

## Review

# Causal Link between Microsatellite Instability and hMRE11 Dysfunction in Human Cancers

Xiling Wu, Yang Xu, Weihang Chai, and Chengtao Her

## Abstract

Maintenance of genomic integrity is essential for cell survival, and genomic instability is a commonly recognized intrinsic property of all cancers. Microsatellite instability (MSI) represents a frequently occurring and easily traceable simple form of sequence variation, signified by the contraction or expansion of specific DNA sequences containing short tandem repeats. MSI is frequently detected in tumor cells with DNA mismatch repair (MMR) deficiency. It is commonly conceived that instability at individual microsatellite loci can arise spontaneously in cells independent of MMR status, and different microsatellite loci are generally not affected uniformly by MMR deficiency. It is well recognized that MMR deficiency *per se* is not sufficient to initiate tumorigenesis; rather, the biological effects have to be exerted by mutations in genes controlling cell survival, DNA damage response, and apoptosis. Recently, shortening of an intronic hMRE11 poly(T)<sub>11</sub> tract has been associated with MMR deficiency, raising the possibility that hMRE11 may be inactivated by defective MMR. However, the molecular nature underlying this association is presently unknown, and review of the current literature suggests that hMRE11 is most likely involved with the MMR pathway in a more complex fashion than simply being a MMR target gene. An alternative scenario is proposed to better reconcile the differences among various studies. The potential role of hMRE11 in telomere repeats stability is also discussed. *Mol Cancer Res*; 9(11); 1443–8. ©2011 AACR.

## Introduction

Microsatellites are simple tandem nucleotide repeats, reiterated motifs of 1 to 6 nucleotides, which are widespread in the human genome (1). Microsatellite instability (MSI) was initially coined to describe a form of DNA aberration involved with expansion or contraction of short nucleotide tandem repeats in colorectal cancers (2–4). Although sporadic MSI can occur in normal cells at various loci at a low frequency, the extent of instability can be elevated by several orders of magnitude in cells with DNA mismatch repair (MMR) deficiency (5). MSI, in varying frequencies, is often observed in tumors of Lynch syndrome (known previously as hereditary nonpolyposis colorectal cancer) patients (2–4). On the basis of the Bethesda Guidelines and subsequent revisions (6, 7), a reference microsatellite panel, consisting of both mononucleotide and dinucleotide repeats, and guidelines for MSI testing have been established, by which tumors can be characterized as MSI high (MSI-H), MSI low

(MSI-L), or microsatellite stable (MSS). Besides being a marker for nucleotide repeat instability, the MSI-H status has also been associated with better prognosis in certain colorectal, gastric, and pancreatic cancers (8). However, for many other malignancies, the correlation between MSI and prognosis remains unclear.

The maintenance of microsatellite repeat integrity in the genome is provided by the MMR system, which corrects single base-base mismatches and insertion-deletion loops (IDL) on the nascent DNA strand. It is generally accepted that MSI is largely attributable to the failure of repairing IDLs arising from replication slippage. MutS $\alpha$  (hMSH2-hMSH6) recognizes base-base mismatches and small IDLs, whereas MutS $\beta$  (hMSH2-hMSH3) recognizes IDLs of 3 or more nucleotides (9, 10). Upon binding, MutS $\alpha$  (or MutS $\beta$ ) undergoes ATP-dependent configuration change conducive for assembly of downstream repair factors, including MutL $\alpha$  (hMLH1-hPMS2 heterodimer), the main MutL activity, or one of the minor MutL factors, MutL $\beta$  and MutL $\gamma$  (11, 12). The most frequently mutated MMR genes in Lynch syndrome are *hMSH2* and *hMLH1*, with incident rates of 39.5% and 59.2%, respectively (www.insight-group.org; ref. 13).

Unlike hMSH2, which is required for all *in vitro* and *in vivo* MMR reactions, MutL $\alpha$  is only required for the *in vitro* MMR reaction in the 3' to 5' orientation (14). Intriguingly, a single 5' to 3' exonuclease hExo1 has been shown to support *in vitro* MMR in both 5' to 3' and 3' to 5'

**Authors' Affiliation:** School of Molecular Biosciences, Washington State University, Pullman, Washington

**Corresponding Author:** Chengtao Her, Mail Drop 64-7520, Washington State University, Pullman, WA 99164. Phone: 509-335-7537; Fax: 509-335-9688; E-mail: cher@wsu.edu

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orientations (15), of which the action of hExo1 in 3' to 5' MMR requires the endonuclease activity carried by MutL $\alpha$  (14). However, *Exo1*-deficient mice only display moderate phenotypes in comparison to *Msh2*- or *Mlh1*-deficient animals (16). In addition, cells lacking *Exo1* are proficient in repairing IDLs of 2 or more nucleotides (16), implying the presence of Exo1-independent exonuclease activities for the maintenance of microsatellite repeat stability. By interaction with hMLH1, the 3' to 5' exonuclease hMRE11 is shown to play a role in the 3' to 5' MMR reaction, and RNAi-mediated hMRE11 silencing elevates MSI (17). Current evidence indicates that hMRE11 dysfunction is causally linked to MSI in colon cancers (18–27).

### **hMRE11 Is Functionally Associated with the Mismatch Repair Protein hMLH1**

Several lines of evidence support a direct association of hMRE11 with the MMR machinery. The first indication came when mass spectrometry-based analysis revealed that hMRE11 and several MMR proteins coexisted with BRCA1 in a common protein complex referred to as BRCA1-associated genome surveillance complex (BASC), which is suggested to function as a DNA damage sensor and to play a broad role in the maintenance of genomic stability (28). The more direct evidence came from studies revealing physical interaction between hMRE11 and hMLH1 through *in vitro* and *in vivo* approaches (17, 29). The hMRE11 and hMLH1 genes also share similar expression profiles in 68 pairs of matched human normal and tumor samples representing 11 tissue types (kidney, breast, prostate, uterus, ovary, cervix, colon, lung, stomach, rectum, and small intestine) and 9 cancer cell lines (29), attesting a high likelihood for them to act in common cellular processes.

Studies have shown that the hMRE11-hMLH1 interaction is involved in MMR and DNA damage response to anticancer agents (17, 30, 31). RNAi-mediated hMRE11 silencing in HeLa cells led to MSI and defective MMR (17), of which hMRE11 deficiency specifically decreased 3' to 5' MMR activity to approximately 50% of the normal (17), in line with hMRE11 being a 3' to 5' exonuclease, and the remaining 50% of 3' to 5' MMR activity could be provided by hExo1 (14). The participation of hMRE11 in 3' to 5' MMR is likely mediated through its interaction with hMLH1. To this end, expression of hMRE11 aa452-634 polypeptide (i.e., the minimal hMLH1-interacting domain) in hMLH1-complemented MMR-proficient 293T cells decreased the efficiency of 3' to 5' MMR along with a comparable reduction of the 3' to 5' excision activity (31). Intriguingly, the majority of the 38 hMLH1 Lynch syndrome-associated missense mutations, representing 25% of approximately 300 known hMLH1 Lynch syndrome mutations, caused significant (>50%) reduction in hMRE11 interaction (31).

Both the hMLH1 and the hMRE11 complexes are known to play critical roles in mediating cellular DNA damage response. The hMRE11 complex is among the

first to arrive at the site of the DNA double-strand break (DSB); sensing of DSBs by the hMRE11 complex is required for ATM activation leading to subsequent phosphorylation of a series of downstream effectors to facilitate DNA repair and cell-cycle checkpoint control (32). Likewise, hMLH1 deficiency alters cell-cycle regulation in response to DNA damage induced by alkylating agents and cisplatin, thereby rendering cells resistant to anticancer treatments (33). These observations raised a possibility that, by virtue of their physical interaction (17, 29), hMRE11 acts with hMLH1 in the same DNA damage-signaling network. The role of hMRE11 in hMLH1-dependent DNA damage response has been studied in cellular responses to alkylating agents and cisplatin (30, 31). The formation of hMRE11 foci in human glioblastoma U87 cells treated with temozolomide (i.e., clinical analog of the model alkylating agent MNNG) required intact MMR; silencing of hMLH1 greatly decreased temozolomide-triggered G<sub>2</sub> arrest and suppressed hMRE11 foci formation (30). *Vice versa*, RNAi-mediated hMRE11 silencing phenocopied hMLH1 deficiency; both showed reduced temozolomide-triggered G<sub>2</sub> arrest and cytotoxicity (30). Furthermore, blocking hMRE11-hMLH1 interaction with hMRE11 aa452-634 polypeptide sufficiently reduced G<sub>2</sub> arrest and cytotoxicity in cells treated with MNU or cisplatin (31). Possibly, the hMRE11-hMLH1 interaction exerts its action on DNA damage response through recognition of DNA lesion-containing chromatin. The study by Mirzoeva and colleagues suggested that temozolomide could elicit NBS1 and hMLH1 chromatin association (30).

The hMRE11-hMLH1 interaction may also play a role in the regulation of pathway selection during DSB repair. It is known that hMRE11 promotes DSB repair through non-homologous end joining (NHEJ; refs. 34, 35), whereas hMLH1 negatively regulates homologous recombination (36). Thus, the hMRE11-hMLH1 interaction is expected to exert a suppressive effect on homologous recombination through its NHEJ-promoting action.

### **hMRE11 Poly(T)<sub>11</sub> Mutation in Mismatch Repair-Deficient Cancer Cells**

It is articulated that MMR deficiency *per se* is not sufficient to produce profound biological consequences, for example, increasing the risk of cancer. MMR maintains genomic stability through recognition and repair of post-replicative errors that occur at a frequency of 1 for every 10 million replicated nucleotides (37). This rate of genome alteration is too low to account for the level of genomic instability conducive to Lynch syndrome development in MMR-deficient patients. It is believed that MMR defects have to first create mutations in genes that are critical for maintaining genomic stability and proper DNA damage signaling. To this end, 32 putative MMR "target" genes have been identified, of which all genes show instability of coding mononucleotide repeats in MMR-deficient cancer cells (38). This target list currently only features genes

possessing mononucleotide repeats within protein-coding sequences, which include cell-cycle and apoptosis regulatory genes (e.g., *TGF $\beta$ 1R2*, *TCF-4*, *IGF2R*, *CDX2*, *BAX*, *CASPASE 5*, *RIZ*, *FAS*, *APAF*) and genes involved in DNA repair and damage response (e.g., *MBD4*, *BLM*, *CHK1*, *hMLH1*, *hRAD50*, *hMSH3*, *hMSH6*). Although the precise pathogenic effects of many coding mononucleotide repeats' instability have yet to be explored, these mutations are expected to lead to complete gene inactivation, thus impairing their corresponding pathways.

In contrast to coding mononucleotide repeat instability, appreciation of the effects of noncoding mononucleotide repeat mutations is much more complicated because this type of instability is often confounded by incomplete penetrance. Studies in the past several years have revealed a close association of MMR deficiency with mutations of an intronic poly(T)11 repeat in hMRE11 IVS-4 (18–27). This repeat is located 1 nucleotide upstream of the splicing acceptor sequence at the 3' end of hMRE11 intron 4. Deletions of 1 to 2 nucleotides at the poly(T)11 repeats occurred with variable frequencies in MMR-deficient tumors, including colorectal, gastric, endometrial, and urothelial cancer. The frequency of this mutation in MMR-deficient tumors ranges from 67 to 100% for colorectal cancer (18, 21, 24–26), 75 to 92% for stomach cancer (19, 26), 50 to 60% for endometrial cancer (21, 26), and 46 to 75% for urothelial cell carcinomas (22, 26). In addition, the hMRE11 poly(T)11 mutation was common among MMR-deficient and/or MSI-positive tumor cell lines (i.e., colorectal, endometrial, prostate, pancreatic, and leukemia and/or lymphoma; refs. 20, 23, 39). In fact, it seemed that mutations occurred more frequently at the hMRE11 intronic poly(T)11 tract than at the hRAD50 exon 13 poly(A)9 tract in tumor cell lines (20). In contrast, the (A)7 tract in NBS1 was rarely mutated in MSI-positive gastrointestinal carcinomas (40).

Although the functional consequence of hMRE11 poly(T)11 deletion has yet to be mechanistically addressed, it is shown that deletion of 2 nucleotides in the poly(T)11 repeats increases aberrant splicing and thus correlates with the reduction but not abolition of hMRE11 protein expression (18, 21, 39). Shortening of the poly(T)11 tract may interfere with the recognition of splicing signals and thus promote aberrant alternative splicing, especially exon 5 skipping (18). The extent of this event seemed to correlate with the size of poly(T)11 deletion, in which significant reduction of hMRE11 protein expression in MMR-deficient tumors is generally associated with biallelic 2-bp deletion (21). However, most of the poly(T)11 mutations identified in MMR-deficient tumors are monoallelic heterozygous with 1-bp deletion (18–26). In MSI-H tumors and cell lines, the incidence of a 2-bp deletion at the poly(T)11 tract is much less prevalent than a 1-bp deletion (18, 20, 23), suggesting that 2-bp and 1-bp shortening of the poly(T)11 tract are differentially regulated. However, it is presently unknown how the hMRE11 poly(T)11 mutation is mechanistically controlled by MMR deficiency. In contrast to the frequent frame shift deletion and/or insertion

mutations at the hRAD50 exon 13 poly(A)9 tract (20), the size of hMRE11 poly(T)11 deletions rarely exceeds 2 bp even on an MSI-H background.

It would be of interest to know what maintains the stability after 2-bp deletion and how MMR deficiency temporally correlates with the occurrence of hMRE11 poly(T)11 mutation during cancer development. Resolution of this conundrum is especially important for a better understanding of the role of the MMR-dependent hMRE11 poly(T)11 mutation in genomic instability during cancer development. In addition, it will also help us to devise more effective strategies in cancer therapy. For example, MMR-deficient tumor cells that contain mutations at the hMRE11 poly(T)11 tract are very sensitive to combined camptothecin-thymidine treatment (41).

### **hMRE11 Dysfunction and Microsatellite Instability and/or Mismatch Repair Deficiency**

The apparent association of hMRE11 poly(T)11 mutation with MMR deficiency in various tumor types seems to support a scenario that defective MMR could exert its effect on genomic instability by targeting hMRE11 (18, 21). However, the type of poly(T) mutation constituting most of this association is the 1-bp deletion. The overall mutation rate at the hMRE11 poly(T)11 tract was dramatically elevated in MMR-deficient cells, but in a cell-type-specific manner, and colorectal cancer displays by far the highest poly(T)11 mutation rate when both 1- and 2-bp deletions were considered (18, 21, 23, 26). Unfortunately, detection of subtle effects of poly(T)11 deletion on hMRE11 impairment is limited by the sensitivity of currently available techniques, and it is further confounded by the existence of other factors affecting the levels of hMRE11, such as the levels of its binding partner hRAD50. In fact, the lack of a significant correlation between hMRE11 poly(T)11 and hRAD50 exon 13 poly(A)9 mutations and their corresponding protein expression was noted in 27 MSI-H colorectal carcinomas, despite the presence of hMRE11 poly(T)11 mutation in most of the tumors (24). Examination of 10 MMR-deficient cell lines, derived from uterine, endometrial, prostate, and colorectal cancers, revealed that all cell lines contained a 1- or 2-bp deletion at the hMRE11 poly(T)11 tract, but a 2-bp biallelic deletion was only found in 1 cell line (20).

Furthermore, current evidence tends to suggest that the causal link between MSI and hMRE11 poly(T)11 mutation is cell-type specific. A disconnection between MSI and hMRE11 poly(T)11 mutation was observed in a series of leukemia and/or lymphoma cell lines (23). Our sequencing analysis of the hMRE11 poly(T)11 tract in MMR-deficient 293T, MMR-proficient 293TL $\alpha$  (33), and HeLa cells identified a monoallelic 1-bp deletion in 293T and a monoallelic 2-bp deletion in 293TL $\alpha$  cells; conversely, no mutation was found in HeLa cells. The level of hMRE11 expression in 293T cells was equivalent to that of HeLa cells, and it was at least 3-fold higher than that of 293TL $\alpha$  cells. These examples highlight that MMR deficiency is not

the major determining factor for hMRE11 impairment. A similar conclusion was drawn when Kern and colleagues analyzed MMR-deficient gastrointestinal cancer cell lines and xenografts; although shortening of hMRE11 poly(T)11 tract was noted, significant reduction of hMRE11 protein expression was not observed (39). Furthermore, Peltomaki and colleagues have recently reported that deletion at the hMRE11 poly(T)11 tract rarely occurred in brain and kidney tumors of MMR-deficient Lynch syndrome patients (26). This evidence is compatible with the inclination that mutations, predominantly 1-bp deletions at the hMRE11 poly(T)11 tract, accumulate in MMR-deficient tumors in a cell-type-specific manner.

Collectively, aforementioned examples support the view that *hMRE11* is unlikely a critical MMR target gene. Given that changes in microsatellites often arise from replication slippage with an estimated frequency of 1 in every 1,000 to 10,000 cell divisions (1, 5), 1-bp deletion at the hMRE11 poly(T)11 tract could occur first, then subjected to clonal expansion during malignant transformation. Alternatively, the slight reduction of hMRE11 protein or the expression of low-level truncated hMRE11 could potentially render cells susceptible to the risk of disarming the wild-type copy of the concerned MMR genes, therefore converting cells to an MSI-H phenotype and promoting mutations in the coding mononucleotide repeats in various MMR target genes (e.g., *hMSH3*, *hMSH6*, *hRAD50*; Fig. 1). Together, the overall effect of these mutations could lead to gross genomic alterations required for developing full-blown cancers.

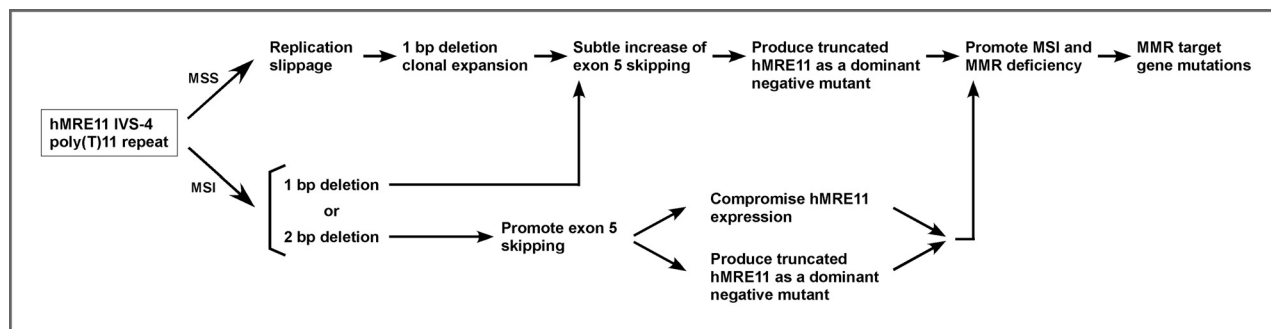
The 2-bp deletion is possibly attributable to MMR deficiency; however, it is unclear whether this deletion derives directly from the wild-type poly(T)11 tract or the 1-bp deletion mutant. Nevertheless, the 2-bp deletion at the hMRE11 (T)11 tract is expected to compromise the role of hMRE11 in the maintenance of microsatellite stability (17). The reduction, but not abolition, of hMRE11 expression may be advantageous for tumor cells to maintain growth potential. Alternatively, reduction of hMRE11 protein expression by shortening of the poly(T)11 tract could impair the function of hMRE11 in DNA replication (i.e., loosen

damage surveillance at the replication fork; refs. 42, 43) and increase the rate of replication slippage at microsatellite sequences. In short, the current literature suggests that hMRE11 is involved in the process of MMR and MMR-dependent tumorigenesis in colorectal cancers.

### hMRE11 and Telomere Repeat Stability

With approximately 3 to 20 kilobases of tandem hexanucleotide (TTAGGG)<sub>n</sub> repeats, telomeres may conceptually be envisioned as super microsatellites (1). The functions of hMRE11 at telomeres are multifaceted, spanning from telomerase regulation and telomere end processing to telomere DNA damage sensing. Telomere dysfunction directly contributes to genome stability. Defective MMR deregulates telomere length control and induces alternative lengthening of telomeres through telomeric DNA recombination (44). Through binding to TRF2, hMRE11 localizes to telomeres and maintains their stability (45, 46). During telomere DNA replication, hMRE11 protects newly replicated leading-strand telomeres from the action of NHEJ by promoting end resection to generate single-strand telomere overhangs, and cells lacking hMRE11 failed to activate DNA damage response induced by uncapped telomeres (46). However, the precise molecular mechanism underlying the involvement of hMRE11 in telomere protection remains to be elucidated.

While telomere end metabolism is a unique molecular event (47, 48), the bulk of telomeres are most probably replicated similar to the rest of chromosomal DNA. Replication of long microsatellite sequences, such as telomeres, is more prone to replication slippage. In sporadic colon cancers, telomeres were shortened in MSI-positive tumors, especially when hMSH2 was deficient (49, 50). Consistent with a role for hMSH2 in the regulation of telomere stability, hMSH2 silencing in normal human cells was shown to accelerate telomere shortening (51). Furthermore, hMSH2 also plays a role in sensing telomere dysfunction, as abrogation of MSH2 eliminated DNA damage response normally triggered by short telomeres (52). In addition,



**Figure 1.** Model for the effect of hMRE11 IVS-4 poly(T)11 mutation on MSI and MMR. In MSS colonic cells, a 1-bp deletion at the hMRE11 poly(T)11 tract randomly arises from replication slippage, resulting in subtle reduction of full-length hMRE11 expression and a slight increase of the truncated hMRE11 protein. This reduction and increase, in turn, lead to MSI and MMR gene inactivation in clonal expanded cells, thereby promoting subsequent MMR target gene mutations and tumorigenesis. In MSI colonic cells, a 1-bp deletion at the hMRE11 poly(T)11 tract is readily accumulated and exerts its biological effects in a similar fashion as stated above, whereas a 2-bp deletion at the poly(T)11 tract has a more direct effect on tumorigenesis.

several types of telomere repeat variants were found to be extremely prone to replication-dependent instability in somatic cells (53), implying that telomere instability may be more common than previously thought.

Although the biological significance of IDLs at telomeres has yet to be explored, one can envision that such aberrant structures are able to trigger DNA repair and damage responses. It is conceivable that hMRE11 may have a role in the maintenance of telomere repeat stability, a trait that can be difficult to analyze and might require the development of more sensitive means. Nonetheless, altering hMRE11 function by poly(T)11 mutation is expected to increase telomere instability while concomitantly decreasing telomere damage response. The combination of these two effects would likely facilitate the accumulation of genomic rearrangements in cancer cells. Further studies are warranted to determine the precise molecular mechanism of action and to elucidate the dynamic interplay between the hMRE11 complex and the MMR pathway in DNA damage surveillance and cancer development.

## Summary and Perspective

A better understanding of the pathogenic effects stemming from MMR deficiency is essential for devising effective anticancer strategies. Identification of potential MMR target genes represents an important approach for reaching that goal. Increasing evidence indicates that hMRE11 is not a typical MMR target gene; reduction of hMRE11 protein

levels or the generation of hMRE11 truncation peptide might be one of the prime risk factors to trigger MMR deficiency in highly proliferative cell types, such as colonic epithelial cells. The subtle functional changes of hMRE11, initiated from poly(T)11 deletion, in fast-growing cells may increase the probability of replication slippage at microsatellite sequences and compromise MMR. To this end, it would be of great interest to delineate the temporal correlation between hMRE11 poly(T)11 mutation and MMR deficiency during cancer development. Given the important role that hMRE11 plays in cellular proliferation, perhaps a fraction of the wild-type poly(T)11 and/or 1-bp deletion alleles observed in various tumor types were actually revertants of 1- or 2-bp deletion mutants; regaining hMRE11 function may help tumor cells to reach a full proliferation potential.

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No potential conflicts of interest were disclosed.

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