, Skolnick MH. A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 1994; **264**: 436-440
8 **Matsuoka S,** Edwards MC, Bai C, Parker S, Zhang P, Baldini A, Harper JW, Elledge SJ. $p57^{KIP2}$ a structurally distinct member of the p2KIP1 cdk inhibitor family ,is a candidate tumor suppressor gene. *Genes Dev* 1995; **9**: 650-662
9 **Orlow I,** Iavarone A, Crider-Miller SJ, Bonilla F, Latres E, Lee MH, Gerald WL, Massague J, Weissman BE, Cordon-Cardo C. Cyclin-dependent kinase inhibitor $p57^{KIP2}$ in soft tissue sarcomas and wilms tumor. *Cancer Res* 1996; **56**: 1219-1221
10 **Lee MH,** Reynisdottir I, Massague J. Cloning of $p57^{KIP2}$, a cyclin dependent kinase inhibitor with unique domain structure and tissue distribution. *Genes Dev* 1995; **9**: 639-649
11 **Kondo M,** Matsuoka S, Uchida K, Osada H, Nagatake M, Takagi K, Harper JW, Takahashi T, Elledge SJ, Takahashi T. Selective maternal allele loss in human lung cancers of the maternally expressed $p57^{KIP2}$ gene at 11p15.5. *Ongen* 1996; **12**: 1365-1368
12 **Matsumoto M,** Furihata M, Ohtsuki Y, Sasaguri S, Ogoshi S. Immunohistochemical characterization of $p57^{KIP2}$ expression in human esophageal squamous cell carcinoma. *Anticancer Res* 2000; **20**: 1947-1952
13 **Noura S,** Yamamoto H, Sekimoto M, Takemasa I, Miyake Y, Ikenaga M, Matsuura N, Monden M. Expression of second class of KIP protein $p57^{KIP2}$ in human colorectal carcinoma. *Int J Oncol* 2001; **19**: 39-47
14 **Ito Y,** Takeda T, Sakon M, Tsujimoto M, Monden M, Matsuura N. Expression of $p57^{kip2}$ protein in hepatocellular carcinoma. *Oncology* 2001; **61**: 221-225
15 **Nakai S,** Masaki T, Shiratori Y, Ohgi T, Morishita A, Kurokohchi K, Watanabe S, Kuriyama S. Expression of $p57^{kip2}$ in hepatocellular carcinoma: relationship between tumor differentiation and patient survival. *Int J Oncol* 2002; **20**: 769-775
16 **Schwarze SR,** Shi Y, Fu VX, Watson PA, Jarrard DF. Role of cyclin-dependent kinase inhibitors in the growth arrest at senescence in human prostate epithelial and uroepithelial cells. *Oncogen* 2001; **20**: 8184-8192
17 **Ito Y,** Yoshida H, Nakano K, Kobayashi K, Yokozawa T, Hirai K, Matsuzuka F, Matsura N, Kuma K, Miyauchi A. Expression of $p57^{Kip2}$ protein in normal and neoplastic thyroid tissues. *Int J Mol Med* 2002; **9**: 373-376
18 **Rosenberg E,** Demopoulos RI, Zeleniuch-Jacquotte A, Yee H, Sorich J, Speyer JL, Newcomb EW. Expression of cell cycle regulators p57(Kip2), cyclinD1, and cyclin E in epithelial ovarian tumors and survival. *Hum Pathol* 2001; **32**: 808-813
19 **Ito Y,** Takeda T, Sasaki Y, Sakon M, Yamada T, Ishiguro S, Imaoka S, Tsujimoto M, Monden M, Matsuura N. Expression of p57/Kip2 protein in extrahepatic bile duct carcinoma and intrahepatic cholangiocellular carcinoma. *Liver* 2002; **22**: 145-149
20 **Lee MH,** Yang HY. Negative regulators of cyclin-dependent kinases and their roles in cancers. *Cell Mol Life Sci* 2001; **58**: 1907-1922
21 **Ito Y,** Takeda T, Wakasa K, Tsujimoto M, Matsuura N. Expression of p57/Kip2 protein in pancreatic adenocarcinoma. *Pancreas* 2001; **23**: 246-250
22 **Keyomarsi K,** Tucker SL, Buchholz TA, Callister M, Ding Y, Hortobagyi GN, Bedrosian I, Knickerbocker C, Toyofuku W, Lowe M, Herliczek TW, Bacus SS. Cyclin E and survival in patients with breast cancer. *N Engl J Med* 2002; **347**: 1566-1575
23 **Georgieva J,** Sinha P, Schadendorf D. Expression of cyclins and cyclin dependent kinases in human benign and malignant melanocytic lesions. *J Clin Pathol* 2001; **54**: 229-235
24 **Oshita T,** Shigemasa K, Nagai N, Ohama K. p27, cyclin E, and CDK2 expression in normal and cancerous endometrium. *Int J Oncol* 2002; **21**: 737-743
25 **Kunimoto Y,** Nakamura T, Ohno M, Kuroda Y. Relationship between immunohistochemical evaluation of thymidylate synthase and proliferating cell nuclear antigen labeling index in gastrointestinal carcinoma. *Oncol Rep* 2004; **12**: 1163-1167
26 **Czyzewska J,** Guzinska-Ustymowicz K, Lebelt A, Zalewski B, Kemona A. Evaluation of proliferating makers Ki-67, PCNA in gastric cancers. *Rocz Akad Med Bialymst* 2004; **49** (Suppl1): 64-66
27 **Tsuboi Y,** Ichida T, Sugitani S, Genda T, Inayoshi J, Takamura M, Matsuda Y, Nomoto M, Aoyagi Y. Overexpression of extracellular signal-regulated protein kinase and its correlation with proliferation in human hepatocellular carcinoma. *Liver Int* 2004; **24**: 432-436
28 **Sato T,** Konishi K, Kimura H, Maeda K, Yabushita K, Tsuji M, Miwa A. Evaluation of PCNA, p53, K-ras, and LOH in endocrine pancreas tumors. *Hepatogastroenterology* 2000; **47**: 875-879
29 **Niijima M,** Yamaguchi T, Ishihara T, Hara T, Kato K, Kondo F, Saisho H. Immunohistochemical analysis and in situ hybridization of cyclooxygenase-2 expression in intraductal papillary – mucinous tumors of the pancreas. *Cancer* 2002; **94**: 1565-1573

Science Editor Wang XL and Guo SY **Language Editor** Elsevier HK