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Frequent Somatic Mutations of the *APC* and *p53* Genes in Sporadic Ampullary Carcinomas

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Although a close relation of somatic mutations of the adenomatous polyposis coli gene with ampullary carcinomas in familial adenomatous polyposis patients has been reported, the possible association with sporadic ampullary neoplasms has not been fully examined. We have therefore investigated loss of heterozygosity at the adenomatous polyposis coli locus and the mutational status of a portion of the adenomatous polyposis coli gene, including the mutation cluster region, in 17 ampullary carcinomas of non-familial adenomatous polyposis patients. Alteration of the adenomatous polyposis coli gene was found in 8 of 17 (47.1%) cases, as missense or insertion mutations, with or without loss of heterozygosity. Additional investigation of *p53* (exons 5–8) and K-*ras* (codons 12 and 13) gene mutations revealed a striking mutational pattern of the *p53* gene. Nine of the 17 cases demonstrated a total of 12 mutations, 6 clustered at codon 189 and 3 at codon 166. Furthermore, 5 of the 12 mutations were nonsense mutations. Regarding the K-*ras* gene, 4 of the 17 (23.5%) cases had mutations in codon 12, 3 of the 4 cases being derived from the intraduodenal bile duct. The findings indicate that alterations of the adenomatous polyposis coli and the *p53* genes are relatively frequent in sporadic ampullary carcinomas. In particular, the clustering at specific *p53* codons might offer an etiological clue to clarify ampullary carcinogenesis. Mutations of the K-*ras* gene, on the other hand, might be characteristic of intraduodenal bile duct origin.

Key words: Ampullary carcinoma — *APC* — *p53*

Ampullary neoplasia is relatively rare, representing 5% of all gastrointestinal tumors.[1)] It presents as benign (5%) and malignant (95%) lesions located in the region of the papilla of Vater.[1, 2)] Because of the high cure rate and good prognosis, in contrast to tumors arising from periampullary structures such as pancreas, duodenum and extraduodenal bile duct, its biological characteristics as well as clinicopathological features are of particular interest. Tumorigenesis of ampullary carcinoma has not yet been fully elucidated at the molecular level.[3–5)] Although sporadic cases are relatively rare, periampullary neoplasia, including carcinoma of the ampulla of Vater, is the most important extracolic manifestation and a major cause of death in patients with familial adenomatous polyposis (FAP).[6)] Periampullary carcinoma occurs in 2.9 to 12% of all FAP patients with an observed/expected mortality ratio for periampullary and small intestinal carcinoma of 250 (95% confidence interval of 112–447, $P<0.001$) in 1050 FAP patients registered at the Research Center for Polyposis and Intestinal Disease.[7, 8)] Ampullary neoplasia in FAP patients has been proven to be frequently associated with biallelic adenomatous polyposis coli (*APC*) gene mutations, without aberrations of the *p53* or K-*ras* genes.[8)] It might therefore be suspected that genetic alteration of the *APC* gene also plays a role in the genesis of sporadic ampullary carcinomas.

The present study was conducted to elucidate the comparative *APC* gene alterations in ampullary neoplasms between FAP and non-FAP patients in Japan, and to clarify the possible role of *APC* gene alterations in sporadic ampullary carcinogenesis. Furthermore, based on the combined *p53* and K-*ras* gene mutational state, we will discuss the mechanism of development and progression of sporadic ampullary carcinoma with special reference to the anatomical characteristics of the ampulla of Vater.

MATERIALS AND METHODS

Materials A total of 17 cases of ampullary carcinoma, 5 obtained at autopsy in the Department of Pathology, University of Tokyo, from 1967 to 1995 (from cases 1–5) and 12 surgically resected at Tokyo Kousei Nenkin Hospital from 1978 to 1992 (from cases 6–17), were studied. Clinicopathological classification and stage grouping were based on General Rules for Surgical and Pathological Studies on Cancer of Biliary Tract (The 3rd edition)[9)] by the Japanese Society of Biliary Surgery. The patients were 12 males and 5 females, ranging from 51 to 85 years

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Table I. Clinicopathological Features for Ampullary Carcinoma Patients

Case	Age	Sex	Size (mm)	Macroscopy	Histology	Direct invasion		Metastasis		Stage	Origin
						pancreas	duodenum	liver	lymph node		
1	79	F	20×15×15	protruding	pap (W/D)	+	+	+	+	IV	common
2	55	F	30×30	protruding	tub (W/D)	+	+	+	+	IV	common
3	76	F	15×15	protruding	tub (P/D)	+	+	+	+	IV	common
4	80	M	30×20×20	ulcerated	tub (M/D)	+	+	+	+	IV	common
5	68	F	30×22×17	protruding	tub (W/D)	−	−	−	−	I	common
6	58	M	10×5	superficial	pap (W/D)	−	−	−	−	I	duo
7	73	M	15×8×8	ulcerated	pap (M/D)	+	+	−	+	III	bile
8	72	M	25×10×8	protruding	tub (W/D)	+	−	−	−	III	bile
9	51	M	13×6	ulcerated	pap (W/D)	+	+	−	−	III	bile
10	75	F	20×10	protruding	pap (W/D)	−	+	−	−	II	common
11	85	M	20×15×7	protruding	sig	−	+	−	−	II	common
12	76	M	11×6	protruding	tub (W/D)	−	−	−	−	I	common
13	72	M	7×6×3	protruding	tub (W/D)	−	+	−	−	III	common
14	63	M	8×6×3	protruding	tub (W/D)	+	+	−	+	III	common
15	67	M	30×20	ulcerated	pap (W/D)	−	+	−	−	II	common
16	52	M	43×33	ulcerated	pap (W/D)	+	+	−	−	II	common
17	70	M	30×20×14	protruding	pap (W/D)	−	+	−	−	II	common

duo and bile, origin from surface duodenal mucosa and intraduodenal bile duct, respectively; M, male; F, female; pap, papillary; tub, tubular; sig, signet ring cell; W/D, well differentiated; M/D, moderately differentiated; P/D, poorly differentiated. Case 17 contains small adenomatous foci. Clinicopathological classification and stage grouping are based on General Rules for Surgical and Pathological Studies on Cancer of Biliary Tract (The 3rd edition) by the Japanese Society of Biliary Surgery.

old (mean, 68.9). All but one (case 6) were advanced cancers, infiltrating beyond the Oddi's sphincter. Microscopically 13 cases were well differentiated, 2 cases moderately differentiated and 2 cases poorly differentiated adenocarcinomas. The entire lesions were formalin-fixed and paraffin-embedded for conventional histological studies. The clinicopathological and detailed pathological features of the cases are summarized in Table I.

DNA extraction Genomic DNA samples of tumor and surrounding normal tissues were extracted from 15-μm-thick sections cut from paraffin-embedded materials as described previously.[10] On the basis of histopathological examination, tumor tissues, where at least 60% of microscopical fields were occupied by neoplastic cells, were excised. Tumor tissues where enrichment of neoplastic cells appeared to be less than 50% were excluded to minimize non-tumor cell contamination. One well differentiated adenocarcinoma (case 17) contained small adenomatous foci which were too small to allow separation from the carcinomatous component. The excised tissues were deparaffinized in xylene, proteinase K-digested and phenol-chloroform-extracted. Then the genomic DNA was ethanol-precipitated, dried and finally dissolved in TE buffer (10 m*M* Tris-HCl (pH 8.0), 1 m*M* EDTA).

Polymerase chain reaction (PCR) Because approximately 60–80% of the somatic mutations of colorectal tumors are reported to be clustered within a small portion of exon 15, this region (codons 1286–1513), designated as the mutation cluster region (MCR), was first examined (designated as *APC* segments 1–12, in the present study).[11] Then, the additional 195-base region towards the 3′ end (codons 1514–1578) where frequent somatic mutations were noted in periampullary carcinomas in FAP patients was examined (*APC* segment 13).[8] The *p53* gene (exons 5–8, where >95% of mutations are clustered) and the K-*ras* gene (exon 1) were also analyzed in all cases. PCR primers for each region are listed in Table II. The PCR reaction, performed in a total volume of 25 μl with 2.5 μl of 10× Taq DNA polymerase buffer (100 m*M* Tris-HCl (pH 8.3), 500 m*M* KCl, 15 m*M* $MgCl_2$, 0.01% gelatin (w/v)), 4 μl of 2.5 m*M* dNTP, 0.75 μl of each primer (20 μM), 1.5 units of Taq DNA polymerase and 0.5 μg of template DNA, consisted of 40 cycles of 1 min at 92°C, 1 min at 52°C and 2 min at 72°C.

DNA sequencing PCR products were subcloned into pBluescript SK(−) (Stratagene, La Jolla, CA) with a mixture containing at least 50 subclones being used as a template for DNA sequencing, as described elsewhere.[12] The PCR primers were also used as the sequencing primers. When mutations of specific genes were observed, the whole procedure including PCR, subcloning and sequencing was repeated again to confirm the original results and to exclude misincorporation by Taq DNA polymerase and sequencing artifacts. Then, DNA derived from the corresponding normal tissue was subjected to the same analysis to confirm the lack of germ line mutations such as FAP or Li-Fraumeni syndromes.

Table II. Primers for PCR Amplification

Gene	Region	Sense	Antisense
APC	Seg. 1	5′-GACTTATTGTGTAGAAGATACT-3′	5′-TTTTCTTTTATTTCTGCTATTTG-3′ (5′-GCAGGGTATTAGCAGAATCT-3′)
	Seg. 2	5′-GAAATAGGATGTAATCAGACG-3′ (5′-AGGATGTAATCAGACGACACA-3′)	5′-TGGTTCTAGGGTGCTGTGA-3′
	Seg. 3	5′-TCAGCTGAAGATCCTGTGAGC-3′ (5′-GAAGATCCTGTGAGCGAAGT-3′)	5′-CTTTGTGCCTGGCTGATTC-3′
	Seg. 4	5′-AGCAGACTGCAGGGTTCTA-3′ (5′-ACTGCAGGGTTCTAGTTTAT-3′)	5′-GAGTGGGGTCTCCTGAAC-3′
	Seg. 5	5′-CCAAAAGTGGTGCTCAGAC-3′	5′-ATGGTTCACTCTGAACGGA-3′
	Seg. 6	5′-TCTGTCAGTTCACTTGATAGT-3′ (5′-CAGTTCACTTGATAGTTTTGA-3′)	5′-GGTTTGTCCAGGGCTATCT-3′
	Seg. 7	5′-GGTAAGTGGCATTATAAGCC-3′	5′-AGGTACTTCTCGCTTGGTTT-3′
	Seg. 8	5′-CCATGCCACCAGCAGAAG-3′ (5′-CCACCAAGCAGAAGTAAAA-3′)	5′-TCTTTTCAGCAGTAGGTGCT-3′
	Seg. 9	5′-TCCATGAAGAGCGAACCAAA-3′	5′-TATCAGCATCTGGAAGAACC-3′
	Seg. 10	5′-AGCACCTACTGCTGAAAAGA-3′ (5′-ACCTACTGCTGAAAAGAGAG-3′)	5′-AAATCCATCTGGAGTACTTTC-3′
	Seg. 11	5′-GGTTCTTCCAGATGCTGATA-3′ (5′-TCTTCCAGATGCTGATACTT-3′)	5′-AGGCATTATTCTTAATTCCACA-3′
	Seg. 12	5′-CCTGAGTGCTCTGAGCCT-3′	5′-CTCTTGGTTTTCATTTGATTCT-3′
	Seg. 13	5′-AAATGACAATGGGAATGAAAC-3′ (5′-GGCATGGCAGAAATAATACAT-3′)	5′-TTGGCATGGCAGAAATAATAC-3′
APC	exon 11	5′-GGACTACAGGCCATTGCAGAA-3′	5′-GGCTACATCTCCAAAAGTCAA-3′
p53	exon 5	5′-TTCAACTCTGTCTCCTTCCT-3′ (5′-CAGTACTCCCCTGCCCTCAA-3′)	5′-CAGCCCTGTCGTCTCTCCAG-3′
	exon 6	5′-TGGTTGCCCAGGGTCCCCAG-3′ (5′-GGCCTCTGATTCCTCACTGA-3′)	5′-TTAACCCCTCCTCCCAGAGA-3′
	exon 7	5′-ACTGGCCTCATCTTGGGCCT-3′	5′-TGTGCAGGGTGGCAAGTGGC-3′ (5′-TGCAGGGTGGCAAGTGGCTC-3′)
	exon 8	5′-TTCCTTACTGCCTCTTGCTT-3′ (5′-CCTATCCTGAGTAGTGGTAA-3′)	5′-AGGCATAACTGCACCCTTGG-3′
K-*ras*	exon 1	5′-TTTTTATTATAAGGCCTGCT-3′	5′-CATATTCGTCCACAAAATGA-3′ (5′-GTCCACAAAATGATTCTGAA-3′)

Primers for nested PCR are shown in parentheses.

Mutant allele specific amplification (MASA) of the *APC* gene To strictly exclude artifactually induced mutations resulting from semi-nested PCR, we confirmed *APC* gene mutations, nearly 70% of which so far reported in tumors were insertion or deletion mutations, by means of the MASA method. PCR sense primers (17 mer) were designed to have the corresponding point mutation to each mutational case at the 3′ end. In addition, primers were so designed as to produce approximately 100-base-pair products and antisense primers (20 mer) were radiolabeled. PCR conditions were the same as described above except for an annealing temperature of 55°C. DNAs from both non-tumor and tumor portions of cases which showed point mutation of the *APC* gene were subjected to PCR simultaneously, then the products were electrophoresed on 0.8% agarose gels, transferred to nylon membranes, and fixed by UV light, and the membrane was exposed to X-ray films.

Loss of heterozygosity (LOH) analysis of the *APC* gene *Rsa* I polymorphism at exon 11 of the *APC* gene was analyzed.[13] PCR was carried out under the same conditions as described above, using the set of primers listed in Table II. The 133-bp PCR products were ethanol-precipitated, air-dried and resuspended in 10 μl of TE. Two μl aliquots were then digested with 20 units of *Rsa* I (Toyobo, Osaka) in a total volume of 20 μl, fractionated and visualized in 1.5% agarose gels containing ethidium bromide under UV light.

RESULTS

Histological findings Fifteen cases were well differentiated, 2 cases moderately differentiated and 2 cases poorly differentiated adenocarcinomas. Detailed histological examination revealed 1 case (case 6) to have originated from the duodenal mucosa and 3 cases (cases 7, 8 and 9)

from the intraduodenal bile duct. Identification of the primary site as the intraduodenal bile duct was done on the basis of the locus of the major part of the tumor. The others were considered to have been derived from the common channel of the ampulla. One well differentiated adenocarcinoma (case 17) contained small adenomatous foci.

Alterations of the *APC* gene The 133-bp PCR products at exon 11 of the *APC* gene consist of two types, one having an *Rsa* I restriction enzyme site and divided into two cut bands, 85 bp and 48 bp, and the other without the site. When PCR products from DNA of the tumor portion yielded only one type, despite the formation of two types of PCR products from DNA of the non-tumor portion, LOH was concluded to be present (Fig. 1). In addition, a mutated allele without the wild type on mutational analysis of exon 15 was also thought to reveal LOH. Five of 17 cases (29.4%) (cases 1, 2, 4, 6 and 17) demonstrated LOH at the *APC* locus, of which 3 also had mutations in the remaining allele (cases 2, 6 and 17). Without evidence of LOH, 3 cases carried a mutated allele (cases 5, 7 and 8) and one showed double mutations (case 16). The alterations were 5 point mutations resulting in amino acid substitution, 1 point mutation without amino acid substitution and 2 insertions causing nonsense mutation just downstream of the mutated codons (Fig. 2). *APC* gene mutations were not observed in any of the corresponding normal tissues (one DNA sample from a non-tumor tissue per one case) in the above-mentioned cases, thus excluding germ line mutation. Mutations were confirmed by repeating the investigation procedure twice and, especially for base substitution cases, the MASA method without nested PCR was further performed. After a single hot PCR using mutation-specific primers, amplifications were noted only in tumor samples, but not in corresponding non-tumor samples. In total, 8 of 17 cases (47.1%) demonstrated alterations of the *APC* gene. The results are summarized in Table III.

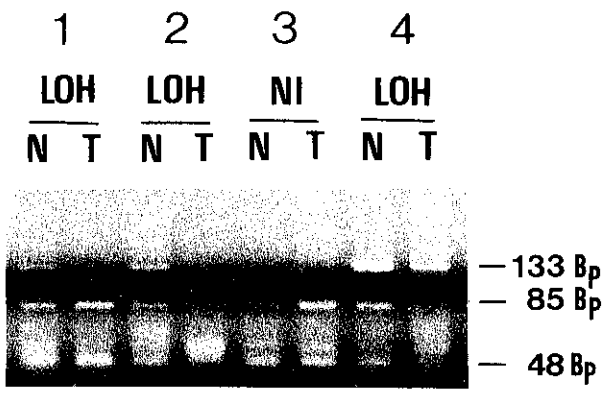

Fig. 1. LOH analysis of *APC* exon 11. Cases 1, 2 and 4 showed LOH for the *APC* gene. NI, not informative; N, normal; T, tumor portion. Numbers (top), case numbers.

Mutations of the *p53* and the K-*ras* genes Nine of the 17 ampullary carcinoma cases were found to have a total of 12 mutations in exons 5, 6 and 8 of the *p53* gene. All of

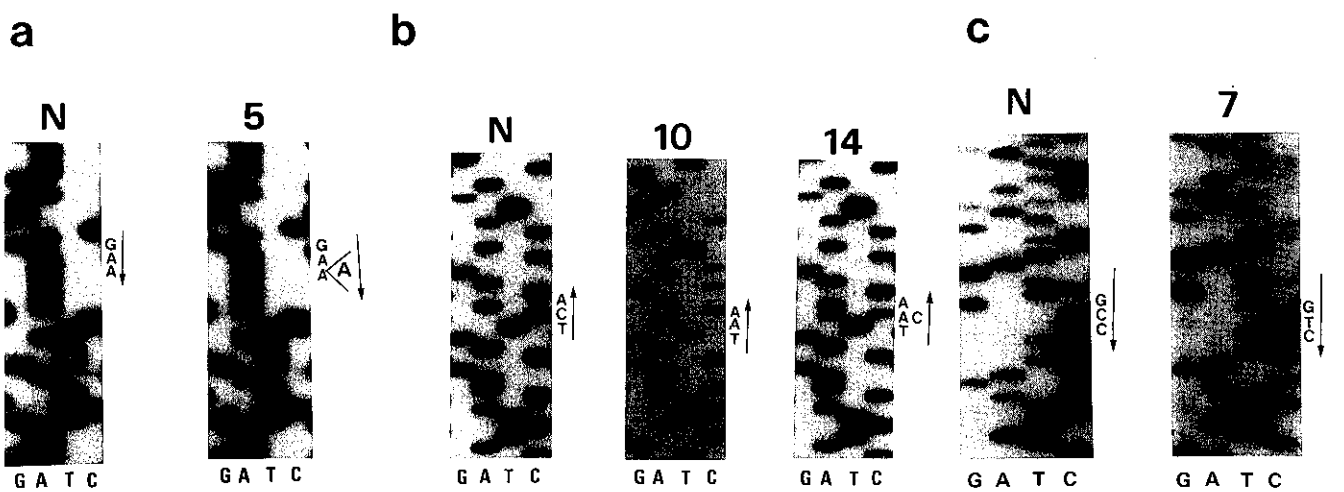

Fig. 2. Sequencing analyses of the *APC* and *p53* genes. Tumor portion of case 5 shows a GAA-to-GAAA insertion mutation at codon 1554 of the *APC* gene (a); cases 10 and 14 contain a TCA-to-TAA nonsense mutation at codon 166 of the *p53* gene (b); case 7 has a GCC-to-GTC missense mutation at codon 189 of the *p53* gene (c). N, normal; 5, 7, 10, and 14, tumorous portions of cases 5, 7, 10, and 14, respectively.

Table III. Somatic Mutations and LOH of the *APC* Gene

Case	Codon	*APC* mutations		LOH	
		Nucleotide change	Amino acid change	exon 11	exon 15
1	WT			LOH	NI
2	1314	AGG to AAG	Arg to Lys	LOH	LOH
3	WT			NI	NI
4	WT			LOH	NI
5	1554	GAA to GAAA/GAA	A insertion	NI	HET
6	1306	GAA to AAA	Glu to Lys	HET	LOH
7	1375	CAC to TAC/CAC	His to Tyr	NI	HET
8	1374	GAA to GAG/GAA	no change	NI	NI
9	WT			HET	NI
10	WT			HET	NI
11	WT			HET	NI
12	WT			NI	NI
13	WT			HET	NI
14	WT			HET	NI
15	WT			NI	NI
16	1374	GAA to AAA/GAA	Glu to Lys	NI	HET
	1499	GGA to AGA/GGA	Gly to Thr		
17	1554	GAA to GAAA	A insertion	NI	LOH

WT, wild type; LOH, loss of heterozygosity; HET, retained heterozygosity; NI, not informative.

Table IV. Somatic Mutations of the *p53* and K-*ras* Genes

Case	*p53* mutations		K-*ras* mutations	
	Codon	Nucleotide change (effect)	Codon	Nucleotide change (effect)
1	WT			
2	WT			
3	WT			
4	WT			
5	WT			
6	146	TGG to TAG/TGG (Try to Stop)		
	189	GCC to GTC/GCC (Ala to Val)		
7	189	GCC to GTC (Ala to Val)	12	GGT to GAT/GGT (Gly to Asp)
8	WT		12	GGT to GAT/GGT (Gly to Asp)
9	WT		12	GGT to GAT/GGT (Gly to Asp)
10	166	TCA to TAA (Ser to Stop)		
	189	GCC to GTC (Ala to Val)		
11	WT			
12	189	GCC to GTC (Ala to Val)		
13	189	GCC to GTC (Ala to Val)		
	292	AAA to AAG (silent)		
14	166	TCA to TAA/TCA (Ser to Stop)		
15	166	TCA to TAA/TCA (Ser to Stop)		
16	189	GCC to GTC/GCC (Ala to Val)	12	GGT to GTT/GGT (Gly to Val)
17	213	CGA to TGA (Arg to Stop)		

WT, wild type.

the *p53* gene mutations were point mutations, 6 being missense mutations at codon 189 (GCC to GTC) substituting Val for Ala, 5, nonsense mutations at codons 146 (TGG to TAG), 166 (TCA to TAA, 3 cases) and 213 (CGA to TGA), and 1, a silent mutation at codon 292 (AAA to AAG) (Fig. 2). Out of the 12 missense mutations, 7 were C-to-T, 1 a G-to-A, and 1 an A-to-G transition mutations, and 3 were C-to-A transversion mutations. With regard to the K-*ras* gene, 4 cases were found to have mutations, all being missense mutations at

codon 12. Three cases were GGT-to-GAT (Gly-to-Asp) transition mutations (case 7, 8 and 9) and 1 case was a GGT-to-GTT (Gly-to-Val) transversion mutation (case 16). Those mutations were not found in any of the corresponding normal-appearing tissues. Mutations were confirmed by repeating the investigation procedure twice. The results are summarized in Table IV.

DISCUSSION

The present study showed that, in line with expectations based on the frequent occurrence of periampullary carcinomas, including carcinomas of the papilla of Vater, in FAP patients, the pathogenesis of sporadic ampullary carcinomas is sometimes related to *APC* gene alteration. In the case of ampullary neoplasms in FAP patients, Toyooka *et al.*[8] earlier examined mutations of the *APC* gene in 11 ampullary neoplasms (2 carcinomas and 9 adenomas) in FAP cases and found 7 with the biallelic mutated *APC* gene (1 carcinoma and 6 adenoma cases). All the somatic mutations were clustered at codons 1554–1556 (poly A minimicrosatellite region), with A or G insertions causing frameshifts and stop-codons in the remaining allele. On the other hand, the findings of alterations of the *APC* gene in 8 of 17 cases (47%) in the present investigation are the first relating to sporadic lesions in Japanese patients. The lack of mutations in corresponding normal tissues and the absence of a family history of polyposis coli indicated a somatic nature for the observed changes. The analyzed region of the *APC* gene covers only approximately 10% of the entire coding region, although the MCR, in which approximately 70% of mutations were detected in previous studies,[11, 14, 15] was included. Therefore, it is possible that there might also have been mutations in the remaining region of the *APC* gene in cases 1 and 4, which showed LOH without detectable mutations. Thus, the true incidence of mutation might be much higher than that observed. During the preparation of this manuscript, an *APC* gene analysis of sporadic ampullary carcinomas performed in an Italian institute was reported.[5] They observed *APC* gene mutation only in 3 of 18 cases (16.7%, two with LOH at 5p21 and one without LOH), while 5q21 LOH was noted in 8 of 16 (50%). Although further screening of codons 279 to 1772 was done by PCR-single strand conformation polymorphism (SSCP) analysis in that report, the mutation incidence was much lower than our result. This discrepancy of mutational incidence is probably due to different sensitivity to detect mutations (PCR-SSCP versus sequencing, which is the most sensitive) and variation in etiological factors such as the environment and the genetic background.

Some of the mutated codons reported here have been found in previous studies.[11] The most common types of alteration (>90%) in several types of cancers and adenomas are truncated-type mutations (such as frameshifts due to insertions or deletions and nonsense mutations). Two of the 8 mutations were of truncated type but 5 of the remaining 6 were missense, resulting in amino acid substitutions. It is uncertain whether those missense mutations significantly affect the biological activity of the APC protein. However, we can speculate that they played an important role in ampullary carcinoma development, because 2 of the 5 missense mutations were accompanied by loss of the other allele and 2 other missense mutations were noted within a single case as double mutations that may indicate involvement of both alleles. In addition, *in vitro* functional studies have revealed that a single amino acid change resulting from a missense mutation might influence the β-catenin-binding activity of *APC* that has been implicated in its function.[16–18]

Considering the relatively high frequency of insertion mutations at codons 1554–1556 (poly A minimicrosatellite region) of the *APC* gene, as shown in the previous and the present study,[8] the underlying mechanism is likely to be linked to replication errors.

As for two mutational hot spots of the *p53* gene, it cannot be completely excluded that artifacts could occur with the presently applied method. However, we concluded that the high frequency of the *p53* gene mutation at codons 189 and 166 in sporadic ampullary carcinoma is unlikely to be an artifact for the following reasons. 1) The relevant PCR controls were always negative. 2) Sequencing was performed bidirectionally on DNA extracted from mixed cultures with at least 50 recombinant colonies. 3) The whole procedure, from PCR to sequencing, was repeated twice to confirm the result. 4) DNA samples isolated from corresponding normal tissue of the patients all demonstrated only the wild-type sequence. 5) Almost all the cases with hot spot mutations also had additional mutations that distinguished them from other cases. Thus, we concluded that artifacts caused by DNA cross-contamination, sequencing artifacts and misincorporation by Taq DNA polymerase were not likely to have occurred.

The high mutational frequency of the *p53* gene is similar to that reported by Scarpa *et al.*,[3] although the mutational pattern is quite different, with 9 of the 12 mutations observed clustering in two specific codons and 5 of the 12 nonsense mutations leading to truncated p53 protein. Scarpa *et al.* analyzed the mutational status of the *p53* and K-*ras* genes in 11 cases of sporadic ampullary carcinomas resected in Italy and found 7 to harbor *p53* genes mutated at exons 5, 6 and 7, with 5 missense mutations and 2 deletion mutations leading to nonsense stop-codons downstream. Furthermore, all of the mutation sites were different from those observed here. One possible explanation for this discrepancy may be varia-

tion in etiological factors such as the environment and the genetic background.

The frequencies of mutations, locations of hot spot regions and types of base substitution are known to differ with the cancer type.[19] Unlike colon cancer cases, where *p53* changes mainly occur at CpG dinucleotides (67%),[20] only one such mutation was observed in our present study. This observation might have important pathogenetic implications. CpG sites are preferential targets for point mutations in mammalian cells, because spontaneous errors during DNA replication could arise due to deamination of methylated cysteine residues.[20,21] Mutations occurring outside of these sites might be related to specific endogenous or exogenous carcinogens.[22,23] Indeed, it has been proposed that ampullary carcinomas occur with a significantly high frequency in patients with particular occupational exposures to as-yet-unidentified chemical carcinogens.[24] In this context, the 4 out of 31 hepatocellular carcinoma cases in Haimen in China (located near Quidong and Shanghai) with the same pattern of *p53* gene mutations at codon 166 are of interest (Shimizu *et al.*, personal communication). Some carcinogen responsible for both hepatocellular and ampullary carcinomas might thus be targeting codon 166 of the *p53* gene. It has also been suggested that the bile itself may enhance cell proliferation and play some role in tumorigenesis, although no specific factor has yet been identified.[25-27]

The incidence of K-*ras* gene mutations (34.5%) found in our series is almost identical to those reported previously.[3,4] Scarpa *et al.* found that 6 out of 17 epithelial tumors of the ampulla of Vater contained *ras* mutations, codon 12 of the K-*ras* gene being affected in 2 adenomas and 3 carcinomas, and that of the N-*ras* gene in 1 adenoma. In their study, 3 of 4 tumors which mainly involved the intraduodenal bile duct had K-*ras* mutations and they therefore inferred that a proportion of K-*ras*-mutated ampullary cancers might originate from the bile duct component of the ampulla. In the present study, all 3 cases arising in the intraduodenal bile duct carried the mutated K-*ras* genes, in contrast to only 1 of 13 cases in the common channel of the ampulla. In addition, neither *APC* nor *p53* gene mutations were found in 2 of these K-*ras* mutated cases. Thus, mutation of the K-*ras* gene could be a marker indicative of an intraduodenal bile duct origin, although many more cases are necessary to confirm the correlation.

Considering the relation between genetic alterations and histopathological findings of the tumor (Table V), mutations of the *APC* gene were observed more frequently in ulcerated (3/5, 60%) than in protruding type (4/11, 36.7%) in the present series, and in papillary adenocarcinomas (5/8, 62.5%) than in their tubular counterparts (3/8, 37.5%). As for the *p53* and K-*ras* genes, mutations were also more frequent in ulcerated type (3/5, 60% and 2/5, 40%, respectively) than in protruding type (5/11, 45.5% and 2/11, 18.2%). Histologically, mutation incidence was much higher in papillary (6/8, 75%, and 3/8, 37.5%) than in tubular adenocarcinomas (3/8, 37.5%, and 1/8, 12.5%). As for cancer differentiation, both of two poorly differentiated adenocarcinomas showed no mutations in any of the examined genes, so that low-grade and high-grade malignancy of the papilla of Vater might arise via different genetic mechanisms. However, further studies of a much larger number of cases is required before any firm conclusion can be drawn. As regards clinicopathological stage, three of the 4 stage IV tumors showed mutations of the *APC* gene, whereas 8 of the remaining 13 stage I, II, and III tumors did not show *APC* gene mutation. On the other hand, *p53* gene mutations were found frequently in stage I, II and III tumors (9/13, 69%), but none was found in any of the 4 stage IV tumors. No clear trend of accumulation of cancer-related gene mutations with the progression of tumor stage could be found. Various genetic pathways may exist for sporadic ampullary carcinogenesis.

Table V. Frequencies of the *APC* and *p53* Gene Alterations in Different Macroscopic and Histopathological Types

Gross appearance	Histological type	*APC*	*p53*	K-*ras*
superficial	papillary	1/1	1/1	0/1
protruding	papillary	2/3	2/3	1/3
	tubular (W/D)	2/6	3/6	1/6
	tubular (M/D)	0/0	0/0	0/0
	tubular (P/D)	0/1	0/1	0/1
	signet ring cell	0/1	0/1	0/1
ulcerated	papillary	2/4	3/4	2/4
	tubular (W/D)	0/0	0/0	0/0
	tubular (M/D)	1/1	0/1	0/1
total		8/17	9/17	4/17

W/D, well differentiated; M/D, moderately differentiated; P/D, poorly differentiated; A/B, number of tumors with gene alteration (A) /total number (B).

In this study, 4 cases showed mutations in only the *APC* gene, 5 cases in only the *p53* gene, and 2 cases, possibly derived from the intraduodenal bile duct, in only the K-*ras* gene. Although all but one (case 6) were relatively advanced cancers which had invaded beyond Oddi's sphincter and early gene mutations are hard to detect, we can conclude that at least some sporadic ampullary carcinomas develop via different genetic mechanisms from those in FAP patients. Meanwhile, the site of origin of ampullary carcinomas can be any one of the three anatomical components of the ampulla of Vater, the ampulla (common channel) or the intraduodenal portions of the bile duct or the pancreatic duct. The

covering duodenal mucosa might also give rise to the same type of tumors. Involvement of various cancer-related genes might reflect the complexity of the origin and early environment of this tumor.

In conclusion, alterations of the *APC* and *p53* genes are relatively frequent in sporadic ampullary carcinomas, with mutational hot spots in the latter case offering a possible clue to the pathogenesis. In addition, the present results point to K-*ras* gene mutations being less frequent, but indicating an intraduodenal bile duct origin.

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