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p53 Mutations in Two Patients with Intraductal Papillary Adenoma of the Pancreas

Miyuki Kaino,[1] Satoshi Kondoh,[1] Satoshi Okita,[1] Syomei Ryozawa,[1] Satoko Hatano,[1] Kei Shiraishi,[1] Seiji Kaino,[1] Tetsuji Akiyama,[1] Kiwamu Okita[1] and Toyokazu Kawano[2]

[1]First Department of Internal Medicine, Yamaguchi University School of Medicine, 1144 Kogushi, Ube, Yamaguchi 755 and [2]Hofu Institute of Gastroenterology, 14-33 Ekinan-cho, Hofu, Yamaguchi 747

There has been no report on p53 gene mutation in benign human pancreatic intraductal tumors. We examined pancreatic juice and tissue specimens from two patients with intraductal papillary adenoma of the pancreas by polymerase chain reaction-single-strand conformation polymorphism analysis and direct sequencing and found point mutations of p53 gene resulting in amino acid substitutions in exons 6 and 8. Thus, p53 gene mutation may be an early event in the neoplastic process of some pancreatic intraductal tumors and may play an important role in tumorigenesis.

Key words: p53 — Mutation — Pancreas — Intraductal adenoma — Tumorigenesis

Inactivation of the p53 gene is a frequent event in a variety of human cancers.[1)] In ordinary pancreatic ductal cell carcinoma, the incidence of p53 aberrations in surgical specimens ranges from 30 to 60%.[2–6)] However, the status of p53 in pancreatic intraductal tumors is not established. We have recently encountered two patients with intraductal adenoma of the pancreas, and took the opportunity to examine the p53 gene in this relatively differentiated tumor by the single-strand conformation polymorphism (SSCP) method and direct sequencing.

One patient was a 78-year-old woman (case 1), and the other was a 65-year-old man (case 2). The tumor sizes were 20 mm and 30 mm, respectively, and both were located in a branch of the pancreatic duct. Pancreatic juice was collected through a cannula into the papilla of Vater by manual suction without secretory stimulation. Samples were centrifuged at 12,000 rpm and the supernatant was discarded. The pellet was fixed with 99.5% ethanol and stored at −20°C until genomic DNA was extracted. Surgical resection specimens from both patients were fixed with 20% formalin and embedded in paraffin. Histologic diagnoses were made on the basis of examination of hematoxylin and eosin-stained specimens. The paraffin blocks were cut into 10 μm sections and the neoplastic tissue was dissected out under light microscopic observation. Genomic DNA was prepared from both pancreatic juice and fragments of the surgical specimen from each patient. Samples underwent treatment with proteinase K and phenol-chloroform extraction, followed by ethanol precipitation.

Four pairs of primers were synthesized according to Murakami *et al.*[7)] for exons 5 to 8 of the p53 gene and labeled with adenosine 5′-[γ-^{32}P]triphosphate using a MEGALABEL kit (TaKaRa, Tokyo). Polymerase chain reaction (PCR) was carried out for each exon and one microliter of the PCR product was diluted in 100 μl of a solution consisting of 96% formamide, 0.1% SDS, 0.04% xylene cyanol, 0.04% bromophenol blue and 20 m*M* EDTA. This solution was denatured at 95°C for 5 min and rapidly chilled on ice, then applied to a 5% nondenaturing polyacrylamide gel containing 5% glycerol. Electrophoresis was performed at 40 watts for 3 to 4 h. The gel was dried and exposed to autoradiographic film with an intensifying screen at −80°C for 1 to 2 days.

Subsequently, genomic DNA was extracted from the PCR-SSCP polyacrylamide gel bands that differed from those of the normal control. The DNA fragments underwent PCR amplification by the method described, and direct sequencing was performed by the dideoxy termination method using a CircumVent Thermal Cycle Dideoxy DNA Sequencing kit (Biolabs, New England, MA). The primer sequences for each exon are as follows: 5′-ACCATGAGCGCTGCTCAGAT-3′ (forward) for exon 6; 5′-CCTATCCTGAGTAGTGGTAA-3′ (forward) for exon 8. The sequencing primers were labeled with adenosine 5′-[γ-^{32}P]triphosphate as noted above. The products were denatured at 95°C for 5 min and applied to a 6% polyacrylamide gel containing 7 *M* urea. Electrophoresis was performed at 40 watts for 2 to 6 h. The gel was dried and exposed to autoradiographic film with an intensifying screen at −80°C for a day.

Both neoplastic lesions had the typical histologic appearance of intraductal papillary adenomas (Fig. 1, A and B). PCR-SSCP analysis of exon 6 from pancreatic juice of case 1 demonstrated a mobility shift as compared to the control, indicative of a mutation (Fig. 2A). Analysis of exon 8 from that of case 2 also demonstrated a specific mobility shift (Fig. 2B). Direct DNA sequencing

[1] To whom correspondence should be addressed.

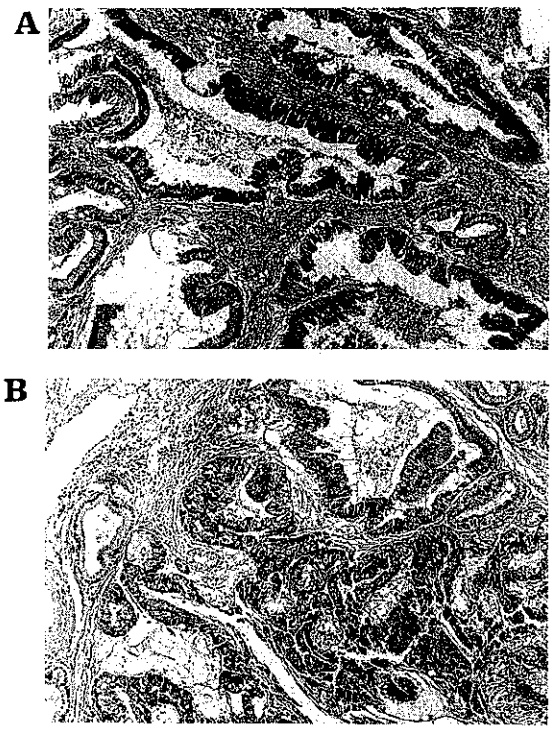

Fig. 1. Low-power view of the intraductal papillary adenomas of the pancreas from cases 1 (A) and 2 (B) (hematoxylin and eosin, ×20).

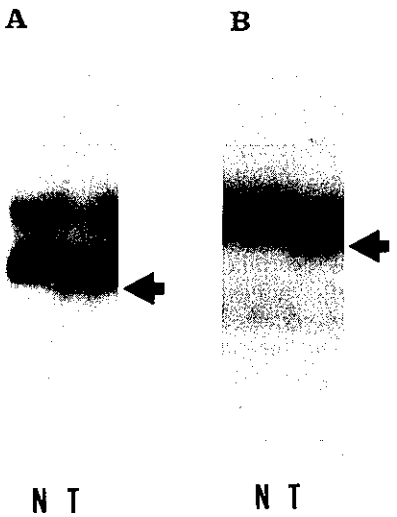

Fig. 2. Polymerase chain reaction-single-strand conformation polymorphism analysis of the p53 gene in pancreatic juice from the patients with intraductal papillary adenomas of the pancreas. Case 1 shows a mobility shift in exon 6 (A), and case 2 shows that in exon 8 (B). N, normal DNA; T, tumor DNA.

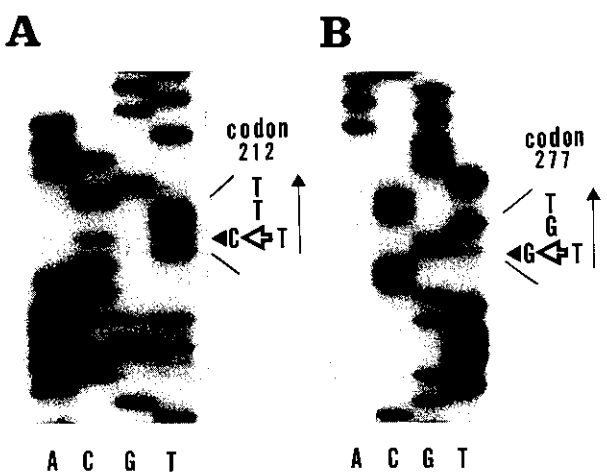

Fig. 3. Amplified DNA from the PCR-SSCP polyacrylamide gel bands was sequenced, and point mutations in the p53 gene were demonstrated at codon 212 in case 1 (A) and codon 277 in case 2 (B). These mutations resulted in substitutions of Phe (TTT) to Leu (CTT) (A) and Cys (TGT) to Gly (GGT) (B).

confirmed the presence of base changes in both cases. Case 1 had a T-to-C point mutation at codon 212, and case 2 had a T-to-G point mutation at codon 277, both resulting in amino acid substitutions (Fig. 3, A and B). The results of PCR-SSCP and sequencing of genomic DNA from surgical specimens were the same as those for pancreatic juice in each patient (data not shown).

The majority of intraductal papillary adenomas and adenocarcinomas of the pancreas are known to correspond to "mucin-producing pancreatic tumors," which have unique features such as mucin hypersecretion, cystic dilatation of the pancreatic ducts, and a benign course after surgical treatment, and which are distinct from ordinary pancreatic ductal cell carcinomas. The number of reports of mucin-producing tumors of the pancreas in Japan has been increasing since the initial report of Ohhashi *et al.* in 1982.[8] However, few investigators have undertaken genetic analysis of these tumors or the pancreatic juice from affected patients.

It is known that the p53 tumor suppressor gene, located on the short arm of chromosome 17, plays an important role in normal cell growth and differentiation. It is frequently inactivated by point mutations and allelic deletions in diverse human cancers, including pancreatic carcinoma.[9–11] Little is known about the association between p53 gene inactivation and the histopathological factors of human pancreatic ductal cell carcinomas. However, recently it has been reported that there is a signifi-

cant association of the presence of these mutations not with histologic type, extent of tumor invasion, or tumor stage, but with poor prognosis.[6] Therefore, we expected that p53 gene mutation might occur at a relatively early stage in some types of pancreatic carcinomas. It has been shown that a p53 mutation can occur at the earliest clinically detectable stage of the neoplastic process in some types of cancer. Nuclear immunostaining of p53 has been observed in mild dysplasias of the esophagus, breast, bronchus and larynx.[13] p53 overexpression has been found in serrated adenomas of the colon with low-grade dysplasia and the commitment of these histologically mild lesions to independent growth was shown.[14]

To date, there has been no report of the presence of any aberration of the p53 gene in the benign variant of human pancreatic tumors, and it has been considered that p53 gene mutations are found only in carcinomas. The regions of the p53 gene we analyzed are highly conserved in different species and include the four 'hot-spots' in which mutations are clustered in diverse human cancers.[10] In this investigation, p53 mutations were detected by PCR-SSCP analysis in both pancreatic juice and surgical specimens from patients with pancreatic intraductal adenoma for the first time. Samples that showed band shifts by PCR-SSCP were analyzed by direct sequencing, and point mutations were demonstrated. There has been one previous study that examined the p53 gene in mucin-producing pancreatic tumors by constant denaturant gel electrophoresis, but no p53 mutation was found in microdissected tumor specimens from six intraductal papillary adenomas.[12] The difference between their result and ours may be due to the methodological difference or the small number of examined samples. Further study is needed to clarify the role of p53 mutation in these tumors.

It has been well documented that mutations in codon 12 of the K-*ras* gene occur in a high proportion of pancreatic cancers.[15] Other studies have demonstrated that the activation of K-*ras* and inactivation of p53 are cooperatively associated in carcinogenesis.[16–19] The samples that we investigated from our two patients with intraductal adenoma have G-to-A point mutations at codon 12 of the K-*ras* oncogene (data not shown), and our results suggest that mutation of both genes may be needed for the development of these tumors, at least in some cases.

In this study, the presence of p53 gene mutations was identified preoperatively by the examination of tumor cells collected from pancreatic juice. Most intraductal papillary tumors are hypersecretors of mucin, and large quantities of pancreatic juice can be easily collected endoscopically from patients. Therefore, the genetic investigation of sloughed tumor cells in pancreatic juice may be clinically useful in addition to routine cytological examination.

In conclusion, we have demonstrated the presence of p53 gene mutations in two patients with intraductal papillary adenomas of the pancreas. Our data suggest that p53 mutation may occur at an early stage of the neoplastic process in at least some pancreatic intraductal tumors and may play an important role in pancreatic tumorigenesis.

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