J Hum Genet (2000) 45:254–256

SHORT COMMUNICATION

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Identification of novel polymorphisms in the *AXIN1* and *CDX-2* genes

Received: April 10, 2000 / Accepted: April 27, 2000

Abstract Axin and Cdx-2 play important roles in the tumorigenesis of human liver and colon. We have identified seven novel single-nucleotide polymorphisms (SNPs) in the *AXIN1* gene and three in the *CDX-2* gene. The identification of SNPs in these cancer-associated genes establishes a basis for future investigations to detect losses of heterozygosity in tumors; these SNPs may also provide genetic background information associated with cancer risk.

Key words Single nucleotide polymorphism · Colorectal cancer · Hepatocellular carcinoma · Axin · Cdx-2

Introduction

Apart from its indispensable role in the determination of dorso-ventral structures during development, the Wnt/wingless signaling pathway can participate in the genesis of a wide range of human cancers, including colorectal carcinoma (CRC) and hepatocellular carcinoma (HCC) (Nakamura 1997). The product of AXIN1, a gene that was first isolated from the murine Fused locus as an inhibitor of Wnt signaling (Zeng et al. 1997), was later shown to reduce β-catenin by forming a complex with β-catenin, GSK-3β (glycogen synthase kinase-3β), and APC (adenomatous polyposis coli) (Kishida et al. 1998, Nakamura et al. 1998). By screening primary HCCs for mutations in the human homologue (*AXIN1*), we previously identified somatic mutations that all resulted in truncation of the predicted protein (Satoh et al. 2000). Moreover, additional experiments showed that the adenovirus-mediated gene transfer of wild-type *AXIN1* inhibited growth and induced apoptosis in cultured HCC cells. These results suggested that Axin functions as a tumor suppressor.

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Cdx-2, a mammalian homologue of Drosophila caudal, appears to play a crucial role in the progression of intestinal tumors. For example, Cdx-2 mutant mice tend to develop multiple intestinal polyps (Chawengsaksophak et al. 1997), and da Costa et al. (1999) observed a 4-bp deletion in the *CDX-2* gene in DNA from a human colorectal-cancer cell line that was not deficient in either wild-type APC or β-catenin.

Recently, others have reported that polymorphisms of two important tumor suppressor genes were associated with susceptibility to the development of cancers in humans. One of the examples involved a polymorphism at codon 72 of p53; Caucasian women homozygous for arginine at codon 72 were shown to carry a seven-times-greater risk for developing human papilloma virus-associated cervical cancers than heterozygotes (Storey et al. 1998). The other example was a missense alteration of codon 1307 of *APC*; the incidence of an *APC* allele that encoded a lysine residue at codon 1307 was significantly more common among a cohort of CRC patients of Ashkenazi Jewish ethnicity than among Ashkenazim who did not have colorectal cancers (Laken et al. 1997). In similar fashion, polymorphisms in other tumor suppressor genes may represent markers of cancer susceptibility in certain populations.

Here we report the identification of single-nucleotide polymorphisms (SNPs) in the *AXIN1* and *CDX-2* genes in a Japanese population sample. These polymorphisms should be useful tools for detecting losses of heterozygosity (LOH) in tumors and for examining potential associations between specific alleles and cancer risk.

Materials and methods

DNA samples

Blood samples were obtained, with informed consent, from 50 healthy Japanese individuals. Genomic DNA was prepared from each sample according to standard methods.

Single-strand conformation polymorphism (SSCP) analysis

A 50-ng aliquot of DNA extracted from each sample was used as a template for the polymerase chain reaction (PCR). Amplifications were carried out in volumes of 25 μl containing 67 μM Tris (pH 8.8), 17 μM NH_4SO_4, 10 μM 2-mercaptoethanol, 150 μM dNTPs, 25 pmol of each primer, 2 mM $MgCl_2$, 10% dimethyl sulfoxide (DMSO), and 1.25 U Taq polymerase. We used seven sets of primers for *CDX-2* (Table 1) and 23 sets of primers to screen the entire coding region of *AXIN1*. Reaction conditions for all primer sets were 5 min at 94°C for initial denaturing, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min, with final extension at 72°C for 5 min, on the GeneAmp PCR system 9600 (PE Applied Biosystems, Foster City, CA, USA). For non-radioactive SSCP analysis, 0.2 μg of each PCR product was diluted with formamide dye solution and electrophoresed in a 12% polyacrylamide (40:1 Acrylamide: Bisacryl-amide) gel containing 10% glycerol, at room temperature and at 4°C. Electrophoretic bands were stained with SYBR™ Green II (FMC BioProducts, Rockland, ME, USA) and detected by means of a fluorescent image analyzer (FMBIO II Multi-View; TaKaRa, Tokyo, Japan).

Table 1. Primer sequences for amplifying the coding region of the *CDX-2* gene

Name of primer	Nucleotide sequences	Product size (bp)
Exon1-1F	5′-AGCCTTCAACGTCGGTCCC-3′	233
Exon1-1R	5′-GAACTTGGACAGCGCGCAG-3′	
Exon1-2F	5′-GACTACGGCGGTTACCACG-3′	212
Exon1-2R	5′-CAGCCATGGGCTACAGCAG-3′	
Exon1-3F	5′-CTCACGCGCTCAACGGTGG-3′	147
Exon1-3R	5′-GCTGCTGCAAACGCTCAACC-3′	
Exon1-4F	5′-CAGACTACCATCCGCACCAC-3′	232
Exon1-4R	5′-GCAGAGGCTGCGCCCCTT-3′	
Exon2F	5′-CTCACTTCTCCTTCCTCCAC-3′	191
Exon2R	5′-GACCGCCTAGCTCCCTCC-3′	
Exon3-1F	5′-CTTTTCTCCACCTTTCCATTTC-3′	226
Exon3-1R	5′-CCTCAGTGTCTGGCTCTGTC-3′	
Exon3-2F	5′-CAGGTCCTCTGAGAAGTGTC-3′	168
Exon3-2R	5′-CAGAGCAATTCCAGGCTGAG-3′	

DNA sequencing

Aberrant PCR products detected by SSCP were purified using the QIAquick PCR Purification Kit (Qiagen, Tokyo, Japan). The DNA sequence of each aberrant sample was determined using an Applied Biosystems model 377 DNA sequencer (PE Applied Biosystems) with a Dye-Terminator Cycle Sequencing FS Ready Reaction Kit (PE Applied Biosystems).

Table 2. Allelic frequencies of SNPs of the *AXIN1* and the *CDX-2* genes in 50 normal independent individuals

Gene	Codon position	Nucleotide position	Genotypes (n = 50)			Allelic frequencies	
AXIN1[a]							
Exon 1	31	93 (A > C)	A/A	A/C	C/C	A	C
			38	11	1	0.87	0.13
Exon 1	111	333 (C > T)	C/C	C/T	T/T	C	T
			49	1	0	0.99	0.01
Exon 1	291	873 (C > T)	C/C	C/T	T/T	C	T
			19	26	5	0.64	0.36
Intron 4		+17[b] (G > A)	G/G	G/A	A/A	G	A
			41	7	2	0.89	0.11
Exon 5	465	1396 (G > A)	G/G	G/A	A/A	G	A
			45	4	1	0.94	0.06
Exon 5	526	1579 (G > T)	G/G	G/T	T/T	G	T
			48	2	0	0.98	0.02
Exon 5	563	1690 (C > T)	C/C	C/T	T/T	C	T
			49	1	0	0.99	0.01
CDX-2[c]							
Exon 1	61	543 (G > C)	G/G	G/C	C/C	G	C
			36	12	2	0.82	0.18
Exon 3	293	1237[d] (T > C)	T/T	T/C	C/C	T	C
			35	14	1	0.84	0.16
Exon 3	3′-UTR	+10[d,e] (G > T)	G/G	G/T	T/T	G	T
			35	14	1	0.84	0.16

SNP, Single nucleotide polymorphism
[a] Numbering system refers to the partial *AXIN1* cDNA sequence in GenBank AF009674
[b] Nucleotide position is identified from the exon-intron boundary
[c] Numbering system refers to the *CDX-2* cDNA sequence in GenBank NM_001265
[d] These two nucleotides showed synchronous substitutions
[e] Nucleotide position is identified from the stop codon

Allelic frequencies of each SNP

The allelic frequencies of each polymorphism were determined by the band pattern of SSCP, using DNA from 50 healthy Japanese volunteers.

Results and discussion

We found a total of ten SNPs in these two tumor-suppressor genes examined. Of the seven SNPs found in the *AXIN1* gene, six were located within the coding region and one in an intronic sequence. None of them would alter the amino-acid sequence of the product. Three SNPs were identified in the *CDX-2* gene coding region; a T-to-C transition at codon 293 in exon 3 would encode proline instead of serine. Table 2 indicates the nature of each SNP in both genes, and the frequency of each allele among the 50 healthy members of the Japanese population.

These SNPs will be useful not only for investigating tumors for LOH in the chromosomal regions where the *AXIN1* and *CDX-2* genes are located (16p13.3 and 13q12–13, respectively; Zeng et al. 1997; Drummond et al. 1997) but also for examining potential associations between specific alleles and cancer susceptibilities. Moreover, these SNPs can also serve as genetic markers for exploring susceptibility to a variety of other common diseases.

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