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# Alterations of the Double-Strand Break Repair Gene *MRE11* in Cancer[1]

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## Abstract

***MRE11* plays a role in DNA double-strand break repair. Hypomorphic mutations of *MRE11* have been demonstrated in ataxia-telangiectasia (AT)-like disorder. *ATM* mutations play a causal role in AT and have been demonstrated in lymphoid malignancies in patients without AT histories. By analogy with the relationship of *ATM* to lymphoid malignancies, it is probable that alterations of *MRE11* are associated with tumor formation. We performed a mutation analysis of *MRE11* in 159 unselected primary tumors. Three missense mutations at conserved positions were found in breast and lymphoid tumors. Additionally, an aberrant transcript without genomic mutation was found in a breast tumor. These findings suggest an occasional role for *MRE11* alterations in the development of primary tumors.**

## Introduction

*MRE11* was originally identified as a gene required for meiotic recombination in *Saccharomyces cerevisiae* (1). In mitotic cells, *Mre11* mutants are sensitive to DSB[3] damage and are hyperrecombinational. Consistent with its role in DSB repair, the MRE11 protein engages in exonuclease and endonuclease activities (2). Homozygous knockout in vertebrate cells revealed that *MRE11* is essential for cell proliferation and the maintenance of chromosome DNA (3).

*MRE11*, *RAD50*, and *XRS2* have been identified in yeast as components of the HR and NHEJ pathways (4). A physical complex with these proteins has been identified. In vertebrates, MRE11 and RAD50 form a complex with NBS1, whose mutation causes NBS (5, 6). The clinical features of NBS overlap with those of AT. They are characterized by chromosome instability, increased hypersensitivity to ionizing radiation, immunodeficiency, and predisposition to cancer. AT is caused by mutations in the *ATM* gene, which encodes a protein kinase homologous with phosphatidylinositol-3 kinase (7). ATM is a key regulator of the cellular response to DSBs. NBS1 is phosphorylated in an ATM-dependent manner after ionizing radiation, suggesting a link between ATM and NBS1 in a common signaling pathway (8). MRE11 phosphorylation upon DNA damage is dependent on NBS1 (9). Therefore, it is highly likely that MRE11 participates in the same pathway in response to DNA damage. Consistent with this functional interaction, hypomorphic mutations in the *MRE11* gene cause ataxia-telangiectasia-like disorder, the phenotypes of which are indistinguishable from those of AT (10).

Several lines of evidence suggest that ATM dysfunction leads to tumor formation. Patients with AT frequently develop cancer, particularly lymphoid tumors. Mutations in the *ATM* gene have been demonstrated in T-prolymphocytic leukemia, chronic lymphocytic leukemia, and mantle cell lymphoma in patients without AT histories (11, 12). Mice homozygous for *ATM* mutation develop thymic lymphoma (13). Heterozygous mutations in the *ATM* gene have been discussed as predisposing factors for breast cancer (14). NBS1 dysfunction is also likely to lead to tumor formation because patients with NBS frequently develop tumors, particularly lymphomas.

Given that MRE11 plays a role in the repair of DSB in the common signaling pathway, it is reasonable to speculate that MRE11 dysfunction may be involved in the development of cancer. A recent finding that gross chromosomal rearrangements, which are often associated with tumor development, were increased by mutations of *MRE11* in *S. cerevisiae* supports this hypothesis (15). Therefore, we screened a panel of 159 unselected human primary tumors for alterations of *MRE11*. Three missense mutations at conserved positions were found in breast and lymphoid tumors. An altered transcript resulting from aberrant splicing was also identified. These findings suggest that alterations of *MRE11* function may be contributing factors in the development of some sporadic tumors.

## Materials and Methods

**Tissue Samples.** Tumor tissues and matched normal tissues were obtained from 159 surgically treated patients. These tumors included 83 breast cancers, 42 colorectal cancers, 13 hepatocellular carcinomas, and 21 malignant lymphomas.

**Reverse Transcriptase-PCR and Single-Strand Conformational Polymorphism Analysis.** RNA was isolated by the acid guanidium-isothianate method. cDNA was reverse transcribed, and nested PCR was performed. The *MRE11* cDNA was divided into two segments to generate the first PCR products. The N-terminal half was amplified with M5 (5′-TCGAAGAGTCCAGCAGTG-3′) and M6 (5′-CTCGCAGTCGTACAAGAG-3′). This region was further divided into four segments for the second PCR. Primer pairs were as follows: (*a*) M7 (5′-AACCTGGTCCCAGAGGAG-3′) and M8 (5′-TCAAACTGGACAGGCCGA-3′); (*b*) M9 (5′-TACATACCTGCCTCGAGT-3′) and M10 (5′-GCCTTTCATCTGGAATGG-3′); (*c*) M11 (5′-GGAAGCACAAAGATTGCG-3′) and M12 (5′-GCTTCTCCTGGGGAAAGA-3′); and (*d*) M13 (5′-CACAACCTGGAAGCTCAG-3′) and M14 (5′-GCTTCTCTGGCTGGTGAG-3′). The COOH-terminal half was amplified with M15 (5′-ACCCAAGCCATACAAAGC-3′) and M16 (5′-CTTACTACAACAACCAGG-3′). This region was also divided into four segments for the second PCR. Primer pairs were as follows: (*a*) M17 (5′-ATGCTGAACGGGAACGTC-3′) and M18 (5′-GTACTGCTTCACCCATCC-3′); (*b*) M19 (5′-GCAGCTCTCACTGCTAAC-3′) and M20 (5′-ACTCATAAGGTCATCAGC-3′); (*c*) M21 (5′-CAGAGGAGTCTGCTTCTG-3′) and M22 (5′-TGTGCTGGACCACCTTTG-3′); and (*d*) M23 (5′-CTACCACTTCAAAGACAG-3′) and M24 (5′-GGAGTTATGCTCAGGAA-3′). Thirty cycles of amplification were performed with Taq DNA polymerase (Amersham), each cycle consisting of 1 min at 94°C, 1 min at the optimally determined annealing temperature for each primer pair, and 1 min at 72°C. The second PCR was performed with primers that were radiolabeled with T4 polynucleotide kinase and [$\gamma$-$^{32}$P]ATP. PCR products were denatured by being boiled in 90%

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[3] The abbreviations used are: DSB, double-strand break; HR, homologous recombination; NHEJ, non-homologous end-joining; NBS, Nijmegen breakage syndrome; AT, ataxia-telangiectasia.

Table 1 *Summary of MRE11 alterations in cancer*

| Sample | Tumor type | Nucleotide change[a] | Mutation/variant[b] | Germline/somatic | Domain |
|---|---|---|---|---|---|
| Mutation | | | | | |
| Br63 | Breast carcinoma | A310T | S104C | ND[c] | Adjacent to phosphodiesterase motif III |
| KBr16 | Breast carcinoma | G1508A | R503H | Somatic | Charged amino acid cluster |
| Ly50 | Lymphoma | G1715A | R572Q | ND | Charged amino acid cluster |
| Aberrant transcript | | | | | |
| Br69 | Breast carcinoma | 2070–2071 63-bp insertion | 690–691 Frameshift | Somatic | Charged amino acid cluster |
| Polymorphism | | A469G | M157V | | |

[a] The MRE11 nucleotide positions are based on the GenBank accession no. AF073362.
[b] Name of mutation indicates a normal residue at the indicated codon and a substituted residue.
[c] ND, not determined. None of the mutations nor the aberrant transcript were seen in 169 normal individuals. All mutations and the polymorphism were confirmed in both DNA and RNA. A putative polymorphism, M157V, was found in two tumors.

formamide, electrophoresed at 15°C on denaturing 5% polyacrylamide gel containing 10% glycerol, and subjected to autoradiography.

**Sequencing.** PCR products were directly sequenced using a Sequenase PCR sequencing kit (Amersham).

**Genomic PCR.** Mutations found in PCR products from mRNA were confirmed by the sequencing of PCR products from genomic DNA. Genomic DNA fragments containing mutated codons were amplified with the following primers: (*a*) codon 104, M9 and M33 (5′-TAGCTTATATGGAAGGC-3′); (*b*) codon 503, M64 (5′-ACATAACTGGAATAGGCAAC-3′) and M65 (5′-CTAGACCTATGGACTGAC-3′); and (*c*) codon 572, M21 and M46 (5′-TGAGACCCTCCTCTCGACT-3′). Genomic DNA fragments corresponding to an aberrant transcript found in Br69 were amplified with the following primers: (*a*) M37 (5′-GAGTCAAGTATCGAAAG-3′) and LINE7 (5′-ATACCACATGTCCTCAC-3′); (*b*) LINE5 (5′-AGTTAACAGTGGGATCGG-3′) and LINE2 (5′-TGAAATAAGCCAGTCAC-3′); (*c*) LINE1 (5′-GTACTTCATAGAAGTAG-3′) and M43 (5′-AGAATGAGCTCTTGCTGC-3′); and (*d*) LINE8 (5′-CCCATAAGAACTTCTGTC-3′) and M24.

## Results

Mutation screening of the entire coding region of *MRE11* revealed alterations in 4 of 159 unselected primary tumors. We looked at matched normal tissue in two of four cases with *MRE11* alterations and found somatic alterations in these two cases (Table 1). A breast tumor, Br63, contained an A to T transversion, which resulted in a Ser to Cys substitution at codon 104 (Fig. 1*A*). This codon is adjacent to phosphodiesterase motif III and conserved from *Caenorhabditis elegans* to humans (Fig. 2*A*). The corresponding normal tissue of Br63 was not available. Two mutations were found in the COOH-terminal domain with charged amino acid clusters. A G to A transition converted Arg to His at codon 503 in breast tumor KBr16 (Fig. 1*B*). This codon is at a position conserved from yeast to humans (Fig. 2*B*). A G to A transition converted Arg to Gln at codon 572 in lymphoma sample Ly50 (Fig. 1*C*). This codon is also at a position conserved from yeast to humans within the clusters of charged amino acids (Fig. 2*C*). Because these tumors exhibit reduced intensities of the wild-type alleles, we evaluated whether they resulted from loss of heterozygosity or from a sequencing problem by creating 1:1 mixtures of the mutated and normal alleles by cloning and mixing. Both alleles exhibited the same intensities at the mutated positions, suggesting that these tumors may have loss of heterozygosity in this region. The mutation in KBr16 was a somatic mutation, whereas the corresponding normal tissue of Ly50 was not available.

These mutations were confirmed by direct sequencing of PCR products from RNAs and from genomic DNAs (Table 1). The absence of these base substitutions in 169 normal individuals indicates that they are unlikely to reflect sequence polymorphisms. Additionally, an A to G transition resulting in a Met to Val substitution at codon 157, which has not been conserved throughout evolution, was found in two tumors (data not shown). This alteration was heterozygous with the wild-type sequence in the tumors and present in the corresponding normal tissues. It is, therefore, highly likely that this substitution represents a sequence polymorphism, though we have not detected this alteration in normal individuals thus far.

Besides these genomic alterations, an aberrant transcript in which a

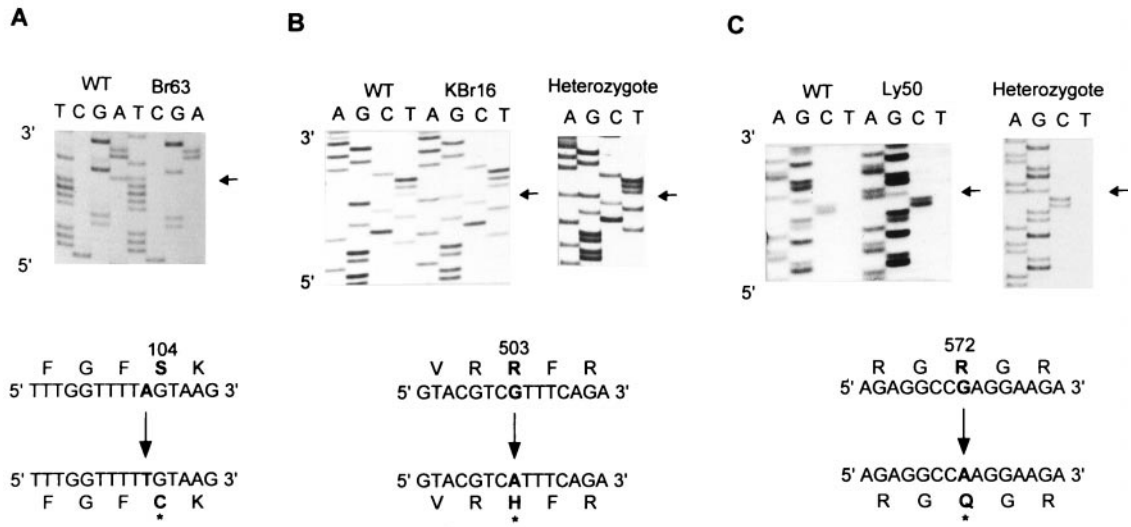


Fig. 1. Missense mutations of *MRE11* demonstrated by direct sequencing of PCR products. Mutated amino acids are marked by *asterisks*. *A*, an A to T transversion at codon 104 in Br63. *B*, a G to A transition at codon 503 in KBr16. *C*, a G to A transition at codon 572 in Ly50. Sequences of 1:1 mixtures of the mutated and normal alleles as heterozygous controls are shown in *B* and *C*.

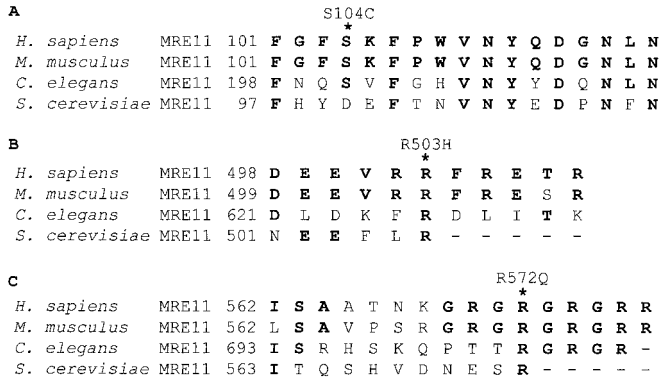


Fig. 2. Location of three MRE11 mutations in relation to conserved domains. *A*, alignment of MRE11 sequences around codon 104 adjacent to phosphodiesterase motif III. *B*, alignment of MRE11 sequences around codon 503 in the charged amino acid cluster. *C*, alignment of MRE11 sequences around codon 572 in the charged amino acid cluster. Identical amino acids are shown in *bold*. Amino acid positions are shown on the *left*. Alignments of MRE11 sequences were reported by Nairz and Klein (19) and Petrini *et al.* (20). Accession nos. of the sequences shown are as follows: *Homo sapiens MRE11*, AF073362; *Mus musculus MRE11*, U58987; *C. elegans MRE11*, Z73978; and *S. cerevisiae MRE11*, D11463.

63-bp sequence was inserted downstream of codon 690 was found in breast tumor Br69 (Fig. 3). The inserted sequence was derived from the intron downstream of codon 690 and flanked by putative splice donor and acceptor sites that are not normally used. Because no genomic alteration was identified in the 318-bp intronic sequence 3′ of codon 690, the 196-bp sequence 5′ of the insertion, the 170-bp sequence 3′ of the insertion, or the 28-bp intronic sequence 5′ of codon 691, this transcript was most likely generated by an abnormal splicing event. The resulting transcript encoded a truncated protein lacking the COOH-terminal-charged amino acids. No wild-type transcript was detected in this tumor. The corresponding normal tissue of Br69 did not express this aberrant transcript. We did not detect this change in 169 normal controls.

## Discussion

The screening of a panel of 159 primary tumors revealed three distinct missense mutations in the *MRE11* gene and an abnormal splicing event in a single tumor. No germline alterations were identified in the two cases where the corresponding normal tissues were available, suggesting somatic alteration of *MRE11* during the development of these tumors. Although functional studies of these mutants remain to be performed, some of the alterations identified in the present study are likely to affect the *MRE11* function. The MRE11 protein consists of two separable functional domains. *MRE11* is a member of a gene family encoding phosphodiesterase functions (16). The $NH_2$-terminal region of MRE11 has significant homology to *Escherichia coli* SbcD, which forms a complex with SbcC. The SbcCD complex exhibits ATP-dependent double-strand DNA exonu-

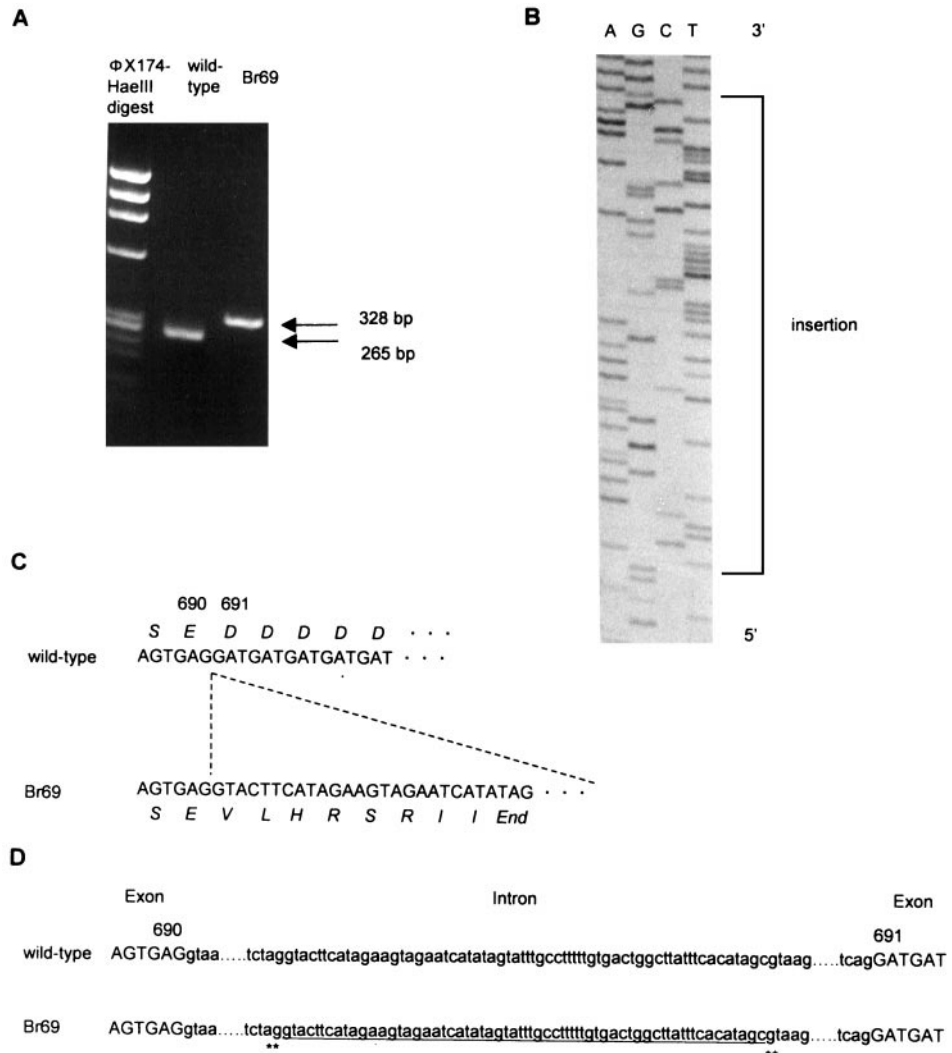


Fig. 3. An aberrant transcript in Br69. *A*, reverse transcriptase-pcr of *MRE11* amplified with primers M23 and M24. *B*, direct sequencing of the PCR product. A 63-bp ectopic sequence was inserted downstream of codon 690. *C*, sequence comparison of the *MRE11* cDNA between wild type and Br69. The insertion resulted in a premature termination. *D*, a genomic structure encompassing the insertion. Alternative splice acceptor and donor sites used in Br69 are marked by *asterisks*. The LINE sequence, which is flanked by these splice sites and marked by underlining, was inserted between codons 690 and 691.

clease activity and ATP-independent endonuclease activity. The COOH-terminal domain with charged amino acid clusters is needed for the DNA-binding activity of MRE11. Four alterations were found within these functional domains.

Hypomorphic mutations in the functional domains have been reported in two families with ataxia-telangiectasia-like disorder (10). Two patients from family 1 harbored a homozygous nonsense mutation at codon 633, resulting in a premature termination. The truncated protein lacked several charged amino acids at the COOH terminus of MRE11. Skin fibroblasts from these patients exhibited higher sensitivity to ionizing radiation and reduced levels of MRE11, NBS1, and RAD50. The mutations in KBr16 and Ly50 converted Arg to noncharged amino acid residues, reducing the number of charged amino acid residues within the COOH-terminal domain. It is therefore possible that these mutations affect the DNA-binding activity of MRE11 mediated by the COOH-terminal-charged amino acid clusters. Similarly, the truncated protein resulting from the abnormal splicing event is likely to affect the DNA-binding activity. Two patients from family 2 harbored a homozygous missense mutation at codon 117 within phosphodiesterase motif III. Clinical and cellular phenotypes of these patients were similar to those in family 1. The mutation in Br63 may have affected the nuclease activity of MRE11, because the mutated codon was adjacent to this motif.

DSBs are repaired either by HR or by NHEJ. The latter pathway is also involved in V(D)J recombination. DNA-dependent protein kinase, Ku70, Ku80, XRCC4, and DNA ligase IV are considered key molecules in this pathway. Evidence that inactivation of a molecule in the NHEJ pathway leads to malignant transformation has been demonstrated. Mouse fibroblasts lacking *Ku70* displayed an increased rate of sister chromatid exchange and a high frequency of malignant transformation. *Ku70*$^{-/-}$ mice developed thymic lymphomas (17). Mouse embryonic fibroblasts lacking *Ku80* displayed a marked increase in chromosome aberrations. The loss of *p53* in the *Ku80*$^{-/-}$ background promoted the development of pro-B-cell lymphomas (18). These findings suggest a role for NHEJ in tumor suppression. Therefore, it is possible that mutated *MRE11* plays a role in tumor formation through the NHEJ pathway. Alternatively, *MRE11* mutants may lead to tumor formation through the HR pathway. A hyperrecombination phenotype observed in yeast *mre11* mutants resembles the genomic instability exhibited by cells from AT and Bloom syndrome patients. *MRE11* null chicken DT40 cells exhibited frequent centrosome amplification. The increased radiosensitivity of these cells has been explained by a defect in HR (3).

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