BRIEF REPORT — POLYMORPHISM REPORT

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A Gln/Arg polymorphism at codon 349 of the *hBUBR1* gene

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Abstract We found a glutamine/arginine polymorphism at codon 349 of the *hBUBR1* gene, encoding a protein kinase required for spindle assembly checkpoint function. The observed heterozygosity was estimated to be 45% in the Japanese population. This polymorphism may be helpful for genetic studies of many cancer types in which chromosomal instability is observed.

Key words SNP · *hBUBR1* gene · checkpoint

Introduction

BUBR1 is a homologue of the *BUB1* gene, (budding uninhibited by benzimidazole 1), which encodes a protein kinase required for spindle assembly checkpoint function (Roberts et al. 1994). Recently, mutations of the *hBUB1* and *hBUBR1* genes were found in human colorectal cancer cells displaying chromosomal instability (Cahill et al. 1998). During screening for possible disease-associated mutations in breast cancer by reverse transcription-polymerase chain reaction-single strand conformation polymorphism (RT-PCR-SSCP) analysis, we identified a polymorphism in the *BUBR1* gene. Here we describe the Gln/Arg polymorphism of the *BUBR1* gene in the Japanese population.

Methods

RT-PCR-SSCP analysis

After reverse transcription of total RNA obtained from breast cancer tissue, the *BUBR1* cDNA was amplified. The PCR

amplification was performed in a 10- μ l volume. PCR primer sequences used were: 109SPF5 (5'-GAGAATGAG-CTGCAAGCAGG-3') and 109SPR5A (5'-ACATGGTGT-CATAACTGGCTGT-3'). Cycling conditions were 35 cycles of 94°C for 30s, 60°C for 30s, and 72°C for 30s. The resulting PCR products (155bp in length) were electrophoresed in a 6% polyacrylamide gel containing 0.5 $\times$ TBE and 5% glycerol at 4°C. The gels were dried and autoradiographed with intensifying screens. After the purification of PCR products, each sample was subjected to cycle sequencing, using a dye terminator cycle-sequencing kit (Perkin-Elmer, Norwalk, CT, USA) according to the supplier's instructions. The same SSCP primers were used to sequence in both directions. Electrophoresis was carried out using a 377 DNA sequencer (Perkin-Elmer).

Allele-specific oligonucleotide (ASO) analysis

We performed hybridization of allele-specific oligonucleotides to investigate the allelic frequency of codon 349 in a normal control population. PCR products (3- μ l) were blotted on a Biotransfer membrane. Oligonucleotides specific for the Gln allele (5'-ACTGCACACACAGCCA-3') and the Arg allele (5'-ACTGCACGCAGCCA-3') were labeled with 32 P, using 10 units of T4 polynucleotide kinase. The membranes were hybridized in 6 $\times$ sodium saline citrate (SSC), 2 $\times$ Denhardt's, 0.5% sodium dodecyl sulfate (SDS), and 50mM Tris-HCl (pH7.5) overnight at 42°C, and washed in 6 $\times$ SSC at 44°C for the Gln allele, 46°C for the Arg allele, for 5min. Autoradiography was carried out at -80°C.

Allelic frequency

The allelic frequencies were estimated to be 45%, from genotyping of a total of 224 Japanese individuals. The allelic frequencies observed were: Gln allele, 0.31 and Arg allele, 0.69.

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References

- Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JKV, Markowitz SD, Kinzler KW, Vogelstein B (1998) Mutations of mitotic checkpoint genes in human cancers. *Nature* 392: 300–303
- Roberts BT, Farr KA, Hoyt MA (1994) The *Saccharomyces* checkpoint gene BUB1 encodes a novel protein kinase. *Mol Cell Biol* 14: 8282–8291