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SHORT COMMUNICATION

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Identification and allelic frequencies of novel single-nucleotide polymorphisms in the *DUSP1* and *BTG1* genes

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Abstract Defects in the activity of the *PTEN* gene, a tumor suppressor, are implicated in many types of cancer in humans. However, not all mediators of PTEN signaling pathways have been clarified, and, during efforts to identify such molecules, we previously induced expression of the *DUSP1* and *BTG1* genes by introducing exogenous *PTEN* into endometrial cancer cell lines. In the course of analyzing these two genes for mutations in ovarian carcinomas, we identified a novel single-nucleotide polymorphism (SNP) in the *DUSP1* gene, and three novel SNPs in the *BTG1* gene, and we have established their allelic frequencies in a Japanese population sample. These polymorphic sites will be useful for detecting losses of heterozygosity (LOH) in tumors and for examining latent associations between specific alleles and disease susceptibility.

Key words Single-nucleotide polymorphism · *DUSP1* · *BTG1* · *PTEN* · Tumor suppressor

Introduction

The *PTEN* tumor suppressor gene encodes a multifunctional phosphatase that plays an important role in inhibiting the phosphatidylinositol-3-kinase pathway, and in downstream functions that include the activation of Akt/protein kinase B, cell survival, and cell proliferation (Maehama and Dixon 1998; Stambolic et al. 1998). Enforced expression of *PTEN* in various cancer cell lines decreases cell proliferation through arrest of the cell cycle, accompanied, in some instances, by the induction of apoptosis (Furnari et al. 1998; Minaguchi et al. 1999). We used cDNA microarrays containing 4009 cDNAs to examine changes in gene expression profiles when exogenous *PTEN* was introduced into *PTEN*-defective endometrial cancer cell lines, and we found that exogenous *PTEN* induced expression of the *DUSP1* and *BTG1* genes (unpublished data).

DUSP1/MKP1/CL100/PTPN10, a dual-specificity phosphatase for tyrosine and threonine, specifically inactivates mitogen-activated protein kinase (MAPK) and suppresses its activation by ras (Alessi et al. 1993). *BTG1* encodes a member of the anti-proliferative Tob/BTG1 family of molecules; transfection experiments have indicated that *BTG1* negatively regulates cell proliferation (Rouault et al. 1992).

Therefore, in view of our results from the microarray experiments, we considered these two genes to be candidates for mediating *PTEN* signaling pathways. We screened both genes for mutations in ovarian cancers and found none, but we did identify four single-nucleotide polymorphisms (SNPs) in non-coding regions. Here we describe these novel SNPs and report their allelic frequencies in a Japanese population sample.

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Subjects and methods

DNA samples. Materials used in this study were obtained, with informed consent, from 132 Japanese individuals. Genomic DNAs were extracted according to standard protocols.

Screening of SNPs. Amplification of genomic DNA fragments by polymerase chain reactions (PCRs) and DNA sequencing of the amplified fragments were performed as described previously (Lin et al. 2000).

SNP typing. SNPs were genotyped by allele-specific oligonucleotide (ASO) hybridization as described previously (Unoki et al. 2000). Allele-specific probes (^{32}P-labeled 13- to 17-mers) of 5′-TACGCCCTCGGGG-3′ or 5′-TACGCC GTCGGGG-3′ for C(IVS + 8)G of *DUSP1*, and 5′-GGGTGGCTGCTCC-3′ or 5′-GGGTGGTTGCTCC-3′ for C(-67T) of *BTG1*, were hybridized at 37°C to the polymerase chain reaction (PCR) products on the membranes

Table 1. Primer sequences for amplifying the coding regions and splice sites of the *DUSP1* and the *BTG1* genes

Gene	Name of primer	Nucleotide sequences	Product size (bp)
DUSP1	DUSP1-F1	5′-CGTCACGTGATCACCATTCA-3′	499
	DUSP1-R1	5′-TGAAGCGCACGTTGACAGAG-3′	
	DUSP1-F2	5′-CCAAAAGCGGCTTTTGGTTC-3′	695
	DUSP1-R2	5′-AGGGCTGGAAGTTTACATCG-3′	
	DUSP1-F3	5′-CGATGTAAACTTCCAGCCCT-3′	420
	DUSP1-R3	5′-AGCCTACAATTGGAGACTCG-3′	
	DUSP1-F4	5′-CCTCCAGCAACAGAACTGAG-3′	492
	DUSP1-R4	5′-ACAGGAATGTTGCCCACACC-3′	
	DUSP1-F5	5′-CATGTGATGGCATGTGGTGA-3′	609
	DUSP1-R5	5′-AATAAGGACCAGCCCTCTCG-3′	
	DUSP1-F6	5′-CACCACCACCGTGTTCAACT-3′	607
	DUSP1-R6	5′-CAGTGCTGAAAACAAACCTGC-3′	
	DUSP1-F7	5′-CCCGACGACACATATACATAT-3′	535
	DUSP1-R7	5′-AGAGTTCAGCTGTAGCGTCC-3′	
BTG1	BTG1-F1	5′-GGTGCGATATTCGGATTGGC-3′	616
	BTG1-R1	5′-GACTCTGACCCAGGGATGTG-3′	
	BTG1-F2	5′-AACAACGGATGCAATCCTGG-3′	932
	BTG1-R2	5′-CTTTCCTATTAAAAGCTGCCG-3′	

Table 2. Allelic frequencies of SNPs of the *DUSP1* and the *BTG1* genes in a Japanese population

Gene	Region	SNPs and their flanking sequence	Allelic frequencies[a]	
DUSP1	Intron1	AAGGTACGCC (C/G) TCGGGGAAGC	C	G
	IVS1 + 8		0.68	0.32
BTG1	5′UTR (exon1)	AGCTATTGAG (A/G) TCTTCGAATG	A	G
	(−278)		0.64	0.36
	5′UTR (exon1)	CCCGGGGTGG (C/T) TGCTCCGCCG	C	T
	(−67)		0.65	0.35
	5′UTR (exon1)	GCCGAGCCCC (G/A) GCCGCCCCGG	G	A
	(−33)		0.67	0.33

SNP, Single-nucleotide polymorphism; UTR, untranslated region
[a] Estimated from the genotyping of 264 chromosomes

and washed in 6 × standard saline citrate (SSC) at 42°C (*DUSP1*) or at 46°C (*BTG1*) according to standard protocols. Genomic DNAs (50-ng each) were amplified by PCR, and each PCR product was digested with *Bgl*II for A(-278)G of *BTG1* or with *Eco*52I for G(-33)A of *BTG1*, according to the manufacturer's protocol (TaKaRa).

Results and discussion

By screening all coding exons and their neighboring introns in the *DUSP1* and *BTG1* genes, using the PCR primers listed in Table 1, we detected four novel SNPs, one in the *DUSP1* gene and three in the *BTG1* gene. To estimate the allelic frequency of each SNP in the Japanese population, we genotyped a panel of 132 subjects, with the results shown in Table 2.

The SNP in the *DUSP1* gene is located in intron 1 and all three SNPs in the *BTG1* gene are located in the 5′ untranslated region. Because none of these polymorphic sites is within a coding region, none is likely to influence the function of the gene product, although we cannot exclude the possibility that certain alleles might alter the efficiency of translation or transcription. Nevertheless, these polymorphisms should be useful for detecting losses of heterozygosity (LOH) in tumors and for examining potential associations between specific alleles and disease susceptibility.

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