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Characterization of *MAD2B* and Other Mitotic Spindle Checkpoint Genes

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Aneuploidy is a characteristic of the majority of human cancers, and recent work has suggested that mitotic checkpoint defects play a role in its development. To further explore this issue, we isolated a novel human gene, *MAD2B (MAD2L2),* which is homologous to the spindle checkpoint gene *MAD2 (MAD2L1).* We determined the chromosomal localization of it and other spindle checkpoint genes, including *MAD1L1, MAD2, BUB3, TTK (MPS1L1),* and *CDC20.* In addition, we resolved the genomic intron–exon structure of the human *BUB1* gene. We then searched for mutations in these genes in a panel of 19 aneuploid colorectal tumors. No new mutations were identified, suggesting that genes yet to be discovered are responsible for most of the checkpoint defects observed in aneuploid cancers.

INTRODUCTION

Genetic instability has long been thought to drive the development of cancer (Boveri, 1914; Hartwell, 1992) and an elevated mutation rate has been proposed to account for the mutational load found in human tumors (Loeb, 1991). Experiments on colorectal tumors have shown that genetic instability arises in two different forms (Lengauer *et al.,* 1997). In a small fraction of cases, mutations in mismatch repair genes give rise to instability at the nucleotide level (Jiricny, 1998; Kolodner, 1995; Perucho, 1996; Modrich, 1997). These cancers, termed MIN because of their characteristic microsatellite instability, accumulate mutations in oncogenes and tumor suppressor genes due to an elevated nucleotide mutation rate. MIN cancers have a diploid or near-diploid karyotype. In the majority of colon cancers, and likely in most other tumor types, a different kind of instability is observed (Lengauer *et al.,* 1997). This instability is manifest at the chromosomal level, and is characterized at the cytogenetic level by marked aneuploidy.

It has recently been shown that the mitotic checkpoint is consistently defective in cancer cells with chromosomal instability (CIN). In a small fraction of CIN cases, this inactivation was associated with mutations in the human mitotic checkpoint gene *BUB1* (Cahill *et al.,* 1998). Many genes whose products play a role in the mitotic spindle checkpoint have been identified in microbes. In the yeast *Saccharomyces cerevisiae,* the BUB (budding uninhibited by benzimidazole)-family genes *Bub1, Bub2,* and *Bub3* (Hoyt *et al.,* 1991; Roberts *et al.,* 1994) and MAD (mitotic arrest deficient)-family genes *Mad1, Mad2,* and *Mad3* (Hardwick and Murray, 1995; Li and Murray, 1991) were initially identified in screens for mitotic checkpoint defects. Orthologs of *Mad1, Mad2, Bub1,* and *Bub3* have been characterized in a number of organisms (Chen *et al.,* 1996; He *et al.,* 1997; Taylor and McKeon, 1997; Efimov and Morris, 1998), including humans (Li and Benezra, 1996; Jin *et al.,* 1998; Taylor *et al.,* 1998). While human orthologs of *Bub2* have not yet been identified, *Mad3* is homologous to the N-terminal portion of the human *BUBR1* gene, suggesting that *BUBR1* may be the human equivalent of yeast *Mad3* (Chan *et al.,* 1998; Taylor *et al.,* 1998).

Additional work has shown that the yeast genes *Mps1* (Hardwick *et al.,* 1996; Weiss and Winey, 1996) and *Cdc20/slp1+* (Hwang *et al.,* 1998; Kim *et al.,* 1998; Schott and Hoyt, 1998) also function in the spindle checkpoint pathway. A human homolog of *Cdc20,* known as p55CDC or *CDC20,* has been implicated as a spindle checkpoint mediator in mammalian cells (Fang *et al.,* 1998; Kallio *et al.,* 1998). *Mps1* is highly homologous to a previously identified human gene, *TTK* (here referred to as *MPS1L1*) (Mills *et al.,* 1992). The high degree of sequence relatedness among these genes suggests that they have a conserved, functional role in the spindle checkpoint in human cells (Hardwick, 1998).

Because the mitotic checkpoint appears to be widely inactivated in aneuploid colorectal tumors, we sought

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TABLE 1

Mapping Locations of Human Mitotic Spindle Checkpoint Genes[a]

Human gene	Accession No.	Primer set	Linked framework marker/STS	Corresponding cytogenetic location
MAD1L1	U33822	CCCAGGTTCTCTGCAGATGC GCTGCCAGGCACTCTCAG	SHGC-33698	7pter–7p15
MAD2 (MAD2L1)	U65410	CGCGTGCTTTTGTTTGTGTC AGGTTTCAGATGGATATATGC	D4S2347	4q27
MAD2B (MAD2L2)	AF139365	CCTCCACTGCTGTCCATC ATTGAAACAACTCACAGCACAG	D1S2740	1p36
		ACCATGACCACGCTCACACGAC TCGCGCACGTAGAGGATG	D1S434	1p36
BUB1	AF046078	TCCCTCCCTGGAGGTTCAGC CAAAACTAGAAGGATTTCCCTG	SHGC-37233	2q14
BUBR1	AF046079	GATTGTAACAAGAACAATCAAGC GTGTCCTAAATTAAATTACAGCAC	D15S146	15q14
BUB3	AF053304	GGTACCCCACGAGCATCG ATCCACCATTGGGGAGTACG	SHGC-13269	10q24–q26
TTK (MPS1L1)	M86699	GAAATATTGATCTTAATAGTTGGC GCCTCTAACATATTTTTCCAG	D6S1866	6q13–q21
HCDC20	U05340	GTAGGCACCAACTGCAAGG GGGGATGGGTGCATCCAGC	D9S1680	9q12–q22

[a] Amplimers from the genomic locus of each gene were scored against the Stanford G3 radiation hybrid panel. The markers that were most strongly linked, and their corresponding cytogenetic locations, are shown.

to further identify and characterize the genes in this pathway in the human system.

MATERIALS AND METHODS

Identification of MAD2B (MAD2L2). The sequences of human *MAD2* (nucleotide) and yeast *MAD2* (protein) were used to search the NCBI expressed sequence tag (EST) database, using the Gapped-BLAST blastn and tblastn algorithms, respectively. Multiple overlapping EST clones (Accession Nos. AA076651, AA079559, AA172174, AA172327, AA293812, AA662845, D20372, F20535, R02577, R02696, R54743, W77999, and W79458) were identified, and a preliminary sequence contig was assembled. Polymerase chain reaction (PCR) primers were designed on the basis of the presumptive cDNA sequence, and the gene was amplified by reverse transcription PCR (RT-PCR) from multiple human tissues. RACE (Marathon, Clontech) was performed to isolate the 5′ end of the gene. Sequencing of these products provided corrections to the EST contig, allowing the final sequence to be determined.

Characterization of BUB1 genomic structure. The intron–exon structure of *BUB1* (except for exon 1) was determined using three techniques: direct sequencing of a BAC clone containing the *BUB1* gene, sequencing of genomic DNA PCR products that spanned introns of the gene, and inverse PCR to isolate adjacent intronic sequences. The primer pairs used to amplify each exon are listed in Fig. 2B.

Radiation hybrid mapping. Primer sets were designed to amplify a genomic DNA fragment from each target gene, as indicated in Table 1. Each genomic PCR product was sequenced to ensure that the amplification originated from the genuine genomic locus rather than from a pseudogene. This gene-based sequence-tagged site (STS) was then scored against the Stanford G3 radiation hybrid panel (lod scores > 6.0) and the corresponding map location determined by statistical linkage with a framework marker (see Stanford G3 Radiation Hybrid website at http://www.shgc.stanford.edu/RH/rhserver_form2.html).

Tumor samples. A panel of 19 CIN cell lines were used for this study. Four of the cell lines (SW837, HT29, CACO2, SW480) are well-characterized colon cancer lines in common laboratory use, obtained from ATCC. The remaining 15 lines were early-passage lines derived from primary colon cancers (Markowitz *et al.*, 1994; Cahill *et al.*, 1998). The presence of CIN was established by karyotypic or allelotypic analysis (Cahill *et al.*, 1998). RNA from the lines was prepared by CsCl gradient ultracentrifugation of guanidine isothiocyanate-lysed cells. First-strand cDNA synthesis was performed using random hexamers and Superscript II (Life Technologies), following the manufacturer's recommendations. In each case, a duplicate reaction containing all of the components except reverse transcriptase was used as a negative control to exclude PCR contamination by genomic DNA.

Mutational analysis. PCR was performed with Platinum *Taq* polymerase (Life Technologies) using the following conditions: an initial denaturation of 95°C for 1 min, then 38 cycles of 95°C for 30 s, 56°C for 30 s, and 70°C for 2 min, with a final extension step of 70°C for 5 min. The *MPS1L1* transcript was amplified in two overlapping segments using the following primers: (segment 1) 5′-GTGGCAGAGAATTGACAATTG-3′, 5′-GAACCTCCACTTCCTATCTGC-3′; (segment 2) 5′-CACTCCACTTCAAAATTTACAGG-3′, 5′-AATATATTTTATACCTCCTATCTG-3′. The sequences of these fragments were determined with the following primers: (segment 1) 5′-GTGGCAGAGAATTGACAATTG-3′, 5′-CAGTGTTCCGCTAAGTGATG-3′, 5-AGCTGTAGAACGTGGAGCAG-3′, 5′-AAGATGCAGAAATAGGTTACCG-3′, 5′-AGTCATTTCAAGGAACCTCTG-3′, 5′-AAAGTTAATACAGAGCAGAAAC-3′; (segment 2) 5′-CACTCCACTTCAAAATTTACAGG-3′, 5′-GACTTTATGATTATGAAATCACG-3′, 5′-CCAAATGCAACCAGATACAAC-3′, 5′-AATCATGAAATTGAATTTCCCG-3′.

MAD2 was amplified with the primers 5′-CGCGTGCTTTTGTTTGTGTC-3′, 5′-AATAAAACATATCAACTATAGATGAC-3′, and sequenced using the primers 5′-GTGCTTTTGTTTGTGTCCCTG-3′ and 5′-CCTGATTTCAGGAAAACCAC-3′.

MAD2B was amplified with the primers 5′- GTACCGGGGAAGGATGACCAC-3′ and 5′- ATTGAAACAACTCACAGCACAG -3′, and sequenced using the primers 5′-GAAGGATGACCACGCTCAC-3′, 5′-CCTCCACTGCTGTCCATC-3′, and 5′-TCGCGCACGTAGAGGATG-3′.

CDC20 was amplified with the primers 5′-GTAGGCACCAACTGCAAGG-3′ and 5′-CTGAGGTGATGGGTTGGTC-3′, and sequenced using the primers 5′-GTAGGCACCAACTGCAAGG-3′, 5′-CTGAGGTGATGGGTTGGTC-3′, 5′-GTGGCCAGCTTCCTCCTG-3′, 5′-CTGGGAATGGGCATCCACG-3′, 5′-CCGTGGCACTGGACAACAG-3′.

MAD1L1 was amplified in three overlapping segments with the following primers: (segment 1) 5′-GCCTGTTGTGGGACAGTC-3′, 5′-TTCCTCTCCTCCAACAGCTG-3′; (segment 2) 5′-AGCTTGCCTTGAAGGACAAG-3′, 5′-TGGCGGCTGAAGAGCTCG-3′, (segment 3) 5′-GCGACTGCTCATCTTCAAG-3′, 5′-TTTTAGGGGAGAAGATTTTATTTC-3′. The resultant PCR products were sequenced with the aid of the following primers: (segment 1) 5′-GCCTGTTGTGGGACAGTC-3′, 5′-CCAGGTTCTCTGCAGATGCAG-3′, 5′-AGGAGAAGATGCAGGAGCAG-3′, 5′-ATCCAGGAACTCCAGGCCAG-3′; (segment 2) 5′-AGCTTGCCTTGAAGGACAAG-3′, 5′-CCCAGCTGACGCGGCGATG-3′, 5′-GCGGAGTCGGCTGGAGGAGG-3′, 5′-GCCATCGTCCAAGGAGGTGG-3′: (segment 3) 5′-GCGACTGCTCATCTTCAAG-3′ and 5′-TTTTAGGGGAGAAGATTTTATTTC-3′.

Part of the sequence of *BUB3* was generously supplied by F. McKeon (Harvard Medical School, Boston, MA). Its transcript was amplified in three overlapping segments with the following primers: (segment 1) 5′-AGTGGCGAGTAGTGGAAACG-3′, 5′-CTGTTCCCACAATCAGCCG-3′: (segment 2) 5′-GAGCGAGTGGCGAGTAGTG-3′, 5′-CACTTGGCGAATGAAGATACC-3′, (segment 3) 5′-GGTACCCCACGAGCATCG-3′, 5′-ATCCACCATTGGGGAGTACG-3′. The sequence of the resultant PCR products was determined with the following primers: (segment 1) 5′-CTGTTCCCACAATCAGCCG-3′; (segment 2) 5′-GAGCGAGTGGCGAGTAGTG-3′, 5′-TCTCCTCCGTGAAGTTCAGC-3′, 5′-CACTTGGCGAATGAAGATACC-3′, 5′-TGGATCCCAAATATTTACAAAGC-3′; (segment 3) 5′-GGTACCCCACGAGCATCG-3′.

RESULTS

Isolation of MAD2B

Gene duplication is a common event during the evolution of simpler eukaryotes to humans. With this in mind, we sought to identify novel homologs of spindle checkpoint genes in humans. Expressed sequence tag database searches using *S. cerevisiae Bub1*, *Bub2*, *Bub3*, *Mad1*, *Mad3*, and *Mps1* revealed no significant homologies to novel transcripts other than those previously described. However, a search with yeast *Mad2* sequences identified a human gene, which we termed *MAD2B (MAD2L2)*, with 24% identity and 45% similarity to the yeast gene in the conserved regions, compared with 43% identity and 61% similarity of the previously identified *MAD2 (MAD2L1)* gene (Li and Benezra, 1996) to yeast *Mad2*. This homology was striking as it was conserved among the known orthologs of *Mad2* identified in other model systems (Fig. 1). The two human genes *MAD2* and *MAD2B* were 26% identical and 48% similar to each other in the conserved regions. Because no other *Mad2* homologs were apparent on inspection of the *S. cerevisiae* complete genome sequence, *MAD2* and *MAD2B* likely represent genes derived from a single ancestral *Mad2* gene. To detect differences in the relative expression levels of the *Mad2* family members, we evaluated both *MAD2* and *MAD2B* transcripts by RT-PCR in a panel of cell lines (see below). Both genes were expressed at similarly high levels in each cell line, suggesting that expression of these genes is not controlled in a mutually exclusive fashion.

Map Locations of Human Spindle Checkpoint Genes

To determine the location of the human spindle checkpoint genes, we mapped them with the aid of the Stanford G3 radiation hybrid panel. The results are summarized in Table 1. The approximate cytogenetic location of the genes can be inferred from the integration of radiation hybrid, physical, and cytogenetic mapping information.

The *MAD2* gene has been previously mapped to 5q23.3 by fluorescence *in situ* hybridization (FISH) (Xu *et al.*, 1997) but to 4q27 using a combination of techniques including FISH and radiation hybrid mapping (Krishnan *et al.*, 1998). Our data are consistent with the 4q27 location. A human *Mad2* pseudogene was also identified and linked to D14S586 at 14q21–q23. The sequence of this pseudogene has been deposited in GenBank (Accession No. AF139364).

The remaining map positions were newly assigned with our data. *MAD1L1* was linked to marker SHGC-33698 located near the telomere of chromosome 7p, while *MAD2B* was linked to the markers D1S2740 and D1S434 located at chromosome 1p36. *BUB1* and *BUBR1* were linked to markers positioned at chromosomes 2q14 and 15q14, respectively, consistent with FISH mapping of genomic clones (Cahill *et al.*, 1998) and radiation hybrid mapping of the *BUB1* cDNA 3′ untranslated region (UTR) (Pangilinan *et al.*, 1997). *MSP1L1* had been localized to chromosome 6 using somatic cell hybrid techniques (Mills *et al.*, 1992); our analysis refined its localization to 6q13–q21. *BUB3* and *CDC20* were uniquely linked to markers at chromosomes 10q24–q26 and 9q12–q22, respectively.

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hMAD2B      mttltrq-DLNFGQVVAAVLCEFLEVAVHLILYVREVYPVGIFQKRKKYN   49
hMAD2       malqlsreQGITLRGSAEIVAEFFSFGINSILYQRGIYPSETFTRVQKYG   50
XMad2       magqltr-EGITLKGSAEIVSEFFFCGINSILYQRGIYPSETFTRIQKYG   49
SpMad2      mssvpir-TNFSLKGSSKLVSEFFEYAVNSILFQRGIYPAEDFKVVRKYG   49
ScMad2      ms------QSISLKGSTRTVTEFFEYSINSILYQRGVYPAEDFVTVKKYD   44

hMAD2B      VPVQMSCHPELNQYIQDTLHCVKPLLEKNDVEKVVVVILDKEHRPVekfv   99
hMAD2       LTLLVTTDLELIKYLNNVVEQLKDWLYKCSVQKLVVVISNIESGEVlerw   100
XMad2       LTLLVSTDPALKEYLNKVTDQLKDWLYKCQVQKLVVVITSIDSNEIlerw   99
SpMad2      LNMLVSVDEEVKTYIRKIVSQLHKWMFAKKIQKLILVITSKCSGEDlerw   99
ScMad2      LTLLKTHDDELKDYIRKILLQVHRWLLGGKCNQLVLCIVDKDEGEVverw   94

hMAD2B      feitqppl-----LSISSDSLLSHVEQLLRAFILKISVCDAVLDHNPPGC   144
hMAD2       qfdiecdktakd-DSAPREKSQKAIQDEIRSVIRQITATVTFLPLLEVSC   149
XMad2       qfdiecdktvk--DGIVREKSQKVIQEEIRSVIRQITATVTFLPLLETAC   147
SpMad2      qfnvemvdtadqfQNIGNKEDELRVQKEIQALIRQITATVTFLPQLEEQC   149
ScMad2      sfnvqhisgnsngQDDVVDLNTTQSQIRALIRQITSSVTFLPELTKEGGY   144

hMAD2B      TFTVLVHTREAATRNMEKIQxikdfpwiladeqdvhmhdprliplktmts   194
hMAD2       SFDLLIYTDKDLVVPEKWEEsgpqfitnseevrlrsftttihkvnsmvay   199
XMad2       AFDLLIYTDKDLEVPEKWEEsgpqfvsnseevrlrsftttihkvnsmvay   197
SpMad2      TFNVLVYADKDSEVPTDWVDsdprilrdaeqvqlrsfstsmhkidcqvay   199
ScMad2      TFTVLAYTDADAKVPLEWADsnskeipdgevvqfktfstndhkvgaqvsy   194

hMAD2B      dilkmqlyveerahkgs                                    211
hMAD2       kipvnd----------                                    205
XMad2       kkidtf----------                                    203
SpMad2      rvnp------------                                    203
ScMad2      ky--------------                                    196
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FIG. 1. Sequence alignment of Mad2 protein family. Human MAD2B and MAD2 and *Xenopus laevis* (X), *Saccharomyces pombe* (Sp), and *Saccharomyces cerevisiae* (Sc) *Mad2* sequences were aligned using Macaw Version 2.0.3. The uppercase amino acids represent sequence blocks that are highly related among the genes.

Intron/Exon Structure of BUB1

Because *BUB1* is the only spindle checkpoint gene yet shown to be functionally altered by mutation in a human tumor, we determined its genomic structure, thereby facilitating future analyses. The *BUB1* gene was found to contain 25 exons (Fig. 2A) which could be individually amplified using the primer pairs indicated in Fig. 2B. The human Bub1 protein is predicted to contain at least three functional domains: (i) an N-terminal domain (CD1) highly conserved from yeast to humans, thought to direct binding to Bub3 and kinetochore localization (Taylor and McKeon, 1997; Taylor *et al.*, 1998); (ii) a region containing a nuclear localization signal sequence (NLS); and (iii) a highly conserved C-terminal kinase domain (CD2), thought to convey the cell cycle-inhibitory signaling of the spindle checkpoint. *In vitro*-generated deletions act dominantly to inhibit checkpoint function by separating CD1 from CD2 (Taylor and McKeon, 1997), and one of the mutations found in a human colorectal cancer is thought to act by a similar mechanism (Cahill *et al.*, 1998). CD1 is contained within exons 2–5 and spans approximately 4 kb of genomic DNA. The NLS is found in exons 8–9 and spans approximately 1.5 kb of genomic sequence, and CD2 is contained in exons 20–25 and spans approximately 4 kb of genomic sequence.

Mutational Analysis of Spindle Checkpoint Genes

The entire coding region of each of the eight genes described in Table 1 was amplified by RT-PCR in a panel of 19 aneuploid tumor cell lines. Each of the genes was found to be expressed in each of the 19 tumors, consistent with their function in actively dividing cells. The PCR products were directly sequenced to search for mutations. While numerous polymorphic base changes were noted, no mutations were identified except for the previously identified mutations in the *BUB1* and *BUBR1* genes. These mutations were previously confirmed at the genomic level. The polymorphisms identified in the other spindle checkpoint genes are listed in Table 2.

DISCUSSION

Previous studies have implicated mitotic spindle checkpoint gene defects in the aneuploidy observed in cancers: The first such study suggested that decreased expression of *MAD2* in breast cancers could play a role in this process (Li and Benezra, 1996). A second study indicated that a small fraction of colorectal cancers contained somatic mutations of either *BUB1* or *BUBR1* (Cahill *et al.*, 1998). The mutations in *BUB1* were shown to be functional and dominant negative, as exogenous expression of the mutant, but not wild-type, *BUB1* genes conferred an abnormal spindle checkpoint to diploid MIN cell lines. The third study implicating mitotic checkpoint gene defects in cancer reported the identification of *MAD1L1* as a protein targeted by the HTLV-1 retrovirus Tax protein, inactivating the spindle checkpoint in infected T cells (Jin *et al.*, 1998). Molecular epidemiological studies have shown that HTLV-1 infection gives rise to adult T-cell leukemias, suggesting that Tax-mediated mitotic checkpoint inactivation could contribute to HTLV-1 tumorigenesis.

In the present study, no other mutations in the checkpoint genes *MAD1L1*, *MAD2*, *MAD2B*, *BUB3*, *MPS1L1*, and *CDC20* were identified. Our results therefore indicate that the human checkpoint genes currently known account for relatively few of the presumptive spindle checkpoint defects expected in colon cancer lines. This conclusion, however, must be tempered by the possibility that decreases in expression of some of these genes may play a role, as previously suggested for *MAD2*. The RT-PCR assays used to document expression of these genes were not sufficiently quantitative to exclude the possibility that some of these genes were expressed at lower levels than required to achieve normal checkpoint status. Barring this explanation, however, it would seem that the altered expression or mutation of other genes must be responsible for the checkpoint defects and aneuploidy commonly observed in colorectal cancers.

While the vast majority of tumors have aneuploid karyotypes, a molecular mechanism underlying this aneuploidy has been characterized in only a small fraction. There is certainly no surfeit of genes that might play a role in generating chromosomal instability (Lengauer *et al.*, 1998). Even within the spindle checkpoint pathway, many of the functional components remain to be identified, as no organism has yet been saturated in a genetic study of the pathway. As the human counterparts of the canonical set of yeast genes are sought, one can expect duplication of these sequences through evolution into gene families. *MAD2B* and *MAD2* represent such a case, apparently divergent from a single yeast *Mad2*. In addition to *Bub* and *Mad* homologs, there are numerous other genes that have been shown to play a role in controlling mitosis and/or leading to missegregation when altered (reviewed in Lengauer *et al.*, 1998).

Though our new results do not shed additional light on the mitotic checkpoint defects in colorectal cancers, the sequence and mapping results should facilitate study of these genes in other tumor types. Interestingly, *MAD2B* and *BUB3* are on chromosomes 1p36 and 10q25, respectively, regions that are commonly deleted in a variety of cancers. The characterization of the genomic locus of *BUB1*, which is somatically mutated in colorectal cancers, should permit the study of its involvement in other tumor types in which DNA, but not RNA, is available. Based on our studies of colorectal cancers, we expect

A

atcccatgtgcataccttctctcttctagGATGCTTGAA	Exon 1	*-26	ATGTCCTTCA
ggaaaattaacattatccatatttttctagATACATACAG	Exon 2	27-86	AATGGGAAAGgtcagcatttagttattttatttcccttaa
cttatgtctttttgttttgggttcgtgtagGCTGAGTACA	Exon 3	87-225	TTTAAAATTTgtaagtatacttaagatgtataatcaaatc
tacattgatgccttttctgctgtcatttagGTTATTTCAG	Exon 4	226-422	AACAATACAGgtagttacaaagtcaaactctctgtgatga
ctccgtattttatcctctttccctcatagCTAGAACCTC	Exon 5	423-466	CCAGCTCAAGgtaaaatcaggctttctaaaatgcctgttg
gaatgctcatgtttgttttctgttttttagGGTTCAGAGC	Exon 6	467-567	TAAGAATCAGgtaataatgacatttgtagcttgtgtggaa
ttgtaaataatgtattttcccccattgtagGGAACGAAGA	Exon 7	568-620	AGTCAAATATgcaagtataatcagtgcattaatctattat
gcatgcaaaccatttctatctttcctaaagTAAATGAAGA	Exon 8	621-805	GAGCAATGGGgtacttgcaatcttgtatttttagttattctt
tttttgttgtttgtttgtttaatatgaaagGTTAATCCAG	Exon 9	806-957	AAGGTCCGAGgtatgttgtgctcctcatgacacatcctgt
ctagtgtgtctcaagagttttctcttccagATGTGTGAAT	Exon 10	958-1217	AAGATGCTGGgtaagctgaaggtagtcctagagatcacag
taccacattttgtctccatttctttgctagGGTGTGAAAC	Exon 11	1218-1276	ATCAAAGAAGgtaaaatgtttaattataataaatgtgttt
tgtttacaattattctttttgttctttaagGTTTCATCAT	Exon 12	1277-1405	GAAGCATTAGgtaaagtattttatctgagtgtgtagcacc
aattagatgctatttttttattctgttgcagAAAATGTAAG	Exon 13	1406-1516	CAGTTTCAAAgtaataactatttaaaagctactaagtgca
ctatttataatttagcaaatctttccttagATTACCACAG	Exon 14	1517-1616	AAAATTATGGgtaaaaaaataccatctagttaatgggatg
gctttttttttcatttcatcttctgttatagGAGGAAGTGC	Exon 15	1617-1698	AAAACCAAAGgtaaacaaacctgttttatctatgaaatgg
tatcttgtctctaatttttgaatctttcagAAAATGTGGT	Exon 16	1699-1876	GAAGATAAAGgtgtgtgttacactgttttagtgtctgtct
gttttggtttctttattccttcacacgtcCAGTCCAATT	Exon 17	1877-1961	ATGGAAAATTcaggtattgtgagggtttatttggttctgc
nnnnnnttttttttttctcattccttaagACTTCATTGT	Exon 18	1962-2203	GATGCTCCAAgtacgaagactcactctttgttatgccctt
cataaattaaaattttcaattatcttctaaGTTCTAAGCT	Exon 19	2204-2347	TTTCAATTGGgtaagaattatttggataaatataactttaa
ggatatgaatttcacatacctgatttgcagGTCCAAAAGC	Exon 20	2348-2463	TGTTTTAAAGgtaaatgacctgagttgtattttgtatgat
actcaggattccactttccattgttcctagAATGCCATTA	Exon 21	2464-2625	AACATTATTAgtaagtattcatttttatgatgagcacatg
gcagcctggctttgtttgtgtttaatatagATTTTTGGAA	Exon 22	2626-2783	TTGGAAACGGgcaagtgttatgcttttaacagggttatta
aagtcatatatacctctatcttcttcatagATCGATTACT	Exon 23	2784-2955	GAACTACCAGgtataaaactaaagtgagggacttggaaat
tagccattatttatgctttgtcttttttcagGCTTCCTCAT	Exon 24	2956-3062	TTTTTAGAAGgtctttttagaaggtgagtattagcattgg
	Exon 25	3063-*	

B

Primer Pairs	Amplified Exons
None available	1
TGAAGTATATCTTTTGCTTGTAG, AGTTTAAAAAGCAAAAGTACAAC	2 + 3
TCCCTCCCTGGAGGTTCAGC, CAAAACTAGAAGGATTTCCCTG	4
ACTGTACATTCTCTCTTGACG, ACAAATCCAAGACTAAATGGAC	5
ATTATGTGATAATTTTACTTACGG, TGTCAAAGTGGATGTAGAAGG	6
AAATCTACTTGGGATTAAGGTC, GAGATGAGGATTTTTTTATGTGAC	7 + 8
TGTATTCCTATGATGACCTAGG, CAGAATTAACTCTAAAACAGCAC	9
GATGTAATGCCTGATTAGTAG, CACATCACTGTGATCTCTAG	10
AGATCAAGCATTTATTCTCTAG, AAAATACACCTCTGAGTGATAC	11
GTGTCTTTTAAGTTATTCTG, TCTTGATATTTTCTGTGATAACC	12 + 13
ATTAGTTCTTTTCTGTCAGCTG, CATTTGACCATGTGATTGGCAG	14
GTAGAGAACTTTTCTAATGGTC, AGCGGATGGGTTGTAGGAC	15
CTTCTTTAATCCTCATTTCTTTGG, GAATCAAAGTTGGCAGAAGAC	16
ATCATAATGCAAGTCAGTGTC, ATGAAAATGAAGAGAATGGCAG	17
GAGAAGGGAATTTCATGTGAC, AGCTTTCCCCATGCTCCTG	18
AGGCTTATTGTAAGAACCAAG, CGTTACCATCAACTTCTCATAG	19
TCTATGTCTTTGTAAGTTCTTTG, ATTTCTTGGCAAACTCAAGTTC	20
TGCTATCATTTTTCAGAATACG, AAATATTTCCAAGTCCCTCAC	21+22+23
ATGTGTAAATTACTATTTAAGTC, TTTAGTTATTCTCTTTTCACTAC	24
ATCCTGCATATTGAAGGCTAC, CAGTGTGATTTTTAAGGACTGTC	25

FIG. 2. Characterization of *BUB1* genomic structure. (**A**) Intron/exon boundaries of *BUB1*. Intron sequences (lowercase) flanking each of the exons are shown in the 5′-to-3′ orientation. The first and last 10 nucleotides (uppercase) of each exon are shown along with the nucleotide numbers corresponding to each exon based on the sequence of *BUB1* cDNA. Ambiguous nucleotides are designated "n". (**B**) Primer pairs for amplification of exons from genomic DNA. Exons are grouped together in cases where two or more exons could be amplified in one product that spanned the intervening intron(s).

TABLE 2

Sequence Variants[a]

Gene	Accession No.	Nucleotide position	Codon
BUB1	AF046078	157	GAG (Glu)–GAT (Asp)
		394	CTG (Leu)–CTA (Leu)
		481	CAG (Gln)–CAA (Gln)
		709	TCT (Ser)–TCC (Ser)
		991	TCC (Ser)–TCG (Ser)
		1993	CCT (Pro)–CGT (Arg)
		2161	GAC (Asp)–GAT (Asp)
BUBR1	AF046079	324	AAA (Lys)–AAG (Lys)
		1088	CAA (Gln)–CGA (Arg)
		1206	GCA (Ala)–GCG (Ala)
		1665	AAT (Asn)–AAC (Asn)
		1895	GTA (Val)–GCA (Ala)
		3306	AAA (Lys)–AAG (Lys)
BUB3	AF053304		No variants noted
MAD1L1	U33822	843	CAC (His)–CAT (His)
		1849	CGC (Arg)–CAC (His)
		1874	GCG (Ala)–GCA (Ala)
		1891	CGC (Arg)–CAC (His)
		2135	TCG (Ser)–TCA (Ser)
		2507	ACC (Thr)–ACT (Thr)
MAD2 (MAD2L1)	U65410	462	CCA (Pro)–CCG (Pro)
MAD2B (MAD2L2)	AF139365		No variants noted
TTK (MPS1L1)	M86699	2558	ATA (lle)–ATT (lle)
		2219	CGA (Arg)–CGG (Arg)
		2818	AAA (Lys)–AGA (Arg)
		3344	CCA (Pro)–CCC (Pro)
CDC20	U05340	411	TCT (Ser)–CCT (Pro)
		542	TAT (Tyr)–TAC (Tyr)
		1191	GTA (Val)–ATA (lle)

[a] The variant bases of the indicated codons are shown. For most cases, the variant base was noted in many samples, with heterozygosity and homozygosity frequencies consistent with Hardy–Weinberg equilibrium. In some rare cases, a single heterozygous base variant was noted in only 1 or 2 of the 19 tumor samples analyzed, but was shown to be a germline variant on sequence analysis of normal DNA derived from the corresponding patient's lymphoblasts. In one case (*CDC20*, G1191A) a homozygous change was noted in cell line Caco-2. As the corresponding normal tissue was unavailable, we could not definitively rule out a somatic mutation. The sequence variants listed for *BUBR1* were reported previously (Cahill *et al.*, 1998).

that *BUB1* mutations will be found in a small fraction of other tumors.

Further study of the cellular biology of chromosome instability in simple eukaryotes should reveal other candidate genes that may play a role in the aneuploidy so frequently observed in human cancers.

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