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Identification of *MARCKS, FLJ11383* and *TAF1B* as putative novel target genes in colorectal carcinomas with microsatellite instability

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Somatic frameshift mutations in some genes containing coding mononucleotide repeats (cMNRs) are well known characteristics of tumors with high microsatellite instability (MSI-H). We identified 22 novel and 11 known target genes containing cMNRs with a length of 10 or more nucleotides by using a systematic database search. Frameshift mutation analysis was performed with these 33 genes in 39 MSI-H and 24 microsatellite stable (MSS) colorectal carcinomas by assessing the mobility shifts of PCR products in gel electrophoresis and by sequencing. All the 39 MSI-H colorectal carcinomas, except one, showed mutations in more than one gene, while no mutations were found in 24 MSS colorectal carcinomas. Of these MSI-H tumors, 11 genes were mutated in more than 40%. The most frequently mutated novel genes were *MARCKS* (72%), *FLJ11383* (74%) and *TAF1B* (82%). Biallelic inactivation in *MARCKS* and *FLJ11383* was also frequent in MSI-H tumors. The observed mutation frequency of the 11 known target genes was compatible with that found by previous studies. The very high frequency of mutations, biallelic mutations and the predicted truncation of protein products suggests that mutations of *MARCKS*, *FLJ11383* and *TAF1B* are selected, and play a role in the tumorigenesis of MSI-H colorectal carcinomas.
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Introduction

The mismatch repair (MMR) system plays an important role in reducing mutations and maintaining genomic instability. In tumors with inactivation of one of the MMR genes, the heteroduplex DNA formed due to slippage-induced replication errors in the repetitive sequences is not corrected, and this gives rise to insertions and deletions of repeat units after subsequent replication (Strand *et al*., 1993). Such alterations usually occur in microsatellites, which are tandem repeats of oligonucleotide units that are found dispersed throughout the entire genome. Alterations in size of these microsatellite DNA sequences, termed microsatellite instability (MSI), has been observed in most tumors of hereditary nonpolyposis colorectal cancer (HNPCC) syndrome and also in some types of sporadic carcinomas (Perucho, 1996; Eshleman and Markowitz, 1996). Although MSI has been usually demonstrated in the noncoding portion of genes, it has also been recently identified in the protein coding regions of some cancer-related genes, and in some of the DNA repair genes (Markowitz *et al*., 1995; Malkhosyan *et al*., 1996). MSI in the coding region of these genes may cause accumulation of truncated protein and loss of gene function. Many genes have been identified to have coding mononucleotide repeats (cMNRs) and in several genes, mutations in cMNRs have been reported (Souza *et al*., 1996; Rampino *et al*., 1997; Yamamoto *et al*., 1997; Duval *et al*., 1999; Kim *et al*., 2001; Bader *et al*., 1999; Schwartz *et al*., 1999). Genes bearing a high rate of mutation, biallelic inactivation and functional evidence of tumor suppressor are believed to be candidate target genes in high microsatellite instability (MSI-H) tumors (Boland *et al*., 1998). Other genes containing cMNRs with low mutational frequencies are not regarded as target genes. Mutations of the target genes appear to accumulate and to be selected during tumor progression in MSI-H tumors. Mutations are more frequently found in MSI-H carcinomas than in MSI-H adenomas, and mutations of the transforming growth factor beta type II receptor (*TGF-β RII*) and the *TCF-4* genes are strongly selected in gastrointestinal malignancies, but not in endometrial cancer (Duval *et al*., 1999; Kim *et al*., 1999).

Although there has been a tremendous increase in the reported number of candidate target genes in MSI-H tumors, many additional genes containing cMNR sequences and frequent mutations in these regions can

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be identified by a human genome-wide systematic data base search and by a mutational analysis of MSI-H tumors. Currently, three studies are available, and these studies reported several additional candidate target genes (Woerner *et al*., 2001; Mori *et al*., 2001; Park *et al*., 2002). In the present study, we searched a number of genes containing cMNR with a length of 10 or more nucleotides, and analysed frameshift mutations in these repetitive sequences in our 39 MSI-H colorectal carcinomas and eight MMR-deficient cell lines. We found that *MARCKS*, *FLJ11383* and *TAF1B* contain cMNRs, which are also characterized by high mutational frequencies and biallelic mutations, and are thus likely to be additional target genes in MSI-H tumors.

Results

Database search for coding sequences with MNR and confirmation of novel cMNRs

Our database search identified a total of 4071 coding sequences containing MNR with a length of seven or more nucleotides (2248 for A, 690 for T, 369 for G and 764 for C). Among these coding sequences, 102 contained MNR sequences with a length of 10 or more nucleotides (56 for A, 38 for T, five for G and three for C).

Confirmation and selection of candidate target genes containing cMNRs

We selected genes with cMNRs for this experiment based on the following two factors: (a) that the cMNR sequence was confirmed in both the genomic DNA database and the EST database; (b) that variability of the cMNR was present exclusively in the MSI-H tumors and in the MMR-deficient cell lines, and not present in matched normal mucosa, MSS tumors and MMR-proficient cell lines. Since our preliminary experiments and other studies had demonstrated that the number of nucleotides in the MNR was related to the high mutational frequency (Parsons *et al*., 1995), we chose 102 coding sequences containing MNRs with a length of 10 or more nucleotides. After manually inspecting the alignments with human genomic sequence data and other sequence analysis results, 33 full-length cDNA sequences were finally verified to have cMNRs with a length of 10 or more nucleotides.

In the finally selected 33 genes, five genes (*TAF1B, MARCKS, FLJ11186, KIAA1052* and *FLJ13615*) contained (A)11. Of the remaining 28 genes, 27 contained (A)10 and one contained (T)10 in their coding regions (Table 1). These sequences encompassed all of the previously identified 11 candidate target genes (*TGF-β RII, AIM2, SEC63, Caspase 5, OGT, ATR, MBD4, SYCP1, GART, PRKDC* and *MAC30*) in MSI-H tumors (Markowitz *et al*., 1995; Bader *et al*., 1999; Schwartz *et al*., 1999; Woerner *et al*., 2001; Mori *et al*., 2001), the remaining 22 genes had not been identified before.

Frequency of frameshift mutations of genes containing cMNRs

We analysed frameshift mutations of these selected cMNRs in eight MMR-deficient cell lines and 39 MSI-H colorectal cancers. Paired samples of normal mucosal tissue from 39 MSI-H colorectal cancers, paired samples of cancer and normal mucosal tissue from 24 MSS colorectal cancers, and three MMR-proficient cell lines were used as control. All the 39 MSI-H colorectal carcinomas, except one, showed mutations in more than one gene, while no mutations were found in 24 MSS colorectal carcinomas. Examples of frameshift mutations are shown in Figures 1 and 2. Mutational rates of MSI-H colorectal carcinomas are summarized in Figure 3. Among the 33 genes evaluated, frequent frameshift mutations ($>40\%$) in MSI-H colorectal carcinomas were found in 11 genes (Figure 3). Five of these 11 genes, *TGF-β RII, AIM2, SEC63, Caspase 5* and *OGT* have been previously reported to show frameshift mutations (Markowitz *et al*., 1995; Schwartz *et al*., 1999; Woerner *et al*., 2001; Mori *et al*., 2001), but the other six genes had not been evaluated before. The mutational frequencies of the five known genes in our MSI-H colorectal carcinomas were 85% in *TGF-β RII*, 67% in *AIM2*, 56% in *SEC63*, 49% in *Caspase 5* and 41% in *OGT*. These mutational incidences are similar to those previously reported. Among the six genes that were not reported previously as candidate target genes in MSI-H tumors, *MARCKS* (72%), *FLJ11383* (74%) and *TAF1B* (82%) showed frequent mutations in MSI-H colorectal carcinomas. The mutational rates of the eight MMR-deficient cell lines were generally similar to those of the MSI-H colorectal cancers.

We differentiated between monoallelic and biallelic status of the mutations by comparing the intensities of the normal and abnormal (shifted) bands. The percentage of tumor cells, determined on histological slides after cryostat fractionation, amounted to approximately $70-90\%$. Biallelic mutations were evaluated after taking the percentage of tumor cells into account. Of the 33 genes evaluated, 23 showed biallelic mutations in MSI-H colorectal carcinomas and/or MMR-deficient cell lines. In the four genes with mutational frequencies exceeding 70%, biallelic mutations were frequent in *MARCKS* (25.6%), *FLJ111383* (30.8%) and *TGF-β RII* (51.3%) but rare in *TAF1B* (2.6%).

Discussion

Our systematic approach to the identification of candidate target genes in MMR-deficient tumors enabled us to identify many novel genes. We found many genes containing cMNR sequences in their coding region and identified very frequent frameshift mutations at the cMNRs in *MARCKS, FLJ11383* and *TAF1B* in our MSI-H colorectal carcinomas.

Table 1 List and description of genes analysed

GenBank accession number	*Gene description*	*Gene name*	*Type of repeat*	*Chromosomal location*
D50683	Transforming growth factor, beta receptor II	*TGF-β RII*	A(10)	3p22
L39061	TATA box binding protein (TBP)-associated factor, RNA polymerase I, B, 63 kD	*TAF-1B*	A(11)	2p25
NM_024938	FLJ11383		A(10)	1
NM_002356	Myristoylated alanine-rich protein kinase C substrate	*MARCKS*	A(11)	6q22.2
NM_004833	Absent in melanoma 2	*AIM2*	A(10)	1q22
NM_018353	FLJ11186		A(11)	14q13.1–14q21.3
NM_007214	Endoplasmic reticulum translocon component (*S. cerevisiae*) like	*SEC63*	A(10)	6q16–22
NM_004347	Apoptosis-related cysteine protease	*Caspase 5*	A(10)	11q22.2–q22.3
NM_003201	Transcription factor 6-like 1 (mitochondrial transcription factor 1-like	*TCF6L1*	A(10)	7pter-cen
AB040903	KIAA1470		A(10)	1
NM_003605	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine: polypeptide-N-acetylglucosaminyl transferase)	*OGT*	T(10)	X
NM_003369	UV radiation resistance associated gene	*UVRAG*	A(10)	11q13.5
NM_014956	KIAA1052 protein		A(11)	11
NM_006846	Serine protease inhibitor Kazal type 5	*SPINK5*	A(10)	5q32
NM_017685	FLJ20139		A(10)	1
NM_001184	Ataxia telangiectasia and Rad3 related	*ATR*	A(10)	3q22–24
NM_018365	FLJ11222		A(10)	15q11.2
NM_025114	FLJ13615		A(11)	12
NM_003925	Methyl-CpG binding domain protein 4	*MBD4*	A(10)	3q21–22
NM_003176	Synaptonemal complex protein 1	*SYCP1*	A(10)	1p13–12
NM_001090	ATP-binding cassette, sub-family F (GCN20), member 1	*ABCF1*	A(10)	6p21.33
NM_018979	Protein kinase; lysine deficient 1	*PRKWNK1*	A(10)	12p13.3
AB033094	KIAA1268		A(10)	3
NM_002915	Replication factor C (acitvator 1) 3 (38 kD)	*RFC3*	A(10)	13q12.3–q13
NM_000819	Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	*GART*	A(10)	21q22.11
AB037754	FLJ20333 (KIAA1333)		A(10)	14
AL136680	DKFZp564C2478		A(10)	1
U47077	Protein kinase, DNA-activated, catalytic polypeptide	*PRKDC*	A(10)	8q11
NM_024570	FLJ11712		A(10)	3
NM_001271	Chromodomain helicase DNA binding protein 2	*CHD2*	A(10)	15q26
AL096857	KIAA1096		A(10)	1
L19183	Differentially expressed in neuroblastoma (MAC30)		A(10)	17q11.2
AL008635	High-mobility group protein 2-like 1	*HMG2L1*	A(10)	22q13.1

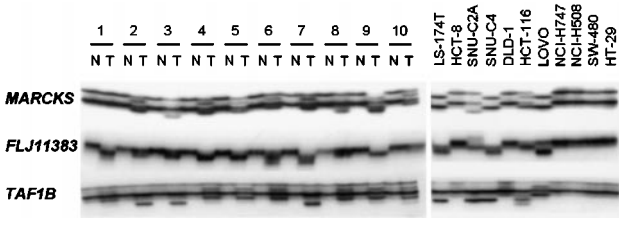

Figure 1 Alteration of the cMNR number of the *MARCKS, FLJ11383* and *TAF1B* genes in MSI-H colorectal carcinomas. N, DNA from normal mucosal tissue; T, DNA from carcinoma tissue. Lanes 1–20 contain PCR products derived from paired tissues of MSI-H colorectal carcinomas. Lanes 21–31 contain PCR products derived from the cell lines. LS-174T, HCT-8, NCI-H747, SNU-C2A, SNU-C4, DLD-1, HCT-116 and LOVO cell lines are MMR-deficient and the remaining three cell lines are MMR-proficient

Frequent nucleotide insertions and/or deletions in the MNRs are well known in MMR-deficient tumors. Mutations in MNRs have been reported in both coding and non-coding regions with variable frequencies (Mori *et al*., 2001; Parsons *et al*., 1995; Zhang *et al*., 2001). In this study, we chose candidate genes containing cMNRs with a length of 10 or more nucleotides, because the number of nucleotides in the MNR have been reported to be related to increased mutational frequency (Chen *et al*., 1995; Eichler *et al*., 1994; Tran *et al*., 1997). We found an average mutational frequency of 55% in the five genes containing 11 nucleotides in cMNRs and an average mutational frequency of 31% in genes containing cMNRs with a length of 10 nucleotides. These mutational frequencies are very high compared to those previously reported for target genes, which suggests that many of them are potentially important target genes. Among these genes, *MARCKS, FLJ11383* and *TAF1B* seem to be most noteworthy as they bear mutational incidences similar to that of the most well known important target gene

of MSI-H tumors, *TGF-β RII*. Moreover, we confirmed the expressions of wild and mutant forms of *MARCKS*, *FLJ11383* and *TAF1B* transcripts in our MSI-H colorectal tumors and of wild forms in matched normal mucosal tissues by RT-PCR analysis (data not shown).

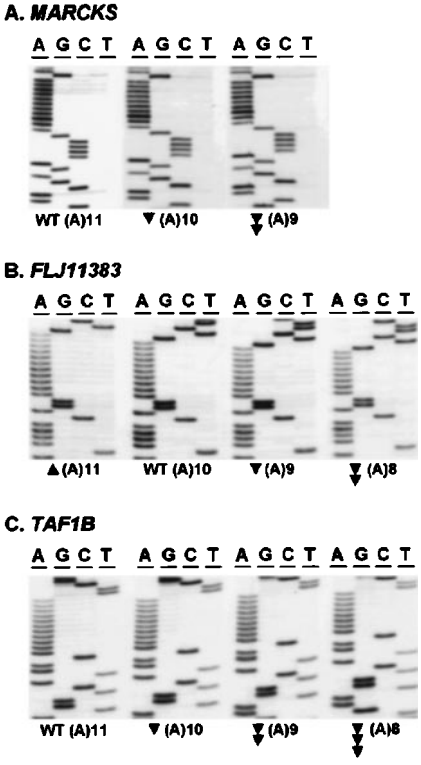

Figure 2 Nucleotide sequence analysis of the representative clones of *MARCKS*, *FLJ11383* and *TAF1B* from MSI-H colorectal carcinomas. Arrowheads pointing up or down indicate insertions or deletions of one nucleotide in the polydeoxyadenosine repeats, respectively. WT denotes wild type

One of the most important significances of these frameshift mutations in cMNR is the functional inactivation of the genes. Mutation in the cMNR of one allele and its inactivation in other allele by a different mechanism (i.e., mutations in a non-MNR tract, deletion or methylation inactivation) provides another possible explanation for its complete functional inactivation. The biallelic inactivation of the *TGF-β RII* gene through mutation in MNR or non-MNR has been reported (Markowitz *et al*., 1995; Parsons *et al*., 1995). In this study, we found biallelic mutations of the cMNR in our MSI-H colorectal carcinomas in 17 of 33 genes, and these were most frequent in *TGF-β RII, FLJ11383* and *MARCKS*. These findings also support the notion that frameshift mutations of these three novel target genes are directly related to functional inactivation and might be related to the tumorigenesis of the MSI-H colorectal carcinomas. Further analysis of the functional inactivation of these genes, and confirmation of their roles in the tumor initiation, and/or progression is necessary to prove that these newly identified genes are important target genes in MSI-H tumors.

A review of the literature regarding the novel candidate target genes of MSI-H tumors suggests that *MARCKS* is potentially important in cell proliferation and/or differentiation. MARCKS (myristoylated alanine-rich protein kinase C substrate; alternate symbols are MACS and 80K-L) is one of the most prominent intracellular substrates for PKC and is abundant and widely distributed in almost all cell types that have been investigated (Blackshear, 1993). The role of MARCKS in the regulation of cell proliferation has been suggested. Moreover, it has been reported that MARCKS concentrations are attenuated in several transformed cell lines (Brooks *et al*., 1996; Manenti *et al*., 1998). This downregulation of MARCKS during proliferation appears to occur via both PKC-dependent and -independent pathways (Brooks *et al*., 1992). The level of MARCKS is low

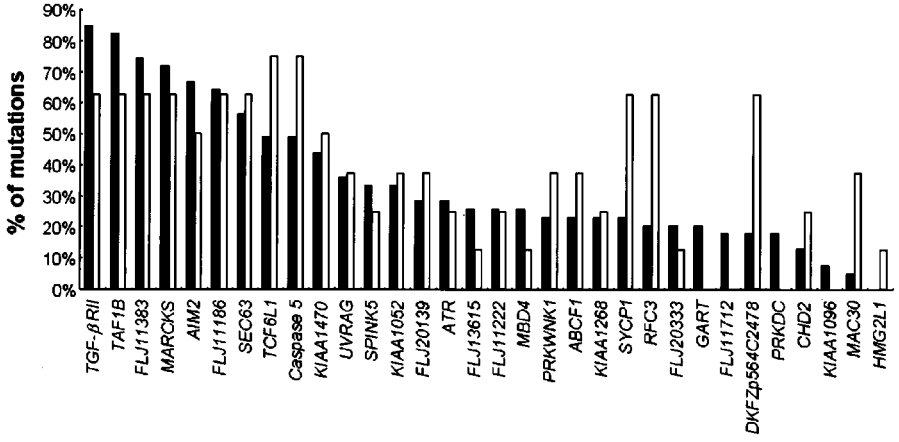

Figure 3 Frequency of frameshift mutations in 33 genes in 39 MSI-H colorectal carcinomas (dark bars) and eight MMR-deficient cell lines (empty bars). Four genes, *TGFβ-RII, TAF1B, FLJ11383* and *MARCKS*, showed mutational rates of greater than 70% in MSI-H colorectal carcinomas

in some cell lines when they are actively proliferating, however, it sharply increases again when they stop dividing (Herget *et al*., 1993). Furthermore, the overexpression of MARCKS strongly suppresses the proliferation of human tumor-derived choroidal melanoma cells (Manenti *et al*., 1998). The (A)11 repeat in *MARCKS* is located in exon 2. Frameshift mutations affecting (A)11 cMNR will lead to truncation of the protein in the effector domain, thereby eliminating its functional activity. Therefore, it is tempting to speculate that the inactivation of MARCKS is associated with tumorigenesis.

Although the incidences of frameshift mutations were very high, the role of mutations of *TAF1B* and *FLJ11383* in tumorigenesis is not as clear as that of *MARCKS*. This is principally due to the fact that these two genes have not been reported to have any function related to tumorigenesis. TAF1B (TAFI63) is the second largest subunit of the TATA box-binding protein (TBP)-containing promoter selectivity factor TIF-1B/SL1 (Comai *et al*., 1994), and *TAF1B* is known to be related to ribosomal transcription, however, no definite role for *TAF1B* in cell proliferation and/or differentiation had been identified. The (A)11 repeat of *TAF1B* is located in exon 3 and the expected truncated proteins of one bp deleted and two bp deleted transcripts are approximately M_r 10 235 and M_r 7 793 in size respectively, as opposed to the M_r 60 873 of the normal TAF1B protein. Therefore, the truncated protein is expected to be in its inactive form. Most of the inactivation of *TAF1B* is presumed to be haplo-insufficient instead of completely insufficient, because biallelic mutations were rare in *TAF1B*. The significance of the haplo-insufficiency of target genes in the tumorigenesis of MSI-H tumors had been proposed previously (Schwartz *et al*., 1999; Ohmiya *et al*., 2001). In the case of the *FLJ11383* gene, no known function has yet been reported. At present two kinds of *FLJ11383* transcripts have been reported, a long form of 2205 bp (GeneBank accession number BC008300) and a smaller form of 1833 bp (GeneBank accession number NM_024938). The (A)10 repeat is only present in the smaller form, and this repeat is located close to the carboxyl terminal (codons 267–270 of 286 amino acids). We confirmed the expressions of wild and mutant forms of the smaller *FLJ11383* transcripts in our MSI-H colorectal tumors and matched normal mucosal tissues by RT–PCR analysis (data not shown). Although the role of *FLJ11383* mutations in tumorigenesis remains enigmatic, frequent frameshift mutations and biallelic mutations in MSI-H tumors suggest that *FLJ11383* might be a potentially important target in MSI-H colorectal carcinomas, and that it requires further functional study.

In conclusion, we found many novel genes containing cMNRs with a length of 10 or more nucleotides and identified a high frequency of frameshift mutations in *MARCKS*, *FLJ11383* and *TAF1B* genes in MSI-H colorectal carcinomas. Further functional study of these target genes will extend our understanding of the molecular pathways of MSI-H tumors and enable novel diagnostic and therapeutic approaches.

Materials and methods

Identification of cMNR

In order to extract genome-wide cMNRs, we scanned the Hs.seq.uniq file (ftp://ncbi.nlm.nih.gov/repository/UniGene), which contains the representative sequences and annotations from each cluster in the human UniGene database (Build 138, February 2001), by using a locally developed computer program (named Exact Tandem Repeats Finder) by one of the authors (H Rhee). After searching the annotated coding sequence with cMNRs, we verified these sequences by comparing alignments with those of the human genomic sequence data at the LocusLink website (http://ncbi.nlm.nih.gov/LocusLink). Among the initially selected coding sequences with mononucleotide repeats (MNRs), sequences containing MNRs in the non-coding region, sequences with unknown genomic DNA sequence, sequences showing polymorphism at repetitive sites, those showing discrepancies with the corresponding genomic DNA sequences, and pseudogenes were excluded from this experiment.

Through this extensive systematic analysis, 33 coding sequences containing MNRs with a length of 10 or more nucleotides were finally selected for mutation data analysis. GeneBank accession numbers, descriptions and names are described in Table 1. Of the 33 genes, 20 genes had some known functions.

Tissue selection and cell lines

In total, 39 cases, confirmed as MSI-H colorectal carcinomas, and 24 cases of microsatellite stable (MSS) colorectal carcinomas were included in this study. Most of the cases had been previously analysed for MSI status (Kim *et al*., 2001). In each case, grossly normal mucosa remote from the tumor was included as a control. All of the cases were identified consecutively for the Gastrointestinal Tumor Working Group Tissue Bank at Yonsei University Medical Center (Seoul, Korea) between December 1996 and November 1999. DNAs were extracted from fresh frozen tissues. Tumor specimens were microdissected on a cryostat and fractionated to enrich the tumor cell population.

Eleven cell lines were obtained from the American Type Culture Collection (ATCC; http://www.atcc.org) and from the Korean Cell Line Bank (KCLB; http://cellbank.snu.ac.kr). Eight (LS-174T, HCT-8, NCI-H747, SNU-C2A, SNU-C4, DLD-1, HCT-116 and LOVO) were MMR-deficient and three (NCI-H508, SW-480 and HT-29) were MMR-proficient in terms of their MSI status, as determined by a previous study (Ku *et al*., 1999; Choi *et al*., 1998; Lengauer *et al*., 1997; Park *et al*., 1987). We confirmed the presence of MSI by using *BAT26* and *BAT25* markers. Cells were grown in RPMI supplemented with 10% FBS (Life Technologies, Inc., Grand Island, NY, USA), penicillin and streptomycin at 37°C in 5% CO_2.

Frameshift mutation analysis and sequencing

Frameshift mutations in the cMNRs were analysed using a PCR-based assay using the primers described in Table 2. PCR reactions were carried out in a 20 μl mixture containing 1.5 mM $MgCl_2$, 20 pmol primer, 0.2 mM of each dATP, dGTP, dTTP, 5 μM dCTP, 1 μCi of [α-^{32}P]-dCTP (3000 Ci/

Table 2 Primers used for the detection of coding mononucleotide repeat length

Gene name	*Forward primer*	*Reverse primer*	*PCR product length*
TGF-β RII	5′-CTTTATTCTGGAAGATGCTGC	5′-GAAGAAAGTCTCACCAGG	73
TAF1B	5′-CCAAATAAAAGCCCTCAACC	5′-TGTCCTGACATCATGAAGGTG	115
FLJ11383	5′-GGAAAATTATGAACAGCCACAA	5′-GCAGCCAAATGCTTGTTATG	121
MARCKS	5′-CCGCCTCCTCGACTTCTT	5′-AGCCGCTCAGCTTGAAAGAC	120
AIM2	5′-CCACTCATCGACTGCATCTC	5′-TGGCTTGAATTGGTCCTTTT	102
FLJ11186	5′-GCAAGAACAGCCATCAAGAA	5′-GGAATGATTTGTTGTTTTCCTT	143
SEC63	5′-AGTAAAGGACCCAAGAAAACTGC	5′-TGCTTTTGTTTCTGTTGCTTTG	104
Caspase 5	5′-CAGAGTTATGTCTTAGGTGAAGG	5′-ACCATGAAGAACATCTTTGCCCAG	141
TCF6L1	5′-TTGGAAAAAGAAATCATGGACA	5′-AATCTCAATTCTTTACCATAAGAAAA	104
KIAA1470	5′-GCATTTGTTCTGGAAGCTCGT	5′-GTGATGAGAAACCGGAGAGAA	143
OGT	5′-TCACTTTTGGCTGGTCAGAG	5′-GGGAGGGAAAGGAGGTAAAG	116
UVRAG	5′-TTTATTTTTAAACATTGTGAGTATG	5′-TTTTTAACTGCAGGCATTCAC	116
KIAA1052	5′-GTCAACTTCTGGGGCCATTA	5′-GAGGCATCCACTGACTCACC	104
SPINK5	5′-TGAGGCGTTTGTTCACTTTG	5′-TGCTCCTGTCTTCATCCTCTT	99
FLJ20139	5′-GCCAACACAAAGTGTCTCCTC	5′-GACTGTTGGATGGATGATGC	89
ATR	5′-GCTTCTGTCTGCAAGCCATT	5′-TGAAAGCAAGTTTTACTGGACTAGG	70
FLJ11222	5′-GCTGCAGAAGACAAACGAAAC	5′-GCAGCTGCTCATAAGCTTCC	106
FLJ13615	5′-GTTGATTATTTTCTTGGCTGAAC	5′-GCACTTCTTTTTCCTCTTTCTTGA	136
MBD4	5′-TGACCAGTGAAGAAACAGC	5′-GTTGTGTTCTGAGTCTTTGG	138
SYCP1	5′-CCCCTTCATCTCTAACAACCC	5′-CACTGATTCTCTGAAATTAAACAAATAAC	153
ABCF1	5′-CCTGGGCTTCATTTTCTCAC	5′-CCTGCCTTTTCGGGTATCTC	73
PRKWNK1	5′-AGTTGGTACGGGAGGAGCA	5′-CTGGGAAGCACTGGATTGTT	83
KIAA1268	5′-GTTTCCTCTGTTTTGCAGGA	5′-GACAGCCAGAGGCTACGAAC	90
RFC3	5′-TTTTCTTTGTCCACAGACTCCA	5′-AAGGTGGTAGTTACTTGCAATGG	70
GART	5′-AGTGTTGAAGAATGGCTCCC	5′-TGTTCCAGATATTAAGACAGCCAC	82
FLJ20333	5′-GGCAAGGCAGCAAATTTAGA	5′-GCATCTAAGGCACTATTCCAGA	124
DKFZp564C2478	5′-GGAGAGATGCCAAGGTGAAA	5′-GCCTTGGGTTAGGATGACAG	143
PRKDC	5′-GACTCATGGATGAATTTAAAATTGG	5′-TTTGAAAATAACATGTAAATGCATCTC	113
FLJ11712	5′-GGCTAAAGTTGACAAGAGTGGAA	5′-GTCAGGAAGGACAAACTGAAACA	147
CHD2	5′-CTATCCCTGTGGACCCTGAA	5′-ACGGTACGACCATCTAAGCA	71
KIAA1096	5′-GGCAAATGAAGAATGGGAAA	5′-GGAGGTAGAACATTCTCTCCAA	129
MAC30	5′-TGTTGCGGAGCCCCTAC	5′-AACCACCCTGTAGGCATCTC	93
HMG2L1	5′-CTCCACACAGATGGGCATAG	5′-CCCCCACCACACTTAAAAGA	103

mmol; NEN DuPont, Boston, MA, USA), 50 ng of sample DNA, 1 × PCR buffer and 1.25 U Taq DNA polymerase (Life Technologies, Inc.) using the following conditions; an initial denaturation at 95°C for 5 min, followed by DNA amplification for 25–30 cycles of denaturation at 95°C for 30 s, primer annealing at 55–60°C for 30 s, and primer extension at 72°C for 15 s. PCR products were separated in 6% polyacrylamide gels containing 5.6 M urea, followed by autoradiography. Frameshift mutations at cMNR sequences were scored according to the mobility shifts of PCR products.

In order to confirm that a shifted band represented a frameshift mutation, genomic DNA fragments exhibiting bandshifts of *MARCKS*, *FLJ11383* and *TAF1B* were excised, eluted from the polyacrylamide gel, and subcloned to pT7Blue vector (Novagen, Madison, WI, USA). Plasmids were sequenced using a T7 sequencing kit (USB, Cleveland, OH, USA) and separated in 8% denaturing polyacrylamide gels.

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References

Bader S, Walker M, Hendrich B, Bird A, Bird C, Hooper M and Wyllie A. (1999). *Oncogene,* **18,** 8044–8047.

Blackshear PJ. (1993). *J. Biol. Chem.,* **268,** 1501–1504.

Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN and Srivastava SA. (1998). *Cancer Res.,* **58,** 5248–5257.

Brooks G, Brooks SF and Gross MW. (1996). *Carcinogenesis,* **17,** 683–689.

Brooks SF, Herget T, Broad S and Rozengurt E. (1992). *J. Biol. Chem.,* **267,** 14212–14218.

Chen J, Heerdt BG and Augenlicht LH. (1995). *Cancer Res.,* **55,** 174–180.

Choi SJ, Kim SK, Kim SJ, Koh CM and Park YS. (1998). *J. Korean Cancer Assoc.,* **30,** 527–535.

Comai L, Zomerdijk JCBM, Beckmann H, Zhou S, Admon A and Tjian R. (1994). *Science,* **266,** 1966–1972.

Duval A, Iacopetta B, Ranzani GN, Lothe RA, Thomas G and Hamelin R. (1999). *Oncogene,* **18,** 6806 – 6809.
Eichler EE, Holden JJ, Popovich BW, Reiss AL, Snow K, Thibodeau SN, Richards CS, Ward PA and Nelson DL. (1994). *Nat. Genet.,* **8,** 88 – 94.
Eshleman JR and Markowitz SD. (1996). *Hum. Mol. Genet.,* **5,** 1489 – 1494.
Herget T, Brooks SF, Broad S and Rozengurt E. (1993). *Proc. Natl. Acad. Sci. USA,* **90,** 2945 – 2949.
Kim JJ, Baek MJ, Kim L, Kim NG, Lee YC, Song SY, Noh SH and Kim H. (1999). *Lab. Invest.,* **79,** 1113 – 1120.
Kim NG, Choi YR, Baek MJ, Kim YH, Kang H, Kim NK, Min JS and Kim H. (2001). *Cancer Res.,* **61,** 36 – 38.
Ku JL, Yoon KA, Kim DY and Park JG. (1999). *Eur. J. Cancer,* **35,** 1724 – 1729.
Lengauer C, Kinzler KW and Vogelstein B. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 2545 – 2550.
Malkhosyan S, Rampino N, Yamamoto H and Perucho M. (1996). *Nature,* **382,** 499 – 500.
Manenti S, Malecaze F, Chap H and Darbon JM. (1998). *Cancer Res.,* **58,** 1429 – 1434.
Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B, Brattain M and Willson JKV. (1995). *Science,* **268,** 1336 – 1338.
Mori Y, Yin J, Rashid A, Leggett BA, Young J, Simms L, Kuehl PM, Langenberg P, Meltzer SJ and Stein OC. (2001). *Cancer Res.,* **61,** 6046 – 6049.
Ohmiya N, Matsumoto S, Yamamoto H, Baranovskaya S, Malkhosyan SR and Perucho M. (2001). *Gene,* **272,** 301 – 313.
Park J, Betel D, Gryfe R, Michalickova K, Di Nicola N, Gallinger S, Hogue CW and Redston M. (2002). *Cancer Res.,* **62,** 1284 – 1288.
Park JG, Oie HK, Sugarbaker PH, Henslee JG, Chen TR, Johnson BE and Gazdar A. (1987). *Cancer Res.,* **47,** 6710 – 6718.
Parsons R, Myeroff LL, Liu B, Willson JKV, Markowitz SD, Kinzler KW and Vogelstein B. (1995). *Cancer Res.,* **55,** 5548 – 5550.
Perucho M. (1996). *Biol. Chem.,* **377,** 675 – 684.
Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC and Perucho M. (1997). *Science,* **275,** 967 – 969.
Schwartz Jr S, Yamamoto H, Navarro M, Maestro M, Reventos J and Perucho M. (1999). *Cancer Res.,* **59,** 2995 – 3002.
Souza RF, Appel R, Yin J, Wang S, Smolinski KN, Abraham JM, Zou TT, Shi YO, Lei J, Cottrell J, Cymes K, Biden K, Simms L, Leggett B, Lynch PM, Frazier M, Powell SM, Harpaz N, Sugimura H, Young J and Meltzer SJ. (1996). *Nat. Genet.,* **14,** 255 – 257.
Strand M, Prolla TA, Liskay RM and Petes TD. (1993). *Nature,* **365,** 274 – 276.
Tran HT, Keen JD, Kricker M, Resnick MA and Gordenin DA. (1997). *Mol. Cell. Biol.,* **17,** 2859 – 2865.
Woerner SM, Gebert J, Yuan YP, Sutter C, Ridder R, Bork P and Doeberitz MVK. (2001). *Int. J. Cancer,* **93,** 12 – 19.
Yamamoto H, Sawai H and Perucho M. (1997). *Cancer Res.,* **57,** 4420 – 4426.
Zhang L, Yu J, Willson JKV, Markowitz SD, Kinzler KW and Vogelstein B. (2001). *Cancer Res.,* **61,** 3801 – 3805.