PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(9):1337-1340
World Journal of Gastroenterology
 ISSN 1007-9327

• BASIC RESEARCH •

Detection of K-ras point mutation and telomerase activity during endoscopic retrograde cholangiopancreatography in diagnosis of pancreatic cancer

Guo-Xiong Zhou, Jie-Fei Huang, Zhao-Shen Li, Guo-Ming Xu, Feng Liu, Hong Zhang

Guo-Xiong Zhou, Jie-Fei Huang, Hong Zhang, Department of Gastroenterology, Affiliated Hospital of Nantong Medical College, Nantong 226001, Jiangsu Province, China
Zhao-Shen Li, Guo-Ming Xu, Feng Liu, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
Correspondence to: Dr. Guo-Xiong Zhou, Department of Gastroenterology, Affiliated Hospital of Nantong Medical College, Nantong 226001, Jiangsu Province, China. zhouguoxiong@pub.nt.jsinfo.net
Telephone: +86-513-5052079
Received: 2003-06-05 **Accepted:** 2003-08-16

Abstract

AIM: To study the value of monitoring K-ras point mutation at codon 12 and telomerase activity in exfoliated cells obtained from pancreatic duct brushings during endoscopic retrograde cholangiopancreatography (ERCP) in the diagnosis of pancreatic cancer.

METHODS: Exfoliated cells obtained from pancreatic duct brushings during ERCP were examined in 27 patients: 23 with pancreatic cancers, 4 with chronic pancreatitis. K-ras point mutation was detected with the polymerase chain reaction and restriction fragment-length polymorphism (PCR-RFLP). Telomerase activity was detected by PCR and telomeric repeat amplification protocol assay (PCR-TRAP-ELISA).

RESULTS: The telomerase activities in 27 patients were measured in 21 exfoliated cell samples obtained from pancreatic duct brushings. D_{450} value of telomerase activities in pancreatic cancer and chronic pancreatitis were 0.446±0.27 and 0.041±0.0111, respectively. Seventy-seven point eight percent (14/18) of patients with pancreatic cancer and none of the patients with chronic pancreatitis showed telomerase activity in cells collected from pancreatic duct brushings when cutoff value of telomerase activity was set at 2.0. The K-ras gene mutation rate (72.2%) in pancreatic cancer was higher than that in chronic pancreatitis (33.3%) ($P<0.05$). In considering of both telomerase activities and K-ras point mutation, the total positive rate was 83.3% (15/18), and the specificity was 100%.

CONCLUSION: Changes of telomerase activities and K-ras point mutation at codon 12 may be an early event of malignant progression in pancreatic cancer. Detection of telomerase activity and K-ras point mutation at codon 12 may be complementary to each other, and is useful in diagnosis of pancreatic cancer.

Zhou GX, Huang JF, Li ZS, Xu GM, Liu F, Zhang H. Detection of K-ras point mutation and telomerase activity during endoscopic retrograde cholangiopancreatography in diagnosis of pancreatic cancer. *World J Gastroenterol* 2004; 10(9): 1337-1340
http://www.wjgnet.com/1007-9327/10/1337.asp

INTRODUCTION

Pancreatic cancer has a poor prognosis, its early diagnosis remains unsatisfactory. Even serum tumor markers, such as CA19-9, are associated with pancreatic cancer, but lack sensitivity and tumor specificity. Recent studies have shown that K-ras oncogene is often activated by specific point mutations restricted to codon 12 in human pancreatic ductal adenocarcinoma. In pancreatic adenocarcinoma, K-ras mutations were found in 75% to 100% of the cases[1-8]. We and others previously showed that k-ras analysis from specimens obtained during ERCP was a sensitive (around 80% to 85%) and specific (close to 100%) method in differentiating benign from malignant pancreatic diseases. Therefore, k-ras mutation identification has been claimed as an useful and early marker in diagnosis of pancreatic cancer[9-12]. Telomerase is a key enzyme with regard to immortalization of cancer cells and increased activity has been demonstrated in various human malignant neoplasms[13-16]. We previously detected telomerase activity in 87% of surgically resected human pancreatic cancers. While in adjacent tissues, the positive rate of telomerase activity was only 10%. Detection of telomerase activity was useful in diagnosis of pancreatic cancer[17].

In the present study, we evaluated the diagnostic role of K-ras mutation and telomerase activity in exfoliated cells obtained from pancreatic duct brushings alone and in combination, in differentiating chronic pancreatitis from pancreatic cancer.

MATERIALS AND METHODS

Patients

From April 1999 to December 2000, 27 patients undergoing ERCP were prospectively investigated at Changhai Hospital, 23 of them were patients with pancreatic cancer, 4 were patients with chronic pancreatitis. There were 17 men, 10 women with a mean age of 58.5 years (range: 22 to 75). The diagnosis of pancreatic cancer was confirmed histologically. The locations of the cancer were pancreatic head (n=13), body (n=5) and tail (n=1), and diffusion over the pancreas (n=4). Chronic pancreatitis was diagnosed by clinical history, ERCP and CT findings. All patients with chronic pancreatitis were followed up clinically and radiologically for at least six months.

Sampling technique

ERCP was performed using Olympus JF-240, 230 and TJF200 duodenoscope (Olympus, Japan). Lesions were identified after injection of iodinated contrast agent, a wire (0.035 inch in length) was introduced carefully into the main pancreatic duct and advanced to the suspected abnormal areas under guidance of fluoroscopic monitoring. Then a pancreatic duct brush (BC-24Q, Olympus) was introduced through the guide wire to the suspicious lesion, and the guide wire was withdrawn. The brush was moved rigorously back and forth across the suspicious lesion, brushes were retracted into the guiding catheter tip and removed together with the catheter to avoid losing a portion of the specimen inside the catheter. Specimens were immediately

collected in 5 mL of sterile saline solution at 4 ℃ for analysis of telomerase activity and DNA or smeared on glass slides, fixed in 95% alcohol and stained by HE for cytological examination, the order of brushing samples studied was always kept consistent.

Detection of K-ras point mutations at codon 12

Samples were centrifuged at 2000 r/mim for 15 min and the pellet was collected. Genomic DNA was isolated using DNA lysis buffer and proteinase K digestion, followed by extraction with phenol and chloroform. K-ras point mutations were detected using PCR and restriction fragment-length polymorphism (PCR-RFLP). Primers were synthesized and supplied by Shanghai Bio-Engineering Research Center. The sequences of primers were: P1=5'-CTTGTGGTAGTTGGACCT-3' containing a mismatched base indicated by the underline, P2=5'-GTCAGAGAAACCTTTATCTG-3', P3=5'-TGCACCAGTAATATG-CATAT-3'. The reaction volume was 50 μL containing 5 μL of 10×Ex Taq buffer, 4 μL of dNTP mixture, 0.5 μL of each primer, 0.25 μL (5 U/μL) of Taq DNA polymerase. The primers for the first PCR were P1 (sense) and P2 (antisense) that flanked codon 12 of the K-ras gene. The first PCR amplification was performed for 30 cycles using a DNA thermal cycler, and each cycle consisted of denaturation at 94 ℃ for 30 s, annealing at 52 ℃ for 30 s, and extension at 72 ℃ for 45 s. A 5 μL sample of the first PCR product was digested with 1 μL (10 U/μL) of Mva I at 37 ℃ for 1 h under the conditions recommended by the supplier of mismatched sense primer described above. Subsequently, 5 μL of the reaction mixture was subjected to a second round of PCR and amplified for 30 cycles under the same conditions using the following primers: sense: P1; antisense: P3. The second PCR products were also digested with 1 μL (10 U/μL) of Mva I for 1 h. Mutations were confirmed by electrophoresis of 10 μL of the second digested sample on a 20 g/L agarose gel.

Detection of telomerase activity

Telomerase activity was detected with telomerase PCR-ELISA (Boehringer Mannheim, Germany). Exfoliated cells obtained from pancreatic duct brushings were collected and washed with PBS, and centrifuged at 10 000 g for 10 min. Then the cells in PBS containing 200 μL Lysis reagent were incubated on ice for 30 min, and centrifuged at 16 000 g for 20 min at 4 ℃. Next, the supernatants (175 μL) were transferred to a Eppendorf tube, and tested for telomerase activity assay. Protein concentration was determined by Coomassie protein assay. Each of TRAP reactions contained 2 μL of cell extract (5-6 μg total protein) and sterile water with a final volume of 50 μL. The reaction mixture was incubated at 25 ℃ for 30 min, heated at 94 ℃ for 5 min. Then PCR amplification was performed for 33 cycles using a DNA thermal cycler, and each cycle consisted of denaturation at 94 ℃ for 30 s, annealing at 50 ℃ for 30 s, and extension at 72 ℃ for 90 s followed by 72 ℃ for 10 min. After PCR, 5 μL of the PCR products was dispensed into a reaction tube and incubated with 20 μL of denaturation reagent at room temperature for 10 min, and then mixed thoroughly by overtaxing briefly with 225 μL hybridization buffer per tube. By transferring 100 μL of the mixture per well of percolated MTP modules, MTP modules were incubated at 37 ℃ on a shaker (300 r/min) for 2 h and washed 3 times with PBS, and enzyme reactions were incubated at room temperature on a shaker (300 r/min) for 30 min by addition of 100 μL anti-Dig-POD per well. After washed 5 times with PBS, 100 μL TMB substrate solution was added to each well and incubated for color development at room temperature for 20 min while shaking at 300 r/min. Using a ELISA reader, the absorbance value of samples was measured at 450 nm within 30 min after termination of reaction.

Statistical analysis

Statistical analysis was performed using the unpaired *t* test and χ^2 test or Fisher's exact test. $P<0.05$ was considered statistically significant. Data were expressed as mean±SD.

RESULTS

Detection of K-ras point mutations at codon 12

K-ras point mutations at codon 12 were detected in 21 exfoliated cell samples obtained from pancreatic duct brushings by PCR-RFLP. Normal DNA had a 114 base pair (bp) band and k-ras DNA mutation remained as a 131 bp band since it was not digested by MvaI (Figures 1,2). K-ras gene mutation rate in pancreatic cancer was 72.2%, which was higher than that of chronic pancreatitis (33.3%, $P<0.05$).

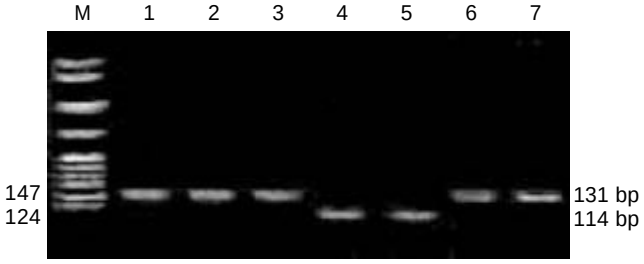

Figure 1 PCR products after restriction enzyme digestion, M: Marker; 1: Positive control; 2-7:Pancreatic cancer.

Detection of telomerase activity

Telomerase activities in 27 patients were measured in 21 exfoliated cell samples obtained from pancreatic duct brushings. D450 values of telomerase activity in carcinoma and chronic pancreatitis were 0.446±0.27 and 0.041±0.0111, respectively. Seventy-seven point eight percent (14/18) of the patients with pancreatic cancer and none of patients with chronic pancreatitis showed telomerase activity in cells from pancreatic duct brushings when cutoff value of telomerase activity was set at 2.0. Both telomerase activity and cytological examination were detected in 66.7% (12/18) of patients.

Table 1 Location of pancreatic cancer and K-ras mutation or telomerase activity

Location	*n*	K-ras mutation		Telomerase activity	
		(+)	%	(+)	%
Head	11	8	72.7	9	81.8
Body	3	2	66.7	2	66.7
Tail	1	1	100	1	100
Diffuse	3	2	66.7	2	66.7
Totol	18	13	72.2	14	77.8

Table 2 Comparison of K-ras point mutations and telomerase activity

	K-ras mutation	Telomerase	Both
Pancreatic cancer (*n*=18)	13	14	12
Chronic pancreatitis (*n*=3)	1	0	0
Sensitivity (%)	72.2	77.8	66.7
Specificity (%)	66.7	100	100

Relationship between K-ras gene or telomerase activity and location of tumors

No significant correlation of positivity of K-ras gene or telomerase activity with tumor location was observed (Table 1).

Comparison of K-ras point mutations with telomerase activity

The total rate of positive telomerase activity and K-ras mutation was 83.3% (15/18). The sensitivity and specificity of K-ras mutation and telomerase in differential diagnosis of pancreatic cancer and chronic pancreatitis was compared (Table 2).

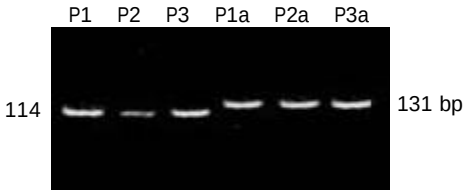

Figure 2 Agarose gel of PCR products, P1, P2, P3 : after restriction enzyme digestion; P1a, P2a, P3a: before restriction enzyme digestion, P1 and P1a, P2 and P2a, P3 and P3a reprent same samples, respectively.

DISCUSSION

Recent application of molecular biologic techniques, such as detection of K-ras mutation, has been proposed for the diagnosis of pancreatic cancer[18-22]. K-ras gene mutation was found in greater than 90% of pancreatic cancers, and detection of K-ras gene mutation-positive cells in pure pancreatic juice or duodenal juice were reported to be useful in the diagnosis of pancreatic cancer[23-26]. K-ras mutation was detected in ERCP brush samples. We used the sensitive RFLP method and found 72.2% of patients with pancreatic cancer had K-ras mutation, which was relatively higher than that in other reports. K-ras mutation was also detected in 1 patient with chronic pancreatitis. Although K-ras mutation has been detected mainly in malignancy, it could be detected in nonmalignant pancreatic lesions[27-29]. However, a recent long-term follow-up report suggested that K-ras mutation in chronic pancreatitis might represent false-positive results[30]. Studies including ours showed that this mutation could also occur in benign condition. Queneau *et al*.[31] found 27.8% (10/36) chronic pancreatitis patients had k-ras mutations. Recently, several investigators failed to detect K-ras mutations in chronic pancreatitis even though they used highly sensitive methods[32,33] and suggested that K-ras mutation was an extremely specific event in pancreatic cancer. Unfortunately, the reason for this discrepancy is not clear.

Telomerase is an enzyme that was found containing an RNA template complementary to the short DNA sequence repeats at chromosomal ends[34]. Telomerase activity has been found in the majority of malignant tissues, such as pancreatic cancers, but not in somatic tissues except for reproductive and hematopoietic cells. Moreover, telomerase activity was detected in an extremely early stage of pancreatic cancer when the tumor could not be detected by other diagnostic methods[35]. Hiyama *et al*.[36] reported that 95% of pancreatic carcinomas but none of 11 benign pancreatic tumors showed telomerase activity. Suehara *et al*.[37] reported that 100% of pancreatic carcinomas had high levels of telomerase activity. None of pancreatitis or normal pancreas extracts had detectable telomerase activity. However, some pancreatic adenomas demonstrated weak telomerase activity. Telomerase activity in pancreatic juice reflected the activity of the enzyme in tissue specimens[38,39]. The telomerase activity in pancreatic juice from patients with pancreatic ductal carcinomas was lower than that in resected tissues. It is possible that various digestive enzymes in pancreatic juice might disturb telomerase assay by inhibiting Taq polymerase[40]. Myung *et al*.[41] and Uehara *et al*.[42]. Have reported their study results in the diagnosis of pancreatic cancer by detecting K-ras mutations and telomerase activity alone and in combination in pancreatic juice. We compared these two methods and evaluated their usefulness alone and in combination for the differentiation of pancreatic cancer from chronic pancreatitis. Using PCR-TRAP-ELISA method, we could give a more accurate diagnosis of pancreatic cancer by evaluating both K-ras mutations and telomerase activity in cells obtained from pancreatic duct brushings, and found that K-ras mutations in cells obtained from pancreatic duct brushings were useful for detecting neoplastic lesions and that telomerase activity was useful for differentiating malignancy from benign lesions of the pancreas, when telomerase activity cutoff was set at 2.0. The sensitivity and specificity of K-ras mutations for the diagnosis of pancreatic cancer were 72.2% and 66.7%, respectively, whereas those of telomerase activity were 77.8% and 100%, respectively. By combining these two methods, the sensitivity was 66.7% and the specificity was 100%.

In conclusion, detection of K-ras mutation and telomerase activity in cells obtained from pancreatic duct brushings may be complementary to each other, and may prove to be a potent tool for the diagnosis of pancreatic cancer.

REFERENCES

1 **Inoue S**, Tezel E, Nakao A. Molecular diagnosis of pancreatic cancer. *Hepatogastroenterology* 2001; **48**: 933-938
2 **Yoshizawa K**, Nagai H, Sakurai S, Hironaka M, Morinaga S, Saitoh K, Fukayama M. Clonality and K-ras mutation analyses of epithelia in intraductal papillary mucinous tumor and mucinous cystic tumor of the pancreas. *Virchows Arch* 2002; **441**: 437-443
3 **Raimondo M**, Tachibana I, Urrutia R, Burgart LJ, DiMagno EP. Invasive cancer and survival of intraductal papillary mucinous tumors of the pancreas. *Am J Gastroenterol* 2002; **97**: 2553-2558
4 **Nakata B**, Yashiro M, Nishioka N, Aya M, Yamada S, Takenaka C, Ohira M, Ishikawa T, Nishino H, Wakasa K, Hirakawa K. Genetic alterations in adenoma-carcinoma sequencing of intraductalpapillary-mucinous neoplasm of the pancreas. *Int J Oncol* 2002; **21**: 1067-1072
5 **Mizumoto K**, Tanaka M. Genetic diagnosis of pancreatic cancer. *J Hepatobiliary Pancreat Surg* 2002; **9**: 39-44
6 **Tada M**, Komatsu Y, Kawabe T, Sasahira N, Isayama H, Toda N, Shiratori Y, OmataM. Quantitative analysis of K-ras gene mutation in pancreatic tissue obtained by endoscopic ultrasonography-guided fine needle aspiration: clinical utility for diagnosis of pancreatic tumor. *Am J Gastroenterol* 2002; **97**: 2263-2270
7 **Pabst B**, Arps S, Binmoeller K, Thul R, Walsemann G, Fenner C, Klapdor R. Analysis of K-ras mutations in pancreatic tissue after fine needle aspirates. *Anticancer Res* 1999; **19**: 2481-2483
8 **Luttges J**, Schlehe B, Menke MA, Vogel I, Henne-Bruns D, Kloppel G. The K-ras mutation pattern in pancreatic ductal adenocarcinoma usually is identical to that in associated normal, hyperplastic, and metaplastic ductal epithelium. *Cancer* 1999; **85**: 1703-1710
9 **Futakawa N**, Kimura W, Yamagata S, Zhao B, Ilsoo H, Inoue T, Sata N, Kawaguchi Y, Kubota Y, Muto T. Significance of K-ras mutation and CEA level in pancreatic juice in the diagnosis of pancreatic cancer. *J Hepatobiliary Pancreat Surg* 2000; **7**: 63-71
10 **Tateishi K**, Tada M, Yamagata M, Isayama H, Komatsu Y, Kawabe T, Shiratori Y, Omata M. High proportion of mutant K-ras gene in pancreatic juice of patients with pancreatic cystic lesions. *Gut* 1999; **45**: 737-740
11 **Watanabe H**, Yamaguchi Y, Ha A, Hu YX, Motoo Y, Okai T, Yoshimura T, Sawabu N. Quantitative determination of K-ras mutations in pancreatic juice for diagnosis of pancreatic cancer using hybridization protection assay. *Pancreas* 1998; **17**: 341-347
12 **Watanabe H**, Sawabu N, Songur Y, Yamaguchi Y, Yamakawa O, Satomura Y, Ohta H, Motoo Y, Okai T, Wakabayashi T. Detection of K-ras point mutations at codon 12 in pure pancreatic juice for the diagnosis of pancreatic cancer by PCR-RFLP analysis. *Pancreas* 1996; **12**: 18-24

13 **Pearson AS**, Chiao P, Zhang L, Zhang W, Larry L, Katz RL, Evans DB, AbbruzzeseJL. The detection of telomerase activity in patients with adenocarcinoma of the pancreas by fine needle aspiration. *Int J Oncol* 2000; **17**: 381-385

14 **Yeh TS**, Cheng AJ, Chen TC, Jan YY, Hwang TL, Jeng LB, Chen MF, Wang TC. Telomerase activity is a useful marker to distinguish malignant pancreatic cystic tumors from benign neoplasms and pseudocysts. *J Surg Res* 1999; **87**: 171-177

15 **Tsutsumi M**, Tsujiuchi T, Ishikawa O, Majima T, Yoshimoto M, Sasaki Y, Fukuda T, Oohigashi H, Konishi Y. Increased telomerase activities in human pancreatic duct adenocarcinomas. *Jpn J Cancer Res* 1997; **88**: 971-976

16 **Mizumoto K**, Suehara N, Muta T, Kitajima S, Hamasaki N, Tominaga Y, Shimura H, Tanaka M. Semi-quantitative analysis of telomerase in pancreatic ductal adenocarcinoma. *J Gastroenterol* 1996; **31**: 894-897

17 **Zhou GX**, Li ZS, Xu GM, Huang JF. Telomerase activity and Telomerase gene expression in pancreatic cancer. *Shiyong Aizheng Zazhi* 2003; **18**: 32-34

18 **Mulcahy H**, Farthing MJ. Diagnosis of pancreatico-biliary malignancy: detection of gene mutations in plasma and stool. *Ann Oncol* 1999; **10**(Suppl 4): 114-117

19 **Wenger FA**, Zieren J, Peter FJ, Jacobi CA, Muller JM. K-ras mutations in tissue and stool samples from patients with pancreatic cancer and chronic pancreatitis. *Langenbecks Arch Surg* 1999; **384**: 181-186

20 **Castells A**, Puig P, Mora J, Boadas J, Boix L, Urgell E, Sole M, Capella G, Lluis F, Fernandez-Cruz L, Navarro S, Farre A. K-ras mutations in DNA extracted from the plasma of patients with pancreatic carcinoma: diagnostic utility and prognostic significance. *J Clin Oncol* 1999; **17**: 578-584

21 **Mulcahy HE**, Lyautey J, Lederrey C, Qi Chen X, Anker P, Alstead EM, Ballinger A, Farthing MJ, Stroun M. A prospective study of K-ras mutations in the plasma of pancreatic cancer patients. *Clin Cancer Res* 1998; **4**: 271-275

22 **Lu X**, Xu T, Qian J, Wen X, Wu D. Detecting K-ras and p53 gene mutation from stool and pancreatic juice fordiagnosis of early pancreatic cancer. *Chin Med J* 2002; **115**: 1632-1636

23 **Kimura W**, Zhao B, Futakawa N, Muto T, Makuuchi M. Significance of K-ras codon 12 point mutation in pancreatic juice in the diagnosis of carcinoma of the pancreas. *Hepatogastroenterology* 1999; **46**: 532-539

24 **Nakamura Y**, Onda M, Uchida E. Analysis of K-ras codon 12 point mutations using duodenal lavage fluid for diagnosis of pancreatic carcinoma. *Pancreas* 1999; **18**: 133-140

25 **Ha A**, Watanabe H, Yamaguchi Y, Ohtsubo K, Wang Y, Motoo Y, Okai T, Wakabayahi T, Sawabu N. Usefulness of supernatant of pancreatic juice for genetic analysis of K-ras in diagnosis of pancreatic carcinoma. *Pancreas* 2001; **23**: 356-363

26 **Wilentz RE**, Chung CH, Sturm PD, Musler A, Sohn TA, Offerhaus GJ, Yeo CJ, Hruban RH, Slebos RJ. K-ras mutations in the duodenal fluid of patients with pancreatic carcinoma. *Cancer* 1998; **82**: 96-103

27 **Tada M**, Ohashi M, Shiratori Y, Okudaira T, Komatsu Y, Kawabe T, Yoshida H, Machinami R, Kishi K, Omata M. Analysis of K-ras gene mutation in hyperplastic duct cells of the pancreas without pancreatic disease. *Gastroenterology* 1996; **110**: 227-231

28 **Rivera JA**, Rall CJ, Graeme-Cook F, Fernandez-del Castillo C, Shu P, Lakey N, Tepper R, Rattner DW, Warshaw AL, Rustgi AK. Analysis of K-ras oncogene mutations in chronic pancreatitis with ductal hyperplasia. *Surgery* 1997; **121**: 42-49

29 **Berndt C**, Haubold K, Wenger F, Brux B, Muller J, Bendzko P, Hillebrand T, Kottgen E, Zanow J. K-ras mutations in stools and tissue samples from patients with malignant and nonmalignant pancreatic diseases. *Clin Chem* 1998; **44**: 2103-2107

30 **Furuya N**, Kawa S, Akamatsu T, Furihata K. Long-term follow-up of patients with chronic pancreatitis and K-ras gene mutation detected in pancreatic juice. *Gastroenterology* 1997; **113**: 593-598

31 **Queneau PE**, Adessi GL, Thibault P, Cleau D, Heyd B, Mantion G, Carayon P. Early detection of pancreatic cancer in patients with chronic pancreatitis:diagnostic utility of a K-ras point mutation in the pancreatic juice. *Am J Gastroenterol* 2001; **96**: 700-704

32 **Hsiang D**, Friess H, Buchler MW, Ebert M, Butler J, Korc M. Absence of K-ras mutations in the pancreatic parenchyma of patients with chronic pancreatitis. *Am J Surg* 1997; **174**: 242-246

33 **Orth M**, Gansauge F, Gansauge S, Beger HG, Adler G, Schmid RM. K-ras mutations at codon 12 are rare events in chronic pancreatitis. *Digestion* 1998; **59**: 120-124

34 **Greider CW**, Blackburn EH. Identification of a specific telomere terminal transferase activity inTetrahymena extracts. *Cell* 1985; **43**: 405-413

35 **Suehara N**, Mizumoto K, Kusumoto M, Niiyama H, Ogawa T, Yamaguchi K, Yokohata K, Tanaka M. Telomerase activity detected in pancreatic juice 19 months before a tumor is detected in a patient with pancreatic cancer. *Am J Gastroenterol* 1998; **93**: 1967-1971

36 **Hiyama E**, Kodama T, Shinbara K, Iwao T, Itoh M, Hiyama K. Telomerase activity is detected in pancreatic cancer but not in benign tumors. *Cancer Res* 1997; **57**: 326-331

37 **Suehara N**, Mizumoto K, Muta T, Tominaga Y, Shimura H, Kitajima S, Hamasaki N, Tsuneyoshi M, Tanaka M. Telomerase elevation in pancreatic ductal carcinoma compared to nonmalignant pathological states. *Clin Cancer Res* 1997; **3**: 993-998

38 **Suehara N**, Mizumoto K, Tanaka M, Niiyama H, Yokohata K, Tominaga Y, Shimura H, Muta T, Hamasaki N. Telomerase activity in pancreatic juice differentiates ductal carcinoma from adenoma and pancreatitis. *Clin Cancer Res* 1997; **3**: 2479-2483

39 **Iwao T**, Tsuchida A, Hiyama E, Kajiyama G. Telomerase activity in pancreatic juice for the preoperative diagnosis of pancreatic cancer. *Nippon Rinsho* 1998; **56**: 1229-1233

40 **Piatyszek MA**, Kim NW, Wwinrich SL, Hiyama K, Hiyama E, Wright WE, Shay JW. Detection telomerase activity in human cells and tumors by a telomeric repeat amplification protocol (TRAP). *Methods Cell Sci* 1995; **17**: 1-15

41 **Myung SJ**, Kim MH, Kim YS, Kim HJ, Park ET, Yoo KS, Lim BC, Wan Seo D, Lee SK, Min YI, Kim JY. Telomerase activity in pure pancreatic juice for the diagnosis of pancreatic cancer may be complementary to K-ras mutation. *Gastrointest Endosc* 2000; **51:** 708-713

42 **Uehara H**, Nakaizumi A, Tatsuta M, Baba M, Takenaka A, Uedo N, Sakai N, Yano H, Iishi H, Ohigashi H, Ishikawa O, Okada S, Kakizoe T. Diagnosis of pancreatic cancer by detecting telomerase activity in pancreatic juice: comparison with K-ras mutations. *Am J Gastroenterol* 1999; **94**: 2513-2518

Edited by Ren SY and Wang XL **Proofread by** Xu FM