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SHORT COMMUNICATION

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Four single-nucleotide polymorphisms in the human *BUB1* gene

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Abstract Four single-nucleotide polymorphisms have been found in the human *BUB1* gene, which encodes a kinase involved in the mitotic spindle checkpoint. A cytosine-to-thymine change in exon 10, corresponding to codon 375 (c.1124C>T), causes an amino acid substitution of serine to phenylalanine. A guanine/cytosine polymorphism in exon 4 (c.279G>C) and a thymine/cytosine polymorphism in exon 12 (c.1293T>C) do not cause amino acid substitution. The other polymorphism, of thymine/cytosine (IVS9-8T>C), is found at 8 bp upstream of exon 10. As mutations of the *hBUB1* gene were reported in a subset of human cancers, these polymorphisms could provide useful tools for the genetic study of susceptibility to various human cancers.

Key words *hBUB1* · Chromosome 2q14 · Polymorphism · Amino acid substitution · Cancer susceptibility

Introduction

The human *BUB1* (*hBUB1*) gene encodes a serine/threonine kinase involved in the mitotic spindle checkpoint that monitors accurate chromosomal segregation during cell division (Pangilinan et al. 1997). Disruption of this checkpoint system has been demonstrated to cause changes in ploidy and the resulting aneuploidy in cancer cells (Cahill et al. 1998). Mutations of the gene in a subset of human colon, lung, and head and neck cancers, and adult T-cell leukemias/lymphomas, indicate that alteration of the *hBUB*1 gene is involved in various human cancers (Cahill et al. 1998; Yamaguchi et al. 1999; Imai et al. 1999; Sato et al. 2000; Ohshima et al. 2000). In the course of single-strand conformation polymorphism (SSCP) and DNA sequencing analysis of the whole coding regions of the *hBUB1* gene in a Japanese population with hepatocellular carcinoma, we identified four previously unreported nucleotide substitutions in the *hBUB1* gene.

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Polymorphism and allele frequency

Primers for the polymerase chain reaction (PCR). For PCR, we used the following primers:
hBUB1ex4F 5′-TCCCTCCCTGGAGGTTCAGC-3′
hBUB1ex4R 5′-CAAAACTAGAAGGATTTCCCTG-3′
hBUB1ex10F 5′-GAGGTAATGCCTGATTAGTAG-3′
hBUB1ex10R 5′-CACATCACTGTGATCTCTAG-3′
hBUB1ex12F: 5′-GTGTCTTTTAAGTTATTCTG-3′
hBUB1ex12R:5′-TCTTGATATTTTCTGTGATAACC-3′

Allele frequency. The estimated allele frequencies of the four polymorphisms in 60 chromosomes from unrelated individuals in the Japanese population are summarized in Table 1.

Chromosomal location. The *hBUB1* gene was mapped to chromosome 2q14 (Pangilinan et al. 1997).

Other comments. Target DNA was amplified using Advantage Klen Taq DNA polymerase (Clontech, Palo Alto, CA, USA), with an initial melting temperature of 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 68°C for 1 min. A final 68°C extension step for 5 min terminated the process. For SSCP analysis, polymerase chain reaction (PCR) products were diluted with ten volumes of loading solution containing 90% formamide, denatured by heating, and subjected to electrophoresis in nondenaturing 5% polyacrylamide gels in 1 × TME (Tris-Mes/EDTA)

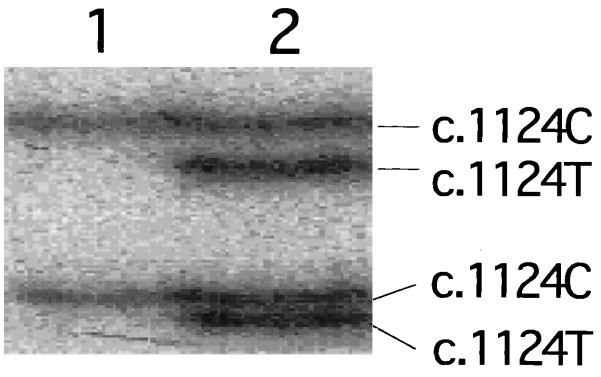

Fig. 1. Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis of exon 10. *Lane 1* indicates a homozygote for Serine/Serine (S/S); *lane 2* is a heterozygote for Serine/Phenylalanine (S/F)

Table 1. Allele frequencies of the polymorphysms in the *hBUB1* gene

Allele[a]	Allele frequency (n = 60)
c.279G	0.983
c.279C	0.017
c.1124C	0.983
c.1124T[b]	0.017
c.1293T	0.95
c.1293C	0.05
IVS9-8T	0.917
IVS9-8C	0.083

[a] Dunnen and Antonarakis (2000)
[b] p.S375F

buffer (Kukita et al. 1997). The gel was dried on filter paper and exposed to X-ray film. DNA sequencing was determined with the ABI PRISM dye terminator cycle sequencing ready reaction kit (Perkin-Elmer, Branchburg, NJ, USA), using an ABI 377 DNA auto-sequencer (Applied Biosystems, Foster City, CA, USA). A polymorphism at codon 375 (c.1124C>T) causes an amino acid substitution from serine to phenylalanine. As this serine residue, as well as three adjacent amino acids, is conserved between human and mouse hBUB1protein, this substitution may confer some functional variation on the protein (Pangilinan et al. 1997; Taylor and McKeon 1997). These single-nucleotide polymorphisms could be useful for the study of susceptibility to various human cancers.

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