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

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. D00 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[9-12]. 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 °C 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 was studied was always kept consistent.

**Detection of K-ras point mutations at codon 12**

Samples were centrifuged at 2000 r/min 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’-GTCAGAGAACCCTTATCTG-3’ , P3=5’-TGCAACCAGTATG-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 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C 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 °C 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 10 000 g for 10 min at 4 °C. 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 °C for 30 min, heated at 94 °C for 5 min. Then PCR amplification was performed for 33 cycles using a DNA thermal cycler, and each cycle consisted of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 90 s followed by 72 °C 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 °C 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 χ² 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).

**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.

**Comparison of K-ras point mutations and telomerase activity**

| Location | K-ras mutation | Telomerase activity |
|----------|----------------|---------------------|
| Head     | 11             | 8                   | 14                  | 81.8                  |
| Body     | 3              | 2                   | 2                   | 66.7                  |
| Tail     | 1              | 1                   | 1                   | 100                   |
| Diffuse  | 3              | 2                   | 2                   | 66.7                  |
| Total    | 18             | 13                  | 14                  | 77.8                  |

**Comparison of K-ras point mutations and telomerase activity**

|                 | K-ras mutation | Telomerase | Both |
|-----------------|----------------|------------|------|
| Pancreatic cancer | 13             | 14         | 12   |
| Chronic pancreatitis | 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 represent 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[23-26]. 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[37-39]. 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, Teznel E, Nakao A. Molecular diagnosis of pancreatic cancer. Hepatogastroenterology 2001; 48: 933-938
2. Yoshizawa K, Nagai H, Sakurai S, Hironaka M, Morinaga S, Saito 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, DiMaggio 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 intraductal papillary-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, Sasehira N, Isayama H, Toda N, Shiratori Y, Omae M. 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, Binoecker M, Thul R, Walsmann G, Fenner C, Klapdor R. Analysis of K-ras gene mutations in pancreatic tissue after fine needle aspirations. Anticancer Res 1999; 19: 2481-2483
8. Luttges J, Schleb B, Menke MA, Vogel I, Henne-Bruns D, Kloppe 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, Isouo 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, Onuma 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
in the diagnosis of carcinoma of the pancreas.
Ha A, Yen, CT, Chen YY, Hwang TL, Jeng LB, Chen MF, Wang TC. Telomerase activity is a useful marker to distin-
guish malignant pancreatic cystic tumors from benign neo-
plasms and pseudocysts. J Surg Res 1999; 87: 171-177

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 adenocarcinos.
mas. Jpn J Cancer Res 1997; 88: 971-976

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

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

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

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

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

Muelcay HE, Lyutey 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

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

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

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

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

Wilentz RE, Chung CH, Sturm PD, Musler A, Sohn TA, Offerhaus GJ, Yee C, Hruban RH, Siebold R. K-ras mutations in the duodenal fluid of patients with pancreatic cancer. Cancer 1998; 82: 96-103

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: 227-233

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 mutation in chronic pancre-
atitis with ductal hyperplasia. Surgery 1997; 121: 42-49

Berndt C, Habobold K, Wengen F, Brux B, Muller J, Bendzko P, Hillebrand T, Kottgen E, Zhao J. K-ras mutations in stools and tissue samples from patients with malignant and nonma-
lignant pancreatic diseases. Chin J Cancer 1998; 44: 2103-2107

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

Queneau PE, Asness 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

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

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

Greider CW, Blackburn EH. Identification of a specific telom-
ere terminal transferase activity in Tetrahymena extracts. Cell 1985; 43: 405-413

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

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

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

Suehara N, Mizumoto K, Tanaka N, Niijima 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

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

Piatyzeck MA, Kim NW, Wwinnich 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 Biol 1995; 1: 1-15

Myung SJ, Kim MH, Kim SY, Kim HJ, Park ET, Yoo KS, Lim
BC, Wan Seo D, Lee SK, Min YL, 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

Uehara H, Nakaiizumi A, Tatsutsa M, Baba M, Takenaka A,
Ueda N, Sakai N, Yano H, Ishii H, Ohigashi H, Ishikiva O, Okada S, Kakizoe T. Diagnosis of pancreatic cancer by detect-
ing 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