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p53 Gene Mutation in Hepatocellular Carcinoma Induced by 2-Amino-3-methylimidazo[4,5-*f*]quinoline in Nonhuman Primates

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2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) is one of several heterocyclic amines formed during the cooking of proteinaceous foods. IQ is a potent carcinogen in rodent bioassays and causes a high incidence of hepatocellular carcinomas in nonhuman primates. We examined 20 hepatocellular carcinomas (HCCs) from nonhuman primates for mutations of the p53 gene using polymerase chain reaction-single strand conformational polymorphism analysis. Mutations in the p53 gene were detected in 4 of 20 HCCs (20%) with 3 showing G-to-T transversions and one a G-to-A transition. Three of these mutations were observed in codons 175 and 248 that are known mutational hot spots in human cancers. These data indicate that part of the IQ-induced HCCs in nonhuman primates may involve inactivation of the p53 gene and suggest that IQ and possibly other heterocyclic amines may participate in human carcinogenesis by a similar mechanism.

Key words: Food mutagen — PCR-SSCP — DNA adduct

2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) is one of several mutagenic heterocyclic amines formed during cooking of proteinaceous foods.[1-3] IQ is a potent mutagen in the Ames *Salmonella* assay,[4] and has been found to be carcinogenic in Fischer 344 (F344) rats,[5] female Sprague-Dawley rats,[6] CDF1 mice[7] and cynomolgus monkeys.[8] It has been suggested that the food-derived heterocyclic amines pose a considerable risk for cancer causation in humans.[9] However, evidence clearly establishing the etiological role of these compounds in human cancer is still lacking.

The p53 tumor suppressor gene is ideally suited for analysis of the mutational spectrum to understand both exogenous and endogenous molecular mechanisms of carcinogenesis.[10,11] Recently, Makino *et al.* reported that 4 of 15 primary Zymbal gland tumors induced by IQ in F344 rats had mutations in the p53 gene. It was further suggested that these mutations might be induced by the formation of DNA adducts of IQ in the p53 gene since all the mutations were found at the guanine base.[12] To explore further the possible involvement of the p53 gene in IQ-induced tumors we have determined the mutation frequency in the p53 gene in 20 IQ-induced hepatocellular carcinomas (HCCs) in nonhuman primates using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis.

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MATERIALS AND METHODS

DNA Preparation from HCCs IQ (purchased from the Nard Institute, Ltd., Osaka) was prepared daily as a suspension in hydroxypropyl cellulose (HPC) and administered to cynomolgus monkeys (*Macaca fascicularis*) by gavage five times a week at a dose of 10 or 20 mg/kg body weight for a period of 21 to 42 months.[8] HCC tissues and adjacent nontumorous liver tissues were obtained from the necropsied monkeys. These tissues were stored at −80°C. Genomic DNA was extracted by the proteinase K-phenol-chloroform extraction method.

PCR-SSCP analysis DNA samples (0.1 μg) were subjected to the polymerase chain reaction[13] in the mixture (5 μl) described previously[14,15] using two appropriate oligonucleotides as primers. The primer sequences for exons 5, 6, 7 and 8 are as follows[16]:
E5a: ACGTGAATTCAACTCTGTGTCCTTCCT;
E5b: TGGATCCAGTCCCAGCTGCTCACC;
E6a: GGTGAGCAGCTGGGACTGGA;
E6b: AGTTGCAAACCAGACCTCAG;
E7a: GGAATTCTGACTGTACCACCATCCA;
E7b: ACGTGGAATTCAGAGGCAAGCAGAGGCTG;
E8a: ACGTGAATTCCTTACTGCCTCCTGCTT;
E8b: ACGTGGAATTCGTGGCAAGGCTCCCCTTT.
The 5′-ends of these primers were labeled by the polynucleotide kinase reaction with [γ-^{32}P]ATP as described previously.[16] The amplified and labeled DNA fragments were subjected to electrophoresis at 40 W for 1–4 h in a 6% non-denaturing polyacrylamide gel with 10% glycerol in a cold room.[16]

DNA sequencing DNA fragments which showed mobility shifts by SSCP analysis were eluted from the gel and amplified by PCR using the same primers, which were phosphorylated with T4 polynucleotide kinase and ATP. The PCR products were either purified with phenol-chloroform, blunt-ended with T4 DNA polymerase and dNTPs, separated in a 3% agarose gel and ligated into *Sma*I-cut dephosphorylated pGEM7 (Promega, Madison, WI) vector[17] or directly subcloned using the TA Cloning™ kit (Invitrogen Corp., San Diego, CA). Two individual clones of each sample which showed a mobility shift were sequenced by the dideoxy chain termination method of Sanger *et al.*[18] All mutations were confirmed by repetition of the entire experimental procedure; PCR-SSCP of genomic DNAs, DNA fragment extraction from the gel, PCR, cloning, and sequencing.

RESULTS AND DISCUSSION

Twenty HCCs induced by IQ were examined for p53 gene mutations utilizing PCR-SSCP analysis on exons 5, 6, 7 and 8. Significant electrophoretic mobility shifts were detected in 4 of 20 HCCs (20%); three in exon 5 and one in exon 7 (Fig. 1). Nucleotide sequence analysis of the shifted bands revealed point mutations of the p53 gene (Fig. 1); at the first position of codon 159 (G-to-T transversion) in case 1425, at the second position of codon 175 (G-to-T transversion) in case 1443, at the second position of codon 175 (G-to-A transition) in case 1393 and at the second position of the codon 248 (G-to-T transversion) in case 1394 (Table I). No mutations were detected in nontumorous tissues around the four HCCs having the p53 mutations.

We detected p53 gene mutations in 4 out of 20 HCCs induced by IQ in nonhuman primates. Three of these mutations were G-to-T transversions resulting in amino acid changes at two hot spots in human tumors.[19] All of the four p53 mutations[12] and three of the four Ha-*ras* mutations[20] detected in rat Zymbal gland tumors induced by IQ were observed at a guanine base. The capacity of IQ to bind covalently to DNA has been shown *in vitro*[21] and *in vivo* in F344 rats,[22] CDF1 mice[23] and cynomolgus monkeys, in which one of the IQ-DNA adducts has been identified as N^2-(deoxyguanosine-8-yl)-IQ.[24] These data suggest that the G-to-T transversions of the p53 gene may be induced by formation of IQ-DNA adducts and that in a fraction of IQ-induced HCCs in nonhuman primates, inactivation of p53 is part of the oncogenic process.

Recently, a significant number of HCCs in patients from Qidong province in China and from southern Africa, where hepatitis B virus (HBV) is endemic and dietary exposure to aflatoxin B1 is high, was found to have a G-to-T transversion at the third position of codon 249 of the p53 gene.[25-27] This suggests that aflatoxin B1 contributes to the development of HCCs through the inactivation of the p53 gene, which might be caused by forming DNA adducts[28] in cooperation with HBV.[16] Our data from the IQ-induced HCCs in nonhuman primates suggest that IQ and possibly other food-derived heterocyclic amines may also contribute to human carcinogenesis by inactivation of the p53 gene via formation of DNA adducts.

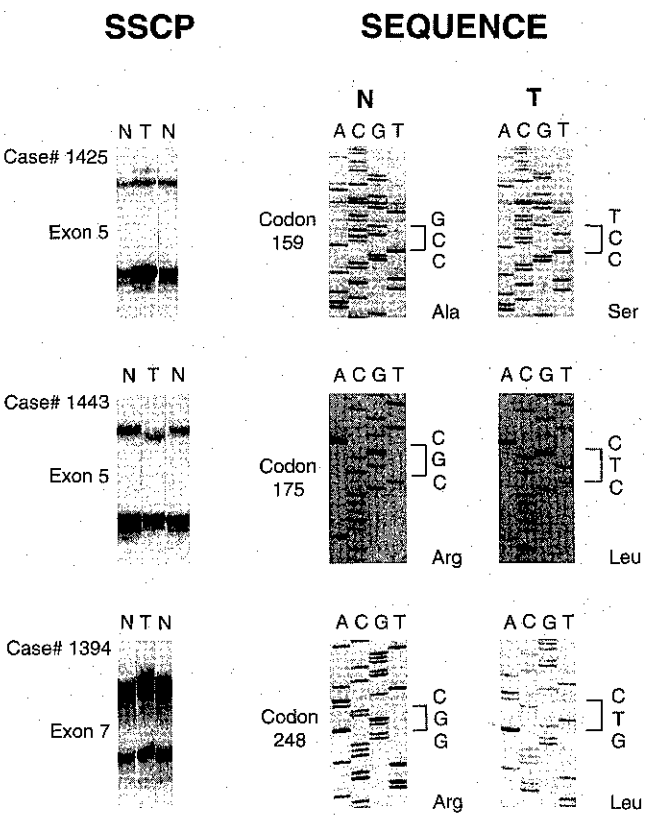

Fig. 1. Detection and identification of p53 gene mutations in nonhuman primate HCCs induced by IQ. Shown are mobility shifts from tumors (T) in comparison to normal tissue (N) as detected by PCR-SSCP of exons 5 and 7 for representative cases. Data are summarized in Table I.

Table I. IQ-induced HCCs in Nonhuman Primates

Case	Codon	Mutation	
		Nucleotide[a]	Amino acid[b]
1393	175	CGC CAC	Arg His
1394	248	CGG CTG	Arg Leu
1425	159	GCC TCC	Ala Ser
1443	175	CGC CTC	Arg Leu

a) Nucleotide change from normal to mutant.
b) Amino acid change from normal to mutant.

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