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BRIEF REPORT — POLYMORPHISM REPORT

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Two single nucleotide polymorphisms of the *hSNF5/INI1* gene

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Abstract We found two single nucleotide polymorphisms at the *hSNF5/INI1* gene located on 22q11.2, encoding a member of the chromatin-remodelling SWI/SNF multiprotein complexes. A guanine/adenine polymorphism at codon 299 in exon 7, and another guanine/adenine polymorphism at 39 bp upstream of exon 9 were identified. As the gene was recently identified as a tumor suppressor gene for malignant rhabdoid tumor, this polymorphism may be useful for the genetic study of susceptibility for human malignancies of various tissue origins.

Key words *hSNF5/INI1* gene · Single nucleotide polymorphism · Malignant rhabdoid tumor · Chromosome 22q11.2 · Tumor suppressor gene

Introduction

The *hSNF5/INI1* gene encodes a member of the chromatin-remodelling SWI/SNF multiprotein complexes and is located at 22q11.2 (Kwon et al. 1994). Recently, inactivation of both alleles of the gene was identified in malignant rhabdoid tumors (MRTs), suggesting that loss-of-function mutations of the *hSNF5/INI1* gene contributed to the oncogenesis of this type of tumor (Versteege et al. 1998). During screening of the entire coding region of the gene for sequence variations by polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis, we identified two single nucleotide polymorphisms in the *hSNF5/INI1* gene in a Japanese population.

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Source/Detection of polymorphisms

In 192 Japanese individuals, all nine exons encoding the entire coding sequence of the *hSNF5/INI* gene were screened by PCR-SSCP analysis for single nucleotide variations of the gene. PCR-SSCP was carried out following procedures described previously (Hirayama et al. 1998). Variant SSCP bands were identified in the PCR products of exon 7 and exon 9. These variants were subcloned and sequenced as described previously (Tsukamoto et al. 1998). A G/A silent substitution at the third nucleotide of codon 299 in exon 7, and another G/A substitution at 39 bp upstream of exon 9 were identified (data not shown).

PCR primers

The PCR primers used were:
Forward (exon7-1) 5′-GCAAAAGCTCTAACTTGTGTC-3′
Reverse (exon7-1) 5′-ATGCTGTATGCGATGGTGGT-3′

Forward (exon9) 5′-TACACTTGGCTGCCCTGTAG-3′
Reverse (exon9) 5′-GCCCAATCTTCTGAGATGC-3′

PCR conditions

Each PCR was performed in a volume of 10 μl containing 20 ng genomic DNA, in a 10-μl solution containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM each of dNTP, 2.5 pmol of each primer, and 0.25 units of Taq polymerase. Each of 35 PCR cycles consisted of 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C (Watanabe et al. 1998).

Allele-specific oligonucleotide (ASO) hybridization analysis. Hybridization to allele-specific oligonucleotides for each variable sequence in exon 7 or exon 9 was carried out as follows. The PCR products (3-μl) were blotted on a Biodyne (Port Washington, NY, USA) transfer membrane. Oligonucleotide specific for the G allele (5′-

CTGTGCTCGGAGCTGGG-3′) or the A allele (5′-CTGTGCTCAGAGCTGGG-3′) for exon 7 variations; or the G allele (5′-CAGGCTGGGAGCTGGCC-3′) or the A allele (5′-CAGGCTGGAAGCTGGCC-3′) for exon 9 variations was end-labeled with [γ-^{32}P] ATP, using 10 units of T4 polynucleotide kinase. The membranes were hybridized in 6 × sodium saline citrate (SSC), 2 × Denhardt's, 0.5% sodium dodecylsulfate (SDS), and 50 mM Tris-Hcl (pH7.5) overnight at 42°C, and washed in 6 × SSC at 45°C for 10 min. Membranes were blotted and exposed to X-ray film at −80°C.

Allele frequency. A total of 192 Japanese individuals were genotyped for sequence variations of exon 7 and exon 9. Allele frequencies of exon 7 were: G allele 0.94, A allele 0.06. Allele frequencies of exon 9 were: G allele 0.89, A allele 0.11. Both polymorphisms were not in linkage disequibilibrum, and combined heterozygosity was 0.17.

Mendelian inheritance. Codominant inheritance was observed in two two-generation families.

Chromosomal localization. The human *SNF5/INI1* gene was assigned to human chromosome 22q11.2 (Versteege et al. 1998).

Other comments. No other variant of the *hSNF5/INI* gene, germline or somatic, was detected in 96 cases of breast cancer, 96 cases of hepatocellular carcinoma, or 48 cases of uterine corpus cancers during screening by PCR-SSCP analysis.

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