Detection of Mutations of p53 Tumor Suppressor Gene in Pancreatic Juice and Its Application to Diagnosis of Patients with Pancreatic Cancer: Comparison with K-ras Mutation

Yasushi Yamaguchi, Hiroyuki Watanabe, Songür Yrdiran, Koushiro Ohtsubo, Yoshiharu Motoo, Takasi Okai, and Norio Sawabu

Department of Internal Medicine and Medical Oncology, Cancer Research Institute, Kanazawa University, Kanazawa 921-8044, Japan

ABSTRACT

Because of the difficulty in obtaining biopsy specimens from pancreatic cancer patients, K-ras mutation analysis in pancreatic juice has been used for specific diagnosis. But recently, false positives have been obtained with this method. To improve the genetic diagnosis of pancreatic cancer, detection of p53 gene mutation in pancreatic juice was studied. Pancreatic juice was sampled endoscopically. Single-strand conformation polymorphism analysis was used for p53 mutation analysis. Furthermore, K-ras mutations at codon 12 were also studied in the same pancreatic cancer patients. Of 26 cases of pancreatic cancer, p53 mutations were detected in 11 (42.3%). No mutations were seen in the cases with mucin-producing adenoma nor with chronic pancreatitis. K-ras mutations were detected in 84.0% of cases by RFLP analysis, which has high sensitivity, and in 65.3% by hybridization protection assay, which has high specificity. Using a combination assay with both genes, specificities of this marker. Actually, selective microdissected studies showed that benign mucous cell hyperplasia of pancreatic ductal epithelium with chronic inflammation had a high occurrence of K-ras mutations at codon 12 (12), suggesting hyperplastic mucous cell flow into PPJ. It is therefore important to reveal the usefulness of other cancer-related gene analyses in PPJ. One of the most common and widely distributed tumor suppressor genes, p53, has been found in 40–76% of pancreatic cancer tissues (3, 13–16). But no reports have focused on p53 in PPJ.

In this report, we analyzed p53 gene mutation of PPJ obtained by duodenal endoscopy from patients with PC and reported the usefulness of this method compared with analysis of K-ras mutations at codon 12.

MATERIALS AND METHODS

Human PC Cell Lines. To investigate the limit of detection of the p53 mutant band by PCR-SSCP analysis, we used two human pancreatic cancer cell lines, MIAPaCa2 and HPAF.

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2To whom requests for reprints should be addressed, at Department of Internal Medicine, Cancer Research Institute, Kanazawa University, 4-86, Yonezumi, Kanazawa 921-8044, Japan. Phone: 81-76-226-2510; Fax: 81-76-226-2539.
HPAF cells were obtained from the American Preservation Committee (Rockville, MD) and MIAPaCa-2 cells from the JCRB Cell Bank (Tokyo, Japan). Cells were cultured in DMEM containing 10% calf serum in a 37°C, 5% CO₂ incubator.

**PPJ and Cancer Tissues.** Twenty-six patients with PC, 4 with MPT that was histologically adenoma, and 16 with CP, who were diagnosed and treated at the Department of Internal Medicine at Kanazawa University Cancer Research Institute Hospital and affiliated hospitals from 1983 to April 1997 were studied. Before the examination, informed consent was obtained from all of the subjects. Among the patients with PC, 12 cases were diagnosed by a histopathological method applied to tissues obtained by operation or autopsy. In the other 14 cases, diagnosis was made by various imaging studies such as ultrasonography, endoultrasanography, computed tomography, and ERCJP. Tumor size, location, and clinical stage were classified according to the criteria in the general rules for clinical and pathological studies on cancer of pancreas (17). In all of four MPTs, the pathological diagnosis in the tissue specimens obtained at operation was adenoma. A diagnosis of CP was based on the pathological diagnosis in the tissue specimens obtained at operation or autopsy. In the other 14 cases, diagnosis was made by various imaging studies such as ultrasonography, computed tomography, and ERCJP. Among the patients with PC, 12 cases were all classified as definite on the basis of clinical diagnostic criteria listed above. PPJ was collected in the fasting state endoscopically through an inserted cannula from the orifice of the papilla of Vater under secretory stimulation by i.v. administration of secretin (1 unit/kg), using a duodenal endoscope (JF-10 or JF-230; Olympus, Tokyo, Japan) as described previously (4–8). DNA was prepared according to methods previously (4–8). DNA was prepared according to methods previously reported (6–8) from centrifuged sediment, extracted with a phenol/chloroform mixture and precipitated with ethanol. In cases 18, 21, 22, 23, and 25 in which PC tissues were obtained by operation or autopsy, only the cancerous parts were removed by microdissection from paraffin-embedded tissues, and DNA was extracted with the same methods and used as the template. Nonradioactive SSCP Analysis. PCR primers were designed for the flanking site of exons 5, 6, 7, and 8 of the p53 gene, in which point mutations have been reported frequently (13). The sequences of primers were as follows: exon 5 sense, TTC TTC TCG CAG TAC TC; antisense, CAG CTG TCT ACC ATC GCT AT; exon 6 sense, CAC TGA TCG TCT TTA GGT CT; antisense, AGT TGC AAA CCA GAC CTC AG; exon 7 sense, GTG TTA CCT ATC AGT GC; antisense, CAA GTG CCT CCT GAC CTG GA; exon 8 sense, CCT ATC CTG AGT AGT CCT A; and antisense, TCC TGC TTG CTT ACC TCG CT. PCR product size was 210 bp in exon 5, 144 bp in exon 6, 144 bp in exon 7, and 165 bp in exon 8. These oligonucleotides were synthesized by Cyanogen plus (Milligan/Biosearch, Milford, MA). PCR reaction was made with AmpliTaq polymerase (Perkin-Elmer Cetus, Foster, CA; 5 units), template DNA (100 ng), and primer (10 pmol), denatured at 95°C for 1 min, annealed at 52°C for 1 min, and extended at 73°C for 1 min, for 25 cycles in the thermal cycler. Reaction products were purified on a Centrisep spin column, and sequence analysis was performed with ABI 373A (Perkin-Elmer Cetus) at 2400 W for 14 h.

**PCR-RFLP for K-ras Mutation at Codon 12.** PCR-RFLP was performed as previously reported (7). Briefly, the template DNA and reaction mixture used were the same as for p53 analysis. For PCR, the modified primers were devised by Levi et al. (20): sense, 5′-ACT GAA ATT AAA CTG GTA GTT GGA CCT-3′; and antisense, 5′-TAA TAT GTC GAC TAA AAG AAT TAC CTC C-3′ flanking codon 12 of K-ras gene. Underlined bases represent mismatches from the K-ras DNA sequence. After PCR was performed, products were digested with restriction enzyme BsrNI and separated by agarose gel electrophoresis with 90 mm Tris-borate buffer (pH 8.3), containing 2.5 mm EDTA and visualized by ethidium bromide staining.

**PCR-HPA for K-ras Mutation at Codon 12.** The PCR-HPA which was first applied by us to detect K-ras mutation at codon 12 using an acridinium ester-labeled DNA probe, was performed as reported previously (8, 9). In brief, after the PCR reaction using the primers corresponding to the wild-type of codon 12 of K-ras gene, products were denatured at 100°C for 10 min, and 50 μl of acridinium ester labeled six probes, the sequences of which corresponded to each point mutation of codon 12 in K-ras gene, and were hybridized with PCR products at 65°C for 15 min. Subsequently, 250 μl of hydrolysis buffer consisting of Triton X and sodium borocatoate were added, and the mixture was incubated at 65°C for 5 min to eliminate the chemiluminescence of nonhybridized acridinium ester-labeled DNA probes. The samples were cooled in ice-water immediately, and their chemiluminescence was measured with a Leader 1 luminometer. The results were measured in relative light units.
RESULTS

p53 Analysis. To investigate the limit of detection of a p53 mutant band by PCR-SSCP, DNA samples from MIAPaCa2 (mutant in exon 7) were mixed with the wild-type DNA from HPAF (no mutant in exon 7) at constant ratios of the wild-type DNA and used as the template for PCR. The PCR products were subjected to nonradioactive SSCP. The mutant band was visible until 0.1% of the mutant in the wild-type as shown in Fig. 1. Among PPJ samples from 26 patients with PC, mutant bands were observed by the PCR-SSCP in 3 cases at exon 5, 2 cases at exon 6, 3 cases at exon 7, and 3 cases at exon 8 (Table 1). In total, 11 of 26 patients (42.3%) were found to be positive for the p53 mutations. On the other hand, in all samples from 4 patients with MPT (adenoma) and 16 patients with CP, mutant bands could not be seen on any exons by the PCR-SSCP (data not shown). Among the PPJ samples from 11 cases in which mutant bands were seen, direct sequencing studies were performed in only three cases in which PCR products could be sufficiently recovered and purified. Fig. 2 shows the DNA sequence of three cases, as follows: case 16, codon 156 of exon 5, CGC to CAC (His); case 21, codon 248 of exon 7, CGG to CCG (Pro); and case 25, codon 144 of exon 5, CAG to TAG (stop). In patients 16 and 25, the mutation pattern was G:C to A:T, and in patients 16 and 25, a codon 156 of exon 5, CAG (Glu)-TAG (stop). In patients 16 and 25, both were positive in 9 and both were negative in 7 of 26 patients, p53 alone was positive in 2 and K-ras alone in 12 patients. In 23 of 25 patients, p53 or K-ras was positive; therefore, the rate of genetic abnormality increased to 92.0% on combination analysis of both genes. By the method of PCR-HPA by which K-ras mutations were analyzed quantitatively, both were positive in 9 and both were negative in 7 of 26 patients. p53 alone was positive in 2 and K-ras alone in 8 patients. p53 or K-ras positivity was detected in 19 of 26 patients so that positivity improved to 73.1% upon their combination.

Case Presentation. A case (patient 16) of PC harboring p53 point mutation without K-ras mutation is presented. The case was a 70-year-old male who came to our hospital with a chief complaint of epigastralgia. No particular physiological finding was observed. On laboratory analysis, despite normal ductal enzymes and amylase, elastase-1, CA19-9, and carcinoembryonic antigen were mildly elevated. On ultrasonography and computed tomography, the pancreatic duct was dilated, and the pancreas head was slightly swollen (Fig. 3, a and b). On ERCP, pancreatic duct was stenotic at the pancreas head, and the distal pancreatic duct of the lesion was dilated (Fig. 3c). On endoutrasonography, low echoic mass was detected at the pancreas head (Fig. 3d). PPJ was aspirated endoscopically, and cytology was diagnosed as class II. K-ras mutation was negative by both the method of PCR-RFLP and HPA. On the other hand, p53 gene was mutated at codon 156 in exon 5. The mutation was CGC to CAC. From these findings, he was confidently diagnosed as having PC. Then, surgery was performed, and the diagnosis was confirmed. This case is thought to be a good example of the usefulness of p53 analysis.

DISCUSSION

According to a review of Greenblatt et al. (14), who compiled p53 mutations in various human tumors from >300 papers published, the frequency of p53 mutations of PC was 44%. The positive rate of p53 mutation in exons 5–8 of the DNA from endoscopically aspirated PPJ of patients with PC was 42.3% (11 of 26 cases) in the present study. This prevalence is no less than that in tissue samples of PC reported previously as above. Moreover, considering that the number of cancer cells dropped into the PPJ was very small in contrast with that in tissue sample, the sensitivity for p53 mutations detected in PPJ in this study appears sufficiently high. However, most of the reports published after 1994 demonstrated p53 mutations in
Detection of $p53$ Mutations in Pancreatic Juice

Table 1  
$p53$ gene and $K$-ras gene mutations in PPJ from patients with PC

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Location</th>
<th>Size (TS)</th>
<th>Stage</th>
<th>SSCP (exon)</th>
<th>Codon</th>
<th>Sequence</th>
<th>PCR-RFLP</th>
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<td>M</td>
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<td>Asp</td>
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<tr>
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<td>70</td>
<td>F</td>
<td>H</td>
<td>2</td>
<td>III</td>
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<td>+</td>
<td>Asp</td>
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<tr>
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<td>H</td>
<td>2</td>
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<td>Arg</td>
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<td>CGC$\rightarrow$CAC</td>
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<td>II</td>
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<td>H</td>
<td>3</td>
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<td>–</td>
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<td>B</td>
<td>4</td>
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<td>Asp</td>
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<td>Asp</td>
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<td>F</td>
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<td>2</td>
<td>IVb</td>
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<td>nd</td>
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$^a$ H, head; B, body; T, tail.

$^b$ TS1 $\leq$ 2.0 cm; 2.0 cm $<$ TS2 $\leq$ 4.0 cm; 4.0 cm $<$ TS3 $\leq$ 6.0 cm; 6.0 cm $<$ TS4.

$^c$, mutation; –, no mutation.

$^d$ Mutational analysis of $p53$ was performed not only in the PPJ but also in the pancreatic tumor tissue.

>50% of PC tissue (21). Furthermore, Rozenblum et al. (3), who analyzed $p53$ in exons 2–11 in first passage xenografts from 47 resected PCs, recently reported $p53$ mutation in 76% of cases. Because most early investigators have analyzed $p53$ chiefly in exons 5–8, which is highly conserved through evolution and presumably of functional importance, 95% of the reported mutations have been found in exons 5–8 (13). However, of 560 mutations in papers in which the entire coding region of $p53$ was sequenced, 87% were in exons 5–8, and most of the others were in exons 4 (8%) and 10 (4%; Ref. 14). Thus, evaluation of only exons 5–8 is likely to underestimate the prevalence of $p53$ mutations. Therefore, it might be possible to improve the sensitivity of detecting $p53$ mutations in PPJ by including exons 4 and 10 of $p53$ in the analysis when mutations are not found in exons 5–8.

A few researchers have already tried to analyze $p53$ mutations in the body fluid for genetic diagnosis. Mao et al. (22) found $p53$ mutations in 2 of 15 sputum samples from patients with lung cancer. Sidransky et al. (23) also reported that by the analysis of urine samples, $p53$ mutations could be detected in 11 of 15 patients with bladder cancer. Kondoh et al. (24) reported only one case showing $p53$ mutation positive in the PPJ from a PC patient. However, there have been no reports on the analysis of $p53$ mutations in any cases of PPJ for the purpose of diagnosing PC as in the present study.

Because the difficulty in obtaining biopsy specimens from patients with PC is a barrier to early and differential diagnosis, cytological examination has been tried to diagnose PC qualitatively with PPJ collected endoscopically. Although skillful cytologists have reported relatively high positive rates in PC patients, the accuracy of diagnosis for PC has not been satisfactory in general, because this procedure tends to be inaccurate due to cell injury and degradation induced by various proteases present in PPJ. Thus, this procedure has not come into wide use. Ishimaru et al. (25) reported that $p53$ immunocytocchemical staining of cells obtained by ERDPDB was positive in 82% of 44 patients with PC from stenotic or obstructive regions of the main pancreatic duct, and $p53$ mutations were detected in 86% of 14 patients who were positive for $p53$ immunocytochemistry. Although this method has the virtue of objectivity in judgment through immunocytocchemical staining, it is not clear whether $p53$-positive cells are specific to cancer, because it has been well known that some cells in noncancerous tissues can be stained with the anti-$p53$ protein antibodies (26). But, in any case, it seems reasonable to take advantage of both ERDPDB and PPJ analysis. That is, we should adopt ERDPDB for collecting cells when inserting a brush is possible and use endoscopic aspiration of PPJ by inserting a cannula into the pancreatic duct when it is not. In addition, it may be possible to enhance the positive rate for detecting $p53$ mutations by genetic analysis if the DNA is extracted selectively from $p53$ immunostained cells by the microdissection method or extracted from centrifuged sediments of whole PPJ in the negative cases immunostained for $p53$. In the present study, $p53$ mutations were negative in PPJ.
from four patients with adenoma exhibiting MPT, which differs in character from common pancreatic duct cancer, but is supposed to be a model of adenoma-carcinoma sequence of PC. The same result was reported on analysis of \( p53 \) mutations in tissue samples from pancreatic adenoma (27), but two cases with pancreatic adenoma showing \( p53 \) mutations were reported (28). Thus, it seems that \( p53 \) mutations may be very rare, if they occur at all, in the initial stage of pancreatic tumor. On the other hand, although significant differences between the positivity of \( p53 \) mutation and clinicopathological factors were not seen as shown in Table 2, the majority of patients had advanced disease. Therefore, additional studies in many cases with stage I tumor are needed to reveal how detection of \( p53 \) in PPJ can be used for the diagnosis of PC at early stage. No \( p53 \) mutations were reported to be detectable in any tissue samples from 15 patients with CP (29). In this study, no mutations of \( p53 \) were seen in any PPJ sample from 16 patients with CP. Thus, \( p53 \) mutations are considered to be highly specific for PC.

In general, the sensitivity of radioactive PCR-SSCP for \( p53 \) analysis has been demonstrated to be 3–5% of the mutant in the wild-type. In the present study, the mutant band could be detected in samples containing only 0.1% mutant using culture cell line DNA; therefore, the sensitivity of the nonradioactive PCR-SSCP method used in this study appears superior to that of the methods used previously. Among the PPJ samples from 11 patients in which mutant bands were seen on SSCP, direct sequencing was possible in only 3 cases in which PCR products could be sufficiently recovered and purified. Most of the PPJ samples in which direct sequence analysis was impossible were old ones stocked for up to 10 years. In five patients in whom we examined the PPJ samples for \( p53 \) mutation, mutational analysis for \( p53 \) of DNA extracted from PC tumors taken by microdissection was performed concurrently. Two showed the same mutant bands of mobility shift on SSCP analysis, with direct sequencing also having the same results in the PPJ samples. In the other three, no mutant bands were seen, and DNA samples exhibited wild-type sequence the same as in PPJ samples. In addition, for two tissue samples of MPT (adenoma), we added \( p53 \) analysis and confirmed the results of PPJ analyses as mutation negative. These results support that exfoliated pancreatic duct cancer cells harboring \( p53 \) mutation may actually flow into the PPJ. PCR error may have occurred as PCR cycles

### Table 2

| Relationship between \( p53 \) gene mutation in pancreatic juice and the size, clinical stage, and location of pancreatic cancers |
|---|---|---|---|---|
| No. of patients with \( p53 \) mutation<sup>a</sup> | + | – | Total |
| Size | | | |
| TS<sub>1</sub> | 0 | 1 | 1 |
| TS<sub>2</sub> | 4 | 5 | 9 |
| TS<sub>3</sub> | 5 | 5 | 10 |
| TS<sub>4</sub> | 2 | 4 | 6 |
| Stage | | | |
| I | 0 | 1 | 1 |
| II | 1 | 0 | 1 |
| III | 1 | 4 | 5 |
| IV | 9 | 10 | 19 |
| Location<sup>b</sup> | | | |
| H | 5 | 7 | 12 |
| B | 4 | 5 | 9 |
| T | 1 | 4 | 5 |

<sup>a</sup> +, mutation; –, no mutation.

<sup>b</sup> H, head; B, body; T, tail.

### Table 3

| Comparison between \( p53 \) and \( K-ras \) mutations in patients with PC |
|---|---|---|---|
| A. \( p53 \) with \( K-ras \) PCR-RFLP method |
| \( p53 \) | + | – | Total |
| \( K-ras \) | | | |
| + | 9 | 12 | 21 |
| – | 2 | 2 | 4 |
| Total | 11 | 14 | 25 |
| B. \( p53 \) with \( K-ras \) HPA method |
| \( p53 \) | + | – | Total |
| \( K-ras \) | | | |
| + | 9 | 8 | 17 |
| – | 2 | 7 | 9 |
| Total | 11 | 15 | 26 |
Detection of \( K\)-ras or mutant allele-specific amplification, suggesting that \( K\)-ras is
portrayed that the prevalence of \( K\)-ras marker (7). Yanagisawa
PCR-RFLP, raising questions as to the cancer specificity of this
eases, especially those determined by sensitive methods, such as
ples from chronic pancreatitis and other benign pancreatic dis-
mutations have been frequently (20–30%) detected in PPJ sam-
mutations in relation to the detection of \( K\)-ras mutation in PPJ
Watanabe et al. (7) and other investigators (10, 11) have re-
reported that the prevalence of \( K\)-ras mutations at codon 12 in PPJ
is >80% as analyzed by sensitive methods such as PCR-RFLP or
mutant allele-specific amplification, suggesting that \( K\)-ras
mutation is a promising potential marker of PC. However, \( K\)-ras
mutations have been frequently (20–30%) detected in PPJ samples
from chronic pancreatitis and other benign pancreatic dis-
eses, especially those determined by sensitive methods, such as
PCR-RFLP, raising questions as to the cancer specificity of this
marker (7). Yanagisawa et al. (12) and Caldas et al. (30)
reported that \( K\)-ras mutations were found in 63% (10 of 16) and
71% (5 of 7), respectively, of the selectively microdissected
specimens of benign mucous cell hyperplasia of the pancreatic
ductal epithelium with chronic inflammation. Therefore, false-
negatives of \( K\)-ras mutations in PPJ have become a serious
problem. However, hyperplastic foci harboring \( K\)-ras mutations
of CP are usually so small that the detection rate of \( K\)-ras
mutations is reduced among mostly normal epithelium cells in
PPJ. In contrast to CP, PC has a large number of cells harboring
\( K\)-ras mutation, exfoliating many mutated cells in the PPJ. In
contrast to CP, PC has a large number of cells harboring
\( K\)-ras mutation, exfoliating many mutated cells in the PPJ. Recently, we have developed the HPA using acridinium ester-
labeled DNA probes that can quantitatively determine \( K\)-ras
mutation in PPJ. We reported that quantitative measurement of
\( K\)-ras mutations in PPJ with this method was useful for differ-
entiating PC from CP using a suitable cutoff value like the
conventional serum tumor markers (8, 9). In the present study,
\( K\)-ras mutations were detected in 84.0% of PPJ from PC cases
(21 of 25) by PCR-RFLP with high sensitivity, and the analysis by
HPA with the advantage of high specificity demonstrated
\( K\)-ras mutations in 65.3% of PC cases (17 of 26). Furthermore,
combination assay for \( p53 \) and \( K\)-ras mutation revealed \( p53 \)
positivity alone in two patients by both methods, and the posi-
tive rate of genetic abnormalities was increased to 92.0% with
PCR-RFLP and 73.1% with HPA. These results suggest that
some cases exhibit \( p53 \) mutation without \( K\)-ras mutation, and
simultaneous analysis of \( p53 \) and \( K\)-ras mutation is useful for
enhancing the genetic diagnosis and screening for PC.

REFERENCES
1. Almoguera, C., Shibata, D., Forrester, K., Martin, J., Arnheim, N.,
and Peercho, M. Most human carcinomas of the exocrine pancreas
2. Smit, V. T. H. B. M., Boot, A. J. M., Fleuren, G. J., Cornelisse, C. J.,
and Bos, J. L. \( K\)-ras codon 12 mutations occur very frequently in
3. Rozenblum, E., Schutte, M., Goggins, M., Hahn, S. A., Panzer, S.,
Zahurak, M., Goodman, S. N., Sohn, T. A., Hruban, R. H., Yeo, C. J.,
and Kern, S. E. Tumor-suppressive pathway in pancreatic carcinoma.
4. Takemori, Y., Sawabu, N., Ohta, H., Satomura, Y., Watanabe, H.,
Yamakawa, O., Kidani, H., Takahashi, H., and Wakabayashi, T. Eval-
uation of cancer-associated carbohydrate antigen NCC-ST-439 meas-
urement in pure pancreatic juice collected by endoscopic aspiration.
5. Ohta, H., Sawabu, N., Takemori, Y., Kidani, H., Wakabayashi, T.,
Satomura, Y., Watanabe, H., Motoo, Y., Okai, T., and Takahashi, H.
Measurement of sialylated stage-specific embryonic antigen-1 in pure
pancreatic juice for the diagnosis of pancreatic cancer. Int. J. Pancreatol.,
6. Watanabe, H., Sawabu, N., Ohta, H., Satomura, Y., Yamakawa, O.,
Motoo, Y., Okai, T., Takahashi, H., and Wakabayashi, T. Identification of
\( K\)-ras oncogene mutations in pure pancreatic juice of patients with


Detection of Mutations of \textit{p53} Tumor Suppressor Gene in Pancreatic Juice and Its Application to Diagnosis of Patients with Pancreatic Cancer: Comparison with K-\textit{ras} Mutation

Yasushi Yamaguchi, Hiroyuki Watanabe, Songür Yrdiran, et al.


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