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Mutation analysis of *hBUB1*, *hBUBR1* and *hBUB3* genes in glioblastomas

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Abstract Glioblastomas, the most malignant human brain tumors, are characterized by marked aneuploidy, suggesting chromosomal instability which may be caused by a defective mitotic spindle checkpoint. We screened 22 glioblastomas for mutations in the mitotic spindle checkpoint genes *hBUB1*, *hBUBR1* and *hBUB3*. DNA sequencing revealed a silent mutation at codon 144 of *hBUB1* (CAG→CAA, Gln→Gln) in one glioblastoma, a silent mutation at codon 952 of *hBUBR1* (GAC→GAT, Asp→Asp) in another glioblastoma, and a silent mutation at codon 388 of the *hBUBR1* gene (GCG→GCA, Ala→Ala) in 8 glioblastomas. We also observed a known polymorphism at *hBUBR1* codon 349 (CAA/CGA, Gln/Arg), with an allelic frequency of 0.75 for Gln and 0.25 for Arg, which is similar to that among healthy Caucasian individuals (0.73 vs 0.27). The coding sequence of the *hBUB3* gene did not contain any mutation, but in 4 glioblastomas (18%), a C→T point mutation was detected at position –6 (6 nucleotides upstream of the ATG initiator codon). Analysis of blood DNA of these patients showed identical sequence alterations, indicating that this is a polymorphism. Again, the frequency in glioblastomas was similar to that in healthy Caucasians (15%). We further screened *hBUB1* in 18 cases of giant cell glioblastoma, a variant characterized by a predominance of bizarre, multinucleated giant cells. There were no changes, except for a silent mutation at codon 144 in two cases. These results suggest that mutations in these mitotic spindle checkpoint genes do not play a significant role in the causation of chromosomal instability in glioblastomas.

Keywords Mitotic checkpoint · *hBUB1/hBUBR1/hBUB3* · Glioblastoma · Giant cell glioblastoma · Polymorphism

Introduction

Genetic instability is a critical phenomenon in the development of malignant human neoplasms and occurs in two different forms, microsatellite instability and chromosomal instability [22, 24, 27, 37]. In subsets of colorectal, gastric and endometrial cancers, defective mismatch repair results in increased mutation rates at the nucleotide level and, consequently, widespread microsatellite instability [22, 24, 27, 37]. Tumors with microsatellite instability usually have a normal complement of chromosomes, i.e., a diploid or near-diploid karyotype [27]. In most other neoplasms, including brain tumors, genetic instability is typically observed at the chromosomal level and may involve gain and loss of whole chromosomes, leading to aneuploidy [27, 35]. The molecular basis of chromosomal instability is not yet fully understood, but one possible mechanism is disruption of the mitotic checkpoint [27, 35], i.e., a conserved function that delays anaphase in the presence of spindle damage [40], so as to increase the probability of successful delivery of an euploid genome to each daughter cell.

Three mitotic-arrest-deficient (*MAD*) genes, *Mad1*, *Mad2*, and *Mad3* [14, 15, 28], three budding-uninhibited-by-benzimidazole (*BUB*) genes, *Bub1*, *Bub2*, and *Bub3*, [15, 39] and *MPS1* [15, 28, 48] have been identified as being required for the execution of the mitotic checkpoint in the yeast *Saccharomyces cerevisiae*. Several human homologues have been identified, including *hMPS1* (TTK/*MPS1L1* located on chromosome 6q13–21) [6], *hMAD1L1* (on chromosome 7pter–7p15) [6, 19], *hMAD2* (*hMAD2L1*, on chromosome 4q27) [29], *hMAD2B* (*hMAD2L2*, on chromosome 1p36) [6], *hBUB1* (on chromosome 2q14) [5, 6], *hBUBR1* (*hBUB1B/MAD3-L* on chromosome 15q14) [5, 8] and *hBUB3* (on chromosome 10q24–26) [6, 25].

Mutational inactivation of mitotic spindle checkpoint genes appears to be involved in the evolution of some human neoplasms, in particular those with aneuploidy.

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Cahill et al. [5] detected *hBUB1* and *hBUBR1* mutations in 2 each of 19 colon cancer cell lines with chromosomal instability. Mutations in the *hBUB1* gene were shown to act in a dominant-negative manner, if the exogenous expression of the mutant allele disrupts the mitotic checkpoint in euploid cell lines [5]. A somatic mutation in the *hBUB1* gene was identified in 1 of 32 sporadic digestive tract cancers [16], in 2/148 lung cancers [12, 41], and in 1/10 cases of 10 adult T cell leukemia/lymphomas [34] that, in addition, contained *hBUBR1* missense mutations and deletion in 3 cases. In contrast, no *hBUB1* mutation was found in head and neck squamous cell carcinomas and lung cancer cell lines [49]. *hBUB1* and *hBUBR1* mutations were also reported absent in 19 aneuploid breast cancer cell lines [31]. *hBUBR1* mutations were not found in 47 lung cancers [41]. In addition, no *hBUB3* mutation was detected in 19 colon cancer cell lines [5], suggesting that mutational inactivation of these checkpoint genes is a rare event in carcinomas and malignant lymphomas.

Glioblastomas are highly aneuploid tumors and frequently show loss of heterozygosity (LOH) on several chromosomes [3, 7, 11, 30, 38, 46, 47] in up to 90% of cases. LOH has frequently been observed on chromosome 10q, where *hBUB3* is located. The objective of the present study was to assess whether mutational inactivation of the *hBUB1*, *hBUBR1* and *hBUB3* genes is involved in the evolution of the glioblastoma phenotype.

Material and methods

Tumor samples

Twenty-two glioblastomas (WHO grade IV) were obtained from patients at the Department of Neurosurgery, University Hospital Zurich, Switzerland. Two glioblastomas (cases 72 and 295) were secondary glioblastomas that had progressed from low-grade astrocytoma. Other glioblastomas were primary (*de novo*) glioblastomas with a clinical history of less than 3 months. The age and sex of the patients are shown in Table 4. Twelve patients were males and ten were females. The mean age of patients at diagnosis was 55±12 years (range 28–71 years).

Tumor samples were immediately frozen in liquid nitrogen and stored at –80°C. RNA and DNA were simultaneously extracted from the same portions (about 200 mg) of tumors using the TRIZOL (Gibco BRL, Cergy Pontoise, France) according to the manufacturer's instructions.

The sources of 18 formalin-fixed, paraffin-embedded specimens of giant cell glioblastomas were reported previously [36]. Tumor samples included only typical cases showing a predominance of multinucleated monstrous, glial fibrillary acidic protein (GFAP)-positive tumor cells in at least one large area of the biopsy specimens. The occurrence of occasional giant cells in a glioblastoma was not considered diagnostic for this variant. Eleven patients were males and 7 were females. The mean age of patients was 42±20 years (range 9–78 years).

hBUB1 mutations

Screening for *hBUB1* mutations was performed by polymerase chain reaction-single-strand confirmational polymorphism (PCR-SSCP) analysis using genomic DNA. PCR amplification of the 25 exons was performed using the primers described in Table 1. Briefly, PCR was performed in a total volume of 10 µl, containing 1 µl DNA solution, 0.75 U Taq DNA polymerase (Sigma, St.

Louis, Mo.), 0.5 µCi [α -³²P]dCTP (ICN Biomedicals, N.V.S.A., Belgium; specific activity, 3,000 Ci/mmol), 1.5–2.5 mM MgCl₂, 0.2 mM of each dNTP, 0.2 µM of both sense and antisense primers, 10 mM TRIS-HCl, pH 8.3, and 50 mM KCl. PCR amplification was performed in a Genius DNA Thermal Cycler (Technique, Cambridge, UK) as follows: initial denaturation for 2 min at 95°C, 35–40 cycles with denaturation at 95°C for 1 min, annealing at 50–58°C for 1 min and extension at 72°C for 1 min, followed by a final extension step for 5 min at 72°C. After PCR, 5 µl of the products was mixed with 12.5 µl of loading buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol and bromophenol blue), denatured at 95°C for 10 min, and quenched on ice. Of the above mixture 4 µl was run on a 6% polyacrylamide non-denaturing gel containing 6% glycerol at 6 W for 14 h. Gels were dried at 80°C and autoradiographed for 12–48 h.

Samples which showed mobility shifts in the SSCP analysis were further analyzed by direct DNA sequencing. PCR products were purified on MicroSpin™ S-300 HR Columns (Pharmacia Biotech, Uppsala) and sequenced using the Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit (USB, Cleveland, USA) according to the manufacturer's instructions.

hBUBR1 mutations

First-strand cDNA of the *hBUBR1* gene was synthesized as follows: 5 µg of total RNA in a total volume of 10 µl was heated to 70°C for 10 min and then incubated at 42°C for 50 min following addition of 10 µl of a mixture containing 1x 1st strand cDNA buffer (Gibco BRL), 10 mM DTT, 1 mM dNTPs, 25 µg oligo-dT primers and 200 U moloney murine leukemia virus reverse transcriptase (M-MuLV RT, Gibco BRL). cDNA samples were then made up to 40 µl with distilled water and kept at –20°C.

Pre-screening for *hBUBR1* mutations was carried out by PCR-SSCP analysis. The entire coding region of the 3,583-bp *hBUBR1* cDNA was amplified as 14 overlapping fragments. PCR was performed in a total volume of 10 µl, containing 0.5 µl cDNA solution, 0.75 U Taq DNA polymerase (Sigma), 0.5 µCi [α -³²P] dCTP (ICN Biomedicals, specific activity, 3,000 Ci/mmol), 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.2 µM of sense and antisense primers, 10 mM TRIS-HCl, pH 8.3, and 50 mM KCl, in a Thermo Cycler (Stratagene, La Jolla, Calif.) as follows: initial denaturation for 2 min at 95°C, 35–40 cycles of denaturation at 95°C for 1 min, annealing at 50–58°C for 1 min followed by extension at 72°C for 1 min, and a final extension step for 5 min at 72°C. Primer sequences are shown in Table 2. After PCR, 5 µl of products were mixed with 12.5 µl loading buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol and bromophenol blue), denatured at 95°C for 10 min, and quenched on ice. Samples of the above mixture (4 µl) were run on a 6% polyacrylamide non-denaturing gel containing 6% glycerol at 6 W for 14 h. Gels were dried at 80°C and autoradiographed for 12–48 h. Samples that showed mobility shifts in the SSCP analysis were further analyzed by direct DNA sequencing as described above.

A polymorphism at codon 349 (CAA/CGA, Gln/Arg) was detected in this study. To assess whether particular alleles are over-represented in glioblastomas, we also analyzed blood DNA obtained from 67 healthy caucasians individuals for this polymorphism.

hBUB3 mutations

First-strand cDNA of the *hBUB3* gene was synthesized as described above.

Pre-screening for *hBUB3* mutations was carried out by PCR-SSCP analysis. The entire coding region of the 1,078-bp *hBUB3* cDNA was amplified as 5 overlapping fragments. Primer sequences are shown in Table 3. Briefly, PCR was performed in a total volume of 10 µl, containing 0.5 µl cDNA solution, 0.75 U Taq DNA polymerase (Sigma), 0.5 µCi [α -³²P] dCTP (ICN Biomedicals, specific activity, 3,000 Ci/mmol), 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.2 µM of sense and antisense primers, 10 mM TRIS-HCl, pH 8.3, and 50 mM KCl, in a Thermo Cycler (Stratagene, La

Table 1 Primers used for SSCP analyses of the *hBUB1* gene

Exon		Primer sequence	Annealing temperature (°C)	Product (bp)
1	Sense	TTCTAGTTTGCGGTT	48	75
	Antisense	TGAAGGACATTTTCC		
2	Sense	GAAGTATATCTTTTGCTTGTAGAA	52	175
	Antisense	GGGGCAGTGTATAGTTTGTT		
3	Sense	TAAACAAACTATACACTGCC	48	248
	Antisense	AAAGCAAAAGTACAACATGA		
4	Sense	TTTTTGTTTTGTTCGTGT	56	247
	Antisense	ATCACAGAGAGTTTGACTTTGTAAC		
5	Sense	ATTGATGCCTTTTCTGCTGT	52	94
	Antisense	GCATTTTAGAAAGCCTGATT		
6	Sense	ATTATGTGATAATTTTACTTAC	53	250
	Antisense	AAAGTGGATGTAGAAGGCAG		
7	Sense	TGTTTTGTGAAGTAATTTGG	54	179
	Antisense	TAAGAGCACTTAAAGGAAAC		
8	Sense	TGCTCTTAGGTACATTGTTG	48	264
	Antisense	AAGAATAACTAAAAATACAAGATT		
9	Sense	ATGCAAACCATTTCTATCTTTC	60	199
	Antisense	CATGAGGAGCACACATAACC		
10	Sense	GATGTAATGCCTGATTAGTAG	54	370
	Antisense	CACATCACTGTGATCTCTAG		
11	Sense	AGATCAAGCATTATTCTCTAG	54	177
	Antisense	AAAATACACCTCTGAGTGATAC		
12	Sense	TTCTGATTTCCCTACTGATACCAC	62	223
	Antisense	CATTAACCTGTCATCAGTGCTAC		
13	Sense	TGATGACAAGTTAATGAAAGCTCT	56	231
	Antisense	TTTCTGTGATAACCACCTATAATG		
14	Sense	TGATATTAGTTCTTTTCTGTCAGCT	58	254
	Antisense	CGGCAGCATCCCATTAAAC		
15	Sense	CAAGTGATAGCATTATTTCCT	58	183
	Antisense	GTAGGACCCATTTCATAGATAA		
16	Sense	CTTCTTTAATCCTCATTTCCTTGG	61	331
	Antisense	GAATCAAAGTTGGCAGAAGAC		
17	Sense	ATCTTGTCTCTAATTTTTGAATCT	52	139
	Antisense	ACCAAATAAACCCCTCACAAT		
18	Sense	GGTTTCTTTATTCCTTTCACAC	62	297
	Antisense	AAGGGCATAACAAAGAGTGAG		
19	Sense	TTTTTTTTTCTCATTCCCTT	48	232
	Antisense	CGTTACCATCAACTTCTCATAG		
20	Sense	TAAAATAAAAGGCGTACATAAA	48	192
	Antisense	TCATACAAAATACAACCTCAGGT		
21	Sense	ATCATTTTTCAGAATACGAG	55	248
	Antisense	ATTCATGTGCTCATCATAA		
22	Sense	AACTCAGGATTCACCTT	56	215
	Antisense	AACCCTGTTAAAAGCATA		
23	Sense	ATAGCAGCCTTTCATCATTTGT	60	261
	Antisense	TATTTCCAAGTCCCTCACTTA		
24	Sense	CATCTTGATATAGTCATGTGTA	44	226
	Antisense	TTAGTTATTCTCTTTTCACTAC		
25	Sense	ATCCTGCATATTGAAGGCTAC	56	289
	Antisense	CAGTGTGATTTTTAAGGACTG		

Jolla, Calif.) as follows: initial denaturation for 2 min at 95°C, 35–40 cycles with denaturation at 95°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min, followed by a final extension for 5 min at 72°C. Samples which showed mobility

shifts in the SSCP analysis were further analyzed by direct DNA sequencing as described above.

A polymorphism at position –6 was detected in glioblastomas in this study. To assess the allelic frequency, we checked for the

Table 2 Primers used for SSCP of the *hBUB1* gene

Fragment		Primer sequence	Annealing temperature (°C)	Product (bp)
1	Sense	CGGAAGAAAGCCCAGGCGGT	58	298
	Antisense	AGGGTCATTTCCAGTGTAA		
2	Sense	CAAGAATCTGCCTGTAAACA	54	269
	Antisense	AAAGGCTCATTGCATAAAC		
3	Sense	AGAAAAACGATATTATAGTGA	50	312
	Antisense	TCCTCCTCTTCTTCTTCTC		
4	Sense	CAGCACCGACAATTCCAAGC	56	277
	Antisense	TAGAAGCCTCATCAGCATTT		
5	Sense	CCTCAACAGATGCAAAATAA	58	279
	Antisense	TGGTGTCACTAAGTGGCTGT		
6	Sense	CTCGTGGCAATACAGCTTC	50	316
	Antisense	CTTTGCTCTTTTAATTTCT		
7	Sense	TTTATGCAGGAGTAGGGGA	50	272
	Antisense	AGTCATTCTGGTATTTTC		
8	Sense	TACAAAGGAGACAATAA	53	323
	Antisense	ATTCATCACAAACATCTGG		
9	Sense	TCAAAACCTCAGAAAGCAT	53	338
	Antisense	AATAATTGGGCTCAGCTTC		
10	Sense	CAGCTTGTGGCACTATCTA	52	313
	Antisense	TCCTTCTCAATTTCCAAC		
11	Sense	TATAGAAGACAGACCAATG	51	311
	Antisense	GAAGATCCTGAAGGGTGAA		
12	Sense	TGGCTGTATTGTTTGGCAC	58	296
	Antisense	CGCTGAGGGTAAAAACATC		
13	Sense	TTCCTACAGTGTGACCTT	56	309
	Antisense	AGCTCCCCAAGAACAGACA		
14	Sense	CTGAATGCCAATGATGAGG	54	236
	Antisense	TAAATTACAGCACATACAG		

Table 3 Primers used for SSCP analyses of the *hBUB3* gene

Fragment		Primer sequence	Annealing temperature (°C)	Product (bp)
A ^a	Sense	GCGAGTGGCGAGTAGTGGA	58	78
	Antisense	CCCGTCCCGCACACC		
1	Sense	CGTTGCTTCTGAGGGGAG	58	252
	Antisense	ATCTAGTCCCTCCACTCCAGGC		
2	Sense	GCCTTCTACGATCCAACGC	58	259
	Antisense	GGTCTCCAGACACTGAGAGGG		
3	Sense	TCCTTGTAATGCTGGGACCT	58	267
	Antisense	TTCTGTACCTCAGGGCTTGG		
4	Sense	CCGAGTGGCAGTTGAGTATTT	58	268
	Antisense	CGTAGTCCCATCATTACTGAAGG		
5	Sense	GGTACCCACGAGCATCG	58	234
	Antisense	ATCCACCATTGGGGAGTACG		

^aA denotes pair of primers for part of genomic 5' region

presence of this variant at genomic level in blood DNA obtained from 60 healthy caucasians individuals.

Gln→Gln) in one of 22 glioblastomas (case 300, Table 4 and Fig. 1) and in 2 out of 18 giant cell glioblastomas. In all cases, the wild-type base G was also present.

Results

hBUB1 mutations

The PCR-SSCP analysis of all 25 exons of the *hBUB1* gene revealed a silent mutation at codon 144 (CAG→CAA,

hBUB1 mutations

SSCP followed by DNA sequencing of the entire coding sequence of *hBUB1* revealed a silent mutation (GAC→GAT, Asp→Asp) at codon 952 in one glioblas-

Table 4 Genetic alterations in mitotic checkpoint genes in glioblastomas

Patient ID	Age/sex	<i>hBUB1</i> alteration	<i>hBUBR1</i> alteration	<i>hBUB3</i> alteration	
			Codon 349	Other codon	
72	28/F	—	Gln/Arg	—	Position -6, C→T ^a
201	63/M	—	Gln/Arg	GCG→GCA, Ala→Ala 388	—
233	59/M	—	Gln/Gln	GCG→GCA, Ala→Ala 388	—
234	43/M	—	Gln/Arg	—	—
256	56/F	—	Gln/Gln	—	—
257	50/M	—	Gln/Arg	—	Position -6, C→T ^a
258	65/F	—	Gln/Gln	GAC→GAT, Asp→Asp 952	Position -6, C→T ^b
287	47/F	—	Gln/Gln	GCG→GCA, Ala→Ala 388	—
288	69/M	—	Gln/Gln	GCG→GCA, Ala→Ala 388	—
289	58/F	—	Gln/Gln	GCG→GCA, Ala→Ala 388	—
290	63/F	—	Gln/Gln	—	—
292	62/M	—	Gln/Arg	GCG→GCA, Ala→Ala 388	—
295	47/M	—	Gln/Gln	—	—
296	63/F	—	Gln/Gln	GCG→GCA, Ala→Ala 388	—
298	30/M	—	Gln/Gln	—	—
299	50/F	—	Gln/Gln	—	—
300	71/F	CAG→CAA, Gln→Gln 144	Arg/Arg	—	—
301	68/F	—	Gln/Arg	—	—
302	64/M	—	Gln/Gln	GCG→GCA, Ala→Ala 388	—
303	67/M	—	Arg/Arg	—	—
314	40/M	—	Gln/Arg	—	—
344	47/M	—	Gln/Gln	—	Position -6, C→T ^b

^aHomozygous mutation^bHeterozygous mutation **Fig. 1** Representative DNA sequencing autoradiographs of silent mutations in the *hBUB1* gene (codon 144, CAG→CAA, Gln→Gln) and the *hBUBR1* gene (codon 952, GAC→GAT, Asp→Asp), and C→T change at -6 upstream of the initiator codon of the *hBUB3* gene. In cases 258 and 300, wild-type alleles are present, while in case 344, the wild-type allele is absent

These frequencies in glioblastomas were similar to those in blood DNA samples from healthy Caucasian individuals (0.73 for Gln and 0.27 for Arg).

hBUB3 mutations

A point mutation (C→T) was detected at 6 nucleotides upstream of the ATG initiator codon (position -6) in 4 of 22 (18%) glioblastomas (Table 4, Fig. 1). Of these, the wild-type base was also present together with the mutated sequence in 2 cases, whereas the other 2 showed only mutated sequences. The analysis of blood DNA from these 4 patients showed identical alterations to those detected in tumor tissues, indicating that this alteration is a polymorphism. Screening for this polymorphism in blood DNA from healthy Caucasian individuals revealed the presence of this mutation in 9/60 (15%) individuals, a similar frequency to that in the glioblastoma patients.

Discussion

toma and another silent mutation (GCG→GCA, Ala→Ala) at codon 388 in 8 glioblastomas (Fig. 1, Table 4).

We also identified a common polymorphism at codon 349 (CAA/CGA, Gln/Arg), with allelic frequencies of 0.75 for Gln and 0.25 for Arg in glioblastomas (Table 4).

Mitotic checkpoint genes play a significant role in chromosome segregation by preventing aneuploidy [1, 5, 13, 35, 40]. During mitosis, *hBUB1*, *hBUBR1* and *hBUB3* proteins localize in kinetochores before chromosome alignment and form a checkpoint kinase complex with

other proteins. The *hBub1* and *hBUBR1* genes encode protein kinases, while the *hBUB3* protein is required for kinetochore localization of *hBUB1* and *hBUBR1* proteins [1, 17, 42, 45]. In *Drosophila*, *BUB1* mutations cause chromosome missegregation and fail to block apoptosis [2]. In mice, *BUB1* is required not only for checkpoint response to spindle damage but also for normal mitotic timing, and for initiation of apoptosis when an apoptotic signal is present [44]. A recent study showed that the *BRCA2* gene, that is associated with inherited susceptibility to breast cancer and plays a role in replication and repair of DNA during S phase, is phosphorylated by *hBUBR1* and may be involved in a mitotic checkpoint [10]. *Bub1* mutations were found in three out of four thymic lymphomas that developed in *Brca2*-deficient mice, suggesting that inactivation of these genes works synergistically in development of these tumors [26]. *Bub3*-null mouse embryos do not survive [20]; the embryos showed accumulation of several mitotic errors in the form of micronuclei, chromatin bridging, lagging chromosomes and irregular nuclear morphology, confirming that *Bub3* is essential for normal mitosis and for early embryonic development in mice [20].

In the present study, we investigated the status of the mitotic spindle checkpoint genes *hBUB1*, *hBUBR1* and *hBUB3* in glioblastomas. Screening of the entire coding sequence and intron-exon boundaries of the *hBUB1* gene revealed a silent mutation at codon 144 (CAG→CAA, Gln→Gln) in one glioblastoma and two giant cell glioblastomas. Although we were unable to analyze normal tissue samples from these patients, this alteration is likely to be a polymorphism, since it has been previously reported in several colon tumors [6]. To date, seven different *hBUB1* polymorphisms have been reported in human tumor cell lines [6, 12, 41, 49]. It remains to be shown whether any particular polymorphism is over-represented in cancer patients.

The analysis of the *hBUBR1* gene in this study showed two silent mutations at codons 952 and at 388, both of which have previously been detected in colon and lung tumors [6, 41], also suggesting a polymorphism. We found another polymorphism at codon 349 (CAA/CGA, Gln/Arg), which has previously been reported in colon and breast carcinomas [6, 21]. The allelic frequencies of this variant in healthy individuals in Japan are 0.31 for Gln and 0.69 for Arg [21]. We found an opposite allelic pattern in glioblastomas (0.75 for Gln and 0.25 for Arg). However, our analysis of DNA from healthy Caucasian individuals showed a frequency similar to that observed in glioblastomas. This indicates that the frequency of these alleles varies significantly between ethnically different populations, but they are not over-represented in glioblastoma patients.

In the present study, C→T mutations were detected at 6 nucleotides upstream of the ATG initiator codon of the *hBUB3* gene in 4 glioblastomas. The same mutation was found in blood DNA of these patients, suggesting that this is a new polymorphism. We also carried out screening for this allelic variant in 60 healthy Caucasian individuals, and

found its frequency to be similar to that in glioblastomas, suggesting that the presence of this polymorphism does not influence susceptibility to development of glioblastomas.

LOH on chromosome 10, in particular, loss of the 10q25-qter region, is the most frequent genetic alteration in glioblastomas, suggesting the presence of putative tumor suppressor gene(s) [38]. The *hBUB3* gene is mapped on 10q24-q26 [6, 25, 45]. However, the absence of miss-coding mutations in *hBUB3* in this study excludes *hBUB3* as a candidate tumor suppressor gene involved in the pathogenesis of glioblastomas. We correlated the data of base change at 6 bp upstream of initiating codon in *hBUB3* and LOH on chromosome 10 published previously [9], but did not find any correlation (data not shown).

Occasional multinucleated neoplastic giant cells are a common histological feature in glioblastomas [4, 23]. Giant cell glioblastomas are defined by the predominance of large bizarre, multinucleated giant cells measuring up to 500 µm [33], with near-haploid or polyploid clones [4]. Our present results show that mutational inactivation of the *hBUB1* gene is not associated with giant cell phenotype. Due to unavailability of frozen samples for the generation of cDNA, we could not perform a mutational analysis of *hBUBR1* and *hBUB3* genes.

In conclusion, this study shows absence of miss-coding mutations in *hBUB1*, *hBUBR1* and *hBUB3* genes in human glioblastomas, suggesting that mutational inactivation of these genes is not significantly involved in the aneuploidy of glioblastomas. Additional studies may be necessary to clarify whether these genes are inactivated through other mechanisms. Jaffrey et al. [18] recently detected microsatellite instability within the *hBUB1* gene in approximately 15% of colorectal cancers, raising the possibility that down-regulation of *hBUB1* may occur through instability. It also remains to be elucidated whether alterations of other mitotic spindle checkpoint genes, including *hMAD1*, *hMAD2*, and *MPS1*, are present in glioblastomas. However, lung, colon and digestive tract human neoplasms have a very low frequency or absence of inactivating mutations of these genes [6, 16, 32, 43].

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