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SHORT COMMUNICATION

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Nine novel single-nucleotide polymorphisms in the integrin β4 (*ITGB4*) gene in the Japanese population

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Abstract We identified nine single-nucleotide polymorphisms (SNPs) in the human integrin β4 (*ITGB4*) gene (17q24–q25), which encodes a cell-surface receptor, by screening all exons and exon-intron boundaries. Seven of these SNPs were present in coding regions and two in intronic sequences; four of the coding SNPs involved amino-acid substitutions. As the gene is implicated in the tumorigenesis of breast cancers, the polymorphic sites will serve as useful markers not only for distinguishing alleles in loss of heterozygosity (LOH) analyses but also for studying genetic susceptibility to malignancies in humans.

Key words Integrin β4 gene · Single nucleotide polymorphism · Chromosome 17q24–25 · Cancer · Japanese

Introduction

Integrins comprise a large family of cell-surface receptors, each of them composed of two subunits, α and β. Integrin β4 (ITGB4) interacts with α6; the α6/β4 heterodimer binds to laminin and is involved in the formation and maintenance of hemidesmosomes. Germline mutations of the *ITGB4* gene have been identified in patients with epidermolysis bullosa, an autosomal recessive genodermatosis (Vidal et al. 1995). Natali et al. (1992) have documented underexpression of the *ITGB4* gene in primary and metastatic breast cancers, suggesting that alteration of this gene contributes to carcinogenesis and/or metastasis in that disease. By screening its entire coding region for sequence variations, using polymerase chain reaction/single-strand conformation polymorphism analysis (PCR-SSCP), we identified nine single-nucleotide polymorphisms (SNPs) in the *ITGB4* gene in a Japanese population sample.

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Subjects and methods

DNA samples. Informed consent, in the formal style of the hospital, was obtained from 48 healthy Japanese volunteers. Genomic DNA was prepared from each blood sample according to the standard protocols.

PCR conditions. Each PCR was performed in a volume of 30 μl, containing 100 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 200 μM of each dNTP, 10 pmol of each primer, and 0.1 unit of Taq polymerase, as described previously (Tsukamoto et al. 1998). We used 54 primer sets to screen the entire coding sequence of the *ITGB4* gene (exons 2–41). The primer sets for the eight regions found to contain SNPs are shown in Table 1 (two variant sites were found in intron 25). Cycling conditions were: 94°C for 3.5 min, then 30 cycles of 94°C for 1 min, 55°C–68°C for 1 min, and 72°C for 1 min, with final extension for 5 min at 72°C, in a Gene Amp PCR 9600 System (Perkin Elmer Cetus, Norwalk, CT, USA). Annealing temperatures were adjusted according to the melting temperature of each pair of primers.

SSCP analysis. A 10 ng aliquot of each PCR product was heat-denatured in the presence of 50% formamide, and electrophoresed for 11 h at 10 V/cm and room temperature in an 8% polyacrylamide gel, with and without 10% glycerol in 0.5 × TBE (1 × TBE; 89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA; pH 8.0) (Harada et al. 1992). DNA fragments were detected with a PlusOne DNA silver-staining kit (Amersham Pharmacia Biotech, Uppsala, Sweden), used according to the manufacturer's instructions.

DNA sequencing. Variant bands were used directly as templates for further amplification. The DNA sequence of each

PCR product was determined using an Applied Biosystems model 377 DNA sequencer (Perkin Elmer Cetus).

Results and discussion

A total of healty 48 Japanese individuals were genotyped for sequence variations that might be present in any exon or exon-intron boundary of the *ITGB4* gene. We identified nine SNPs, of which seven were located in coding regions and two in intronic sequences. Four of the coding SNPs reflected amino-acid substitutions. Genotypes and allele frequencies are summarized in Table 2.

The human *ITGB4* gene was originally mapped to chromosome 17q11-qter by analysis of human-rodent somatic cell hybrids (Hogervorst et al. 1991), but integrated mapping information in Genemap99 now locates this gene at 17q24–25. Human cancers derived from breast, esophagus, or ovary frequently show allelic losses on chromosome band 17q25. Moreover, a locus responsible for hereditary focal non-epidermolytic palmoplantar keratoderma (*TOC*), a condition associated with esophageal cancer, has been mapped to the same band. We ourselves have described frequent allelic losses and a common region of deletion at this chromosomal region in breast cancers (Fukino et al. 1999).

The cytoplasmic domain of ITGB4 activates the p21 pathway of growth arrest and apoptosis (Clarke et al. 1995). As the expression of *ITGB4* is reduced in primary and metastatic breast cancer, the combined data suggest that the *ITGB4* gene is a candidate for involvement in the allelic losses that occur on 17q in some tumors. The polymorphisms documented here will be useful not only for examining loss of heterozygosity (LOH) in the chromosomal regions where the *ITGB4* gene is located (17q24–25) but also for studying associations between specific SNPs and cancer susceptibility.

Table 1. Primer sequences to amplify eight regions which contain SNPs

Name of primer	Nucleotide sequences	Product size (bp)
Exon 5-1F	5′-GCCAGGGAATGGGTGCTGCC-3′	154
Exon 5-1R	5′-TAGCCAGGCTGAGCATGGGG-3′	
Exon 15F	5′-ACAGCTGGCTCACTGGTGC-3′	163
Exon 15R	5′-ACGCCCACACCTCGTAGGT-3′	
Exon 21F	5′-GGTGACCACCTCCATCTCAC-3′	179
Exon 21R	5′-TCCGGCCAGGCCTAGTTCC-3′	
Exon 25-2F	5′-GAAGCAGCTGCTGGTGGAGG-3′	184
Exon 25-2R	5′-CCACGTGGCCAGGTCGCAAT-3′	
Exon 34-2F	5′-GCTAAGCACATCCTCCACCC-3′	145
Exon 34-2R	5′-GAGAGGCAGGGTTGGCTGA-3′	
Exon 35-2F	5′-TCGGGCTCAGATGAAAGGGT-3′	130
Exon 35-2R	5′-AGGACACTGTGGAGACAGGA-3′	
Exon 39F	5′-GCATAGGCTGAAGGCATCTT-3′	224
Exon 39R	5′-TGGGGGTGAAGGAAAGGAC-3′	
Exon 41F	5′-AAGCCAGGTCATTTAATGCCTC-3′	214
Exon 41R	5′-GACGCGCTAGTGGGACATGG-3′	

SNP, Single-nucleotide polymorphism

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Table 2. Genotypes and allele frequencies of the integrin β4 gene

Region	Nucleotide change	Amino acid change	Genotypes (n = 48)			Allele frequencies (%)	
Exon 5	293G > A	R98H	G/G	G/A	A/A	G	A
			47	1	0	99	1
Exon 15	1821G > A	S607S	G/G	G/A	A/A	G	A
			27	14	7	71	29
Exon 21	2531G > T	R844L	G/G	G/T	T/T	G	T
			47	1	0	99	1
Intron 25	2962 + 11 G > A	Intronic variant	G/G	G/A	A/A	G	A
			47	1	0	99	1
Intron 25	2962 + 18 G > C	Intronic variant	G/G	G/C	C/C	G	C
			46	2	0	98	2
Exon 34	4311G > C	P1437P	G/G	G/C	C/C	G	C
			8	29	11	47	53
Exon 35[a]	4496C > T	S1499F	C/C	C/T	T/T	C	T
			36	11	1	87	13
Exon 39	4845G > A	G1615G	G/G	G/A	A/A	G	A
			46	2	0	98	2
Exon 41	5123C > T	P1708L	C/C	C/T	T/T	C	T
			33	11	4	80	20

[a] Integrin β4 contains alternatively spliced exon 35

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