

Therapeutic Targeting of the DNA Mismatch Repair Pathway

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Abstract

The mismatch repair (MMR) pathway is involved in the removal of DNA base mismatches that arise either during DNA replication or are caused by DNA damage. Mutations in four genes involved in MMR, *MSH2*, *MLH1*, *PMS2* and *MSH6*, predispose to a range of tumorigenic conditions, including hereditary nonpolyposis colon cancer, also known as Lynch syndrome. Here we discuss the canonical MMR pathway and the burgeoning evidence for noncanonical roles for the MMR genes, and highlight the therapeutic implications of MMR. In particular, we discuss how the DNA repair defect in MMR-deficient cancers could be exploited by the development of novel therapeutic strategies based on synthetic lethal approaches.

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Background

DNA is constantly exposed to a variety of different insults that can ultimately result in DNA mutation and altered cell behavior. DNA mutations can be caused by DNA damage, initiated by exogenous agents such as ultraviolet light, or endogenous cellular factors such as reactive oxygen species (1). Mutations can also be formed when errors in DNA replication become fixed in the genome. For example, replication errors such as base substitution mismatches (in which the incorrect base is incorporated into newly synthesized DNA) and insertion-deletion mismatches can result in mutations. Although these replication errors are relatively rare and can be reversed by “proofreading” DNA polymerases, if left unrepaired, they can potentially foster tumorigenesis. To limit the severe impact of DNA damage and replication errors, cells have evolved a plethora of molecular processes that maintain the integrity of the genome.

The DNA mismatch repair (MMR) pathway comprises one of these molecular pathways and has a well-established role in the reversal of mutations caused by DNA replication (postreplicative repair). The importance of this pathway is perhaps best illustrated by the fact that MMR-deficient cells adopt a “mutator phenotype” in which the frequency of base substitutions, some of which cause frameshifts or codon alterations in coding sequences, increases. More significantly, when the genes that mediate MMR are mutated or epigenetically silenced, the predisposition to cancer is vastly increased. In this review, we aim to provide the reader with a basic understanding of the MMR pathway.

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We also discuss how MMR dysfunction can determine the response to treatment and may also be viewed as one of the “Achilles' heels” of tumor cells that may be exploited therapeutically.

The Mismatch Repair Pathway

The proteins that mediate the MMR pathway are highly conserved from bacteria through to humans, although MMR in higher eukaryotes has a number of unique features (2).

Types of DNA changes repaired by mismatch repair

MMR is the main pathway responsible for repair of base-base mismatches and insertion and/or deletion loops that are formed during DNA replication. Consequently, mutation rates are 100 to 1,000 fold greater in MMR-deficient tumor cells in comparison to normal cells. These postreplicative repair defects caused by MMR deficiency can be clearly observed in microsatellites. Microsatellites are short tandem repetitive DNA sequences that are found throughout the genome (3). The repetitive nature of microsatellites renders them particularly susceptible to replication errors caused by DNA polymerase slippage over the repeat sequence. Normally these replication errors are repaired by MMR, but in its absence, microsatellite sequences accumulate replication errors, and the number of DNA residues in a single microsatellite reduces or increases (a characteristic known as microsatellite instability or MSI). Tumors with defects in genes that control MMR, such as *MLH1*, *MSH2*, and *PMS2*, generally exhibit MSI. Interestingly, defects in another MMR gene, *MSH6*, do not cause the same high frequency of MSI (4), an effect possibly explained by the ability of other MMR proteins to compensate for *MSH6* deficiency (see below; refs. 4, 5).

The components

The canonical human MMR pathway has two major components, both heterodimers, with names originating

from their lower organism orthologs, MutS and MutL. MutS is present in two basic forms, MutS α and MutS β . The MutS α heterodimer, which encompasses MSH2 in a complex with MSH6, is involved primarily in the repair of base substitutions and small mismatched loops (Fig. 1). MutS β (encompassing MSH2 and MSH3) repairs both small loops as well as large loop mismatches (~10 nucleotide loops; Fig. 1). It is debatable whether MutS β can repair single-base mutations (6, 7). The MutL heterodimer is also present in a number of forms, including the MutL α complex, which is made up of MLH1 and PMS2 proteins, the MutL β heterodimer (MLH1 and PMS1), and MutL γ (MLH1 and MLH3; ref. 8). The primary MutL activity for mismatch correction is MutL α , with MutL β apparently providing a minor role, whereas MutL γ has a rather unclear role in MMR (9–11).

The canonical pathway

The best understood role of human MMR is in the repair of postreplicative mismatches, in which an incorrect base has been incorporated into newly synthesized DNA. Here, MutS initiates a process that removes the DNA from the newly synthesized daughter strand around a mismatch and replaces it with the correct sequence, using the parental DNA strand as a template (Fig. 2). Mismatch recog-

niton is carried out by MutS. The MutS heterodimer does this task by recognizing distortions in the DNA double helix structure caused by mismatched bases (12). MutS initially binds double-stranded DNA at the site of a mismatch and then recruits MutL. MutL seems to act as the mediator for a series of subsequent protein interactions that facilitate MMR. In one of the proposed models of MMR, the MutS/MutL complex leaves the site of mismatch after the initial recognition event and then slides up and down the flanking DNA sequence. This “sliding clamp” conformation is controlled by the exchange of ADP for ATP, such that when MutS is bound to ADP, it remains tightly bound to the DNA at the mismatch, but when this is converted to ATP, MutS converts to its sliding clamp form (13). MutS proteins possess a weak ATP hydrolysis activity that is stimulated in the presence of a mismatch and controlled by the ADP-ATP exchange provoked by the mismatch. The exchange of ADP for ATP changes the affinity of MutS for the mismatch, resulting in the release of the protein. The sliding clamp movement of MutS/MutL complex up and down the DNA double helix eventually allows MutS/MutL to encounter a single-strand gap in the DNA sequence that is bound by accessory proteins such as PCNA and RFC (14). As the function of postreplicative MMR is to reverse errors of replication (i.e., replication errors that are found in newly synthesized DNA), the MMR machinery must be able to distinguish parental DNA from daughter DNA and then remove and replace the (presumably aberrant) daughter DNA sequence (see Fig. 2), and it is thought that intermittent gaps in DNA in between Okazaki fragments on lagging strand DNA could represent a signal that distinguishes parental from daughter strand in mammalian MMR (15). However, the discriminatory signal on leading-strand DNA in mammalian cells is still unclear. Regardless, it seems that the encounter of the sliding clamp with PCNA and RFC, and the presence of a single-strand gap in the DNA, allows the identification of the daughter strand and a DNA exonuclease, EXO1, to enter the DNA structure (16). EXO1, guided by the MutS/MutL sliding clamp, then starts removing daughter-strand DNA towards and then beyond the site of the mismatch. Once the mismatch is removed, the activity of EXO1 is suppressed by MutL, thus terminating DNA excision. At the completion of this process, a DNA polymerase synthesizes DNA in place of the excised sequence. Finally a DNA ligase joins any remaining gaps in the DNA sequence (14).

In addition to the repair of errors during replication, MMR also mediates the response to certain forms of DNA damage-inducing agents that can modify the structure of bases, including methylating agents, thioguanine, oxidizing agents, *cis*- and carboplatin, methyl methanesulfonate, 5-fluorouracil (5-FU), and 5-FudR (17). An example is the response to alkylating agents, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), which cause the alkylation of DNA and the formation of O⁶methylguanine (O⁶mG)-containing mismatches. These adducts are recognized by the MutS heterodimer. Not only does the MMR

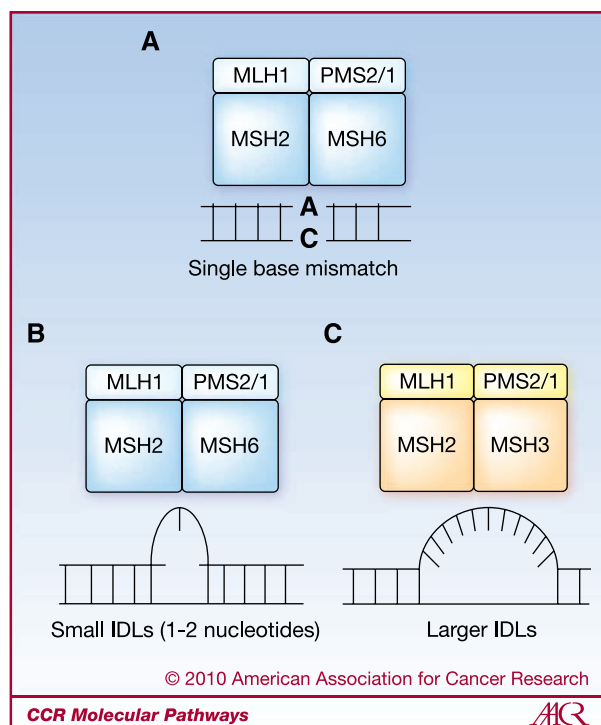


Fig. 1. Schematic of DNA damage recognized by the MMR pathway. A, the MutS α (MSH2/MSH6) heterodimer recognizes base-base mismatches and B, small insertion-deletion loops (IDL). The MutS β (MSH2/MSH3) heterodimer recognizes single nucleotide IDLs and C, longer IDLs (10-nucleotide loops). In association with the MutL heterodimer and other associated proteins, these mismatches are excised and repaired.

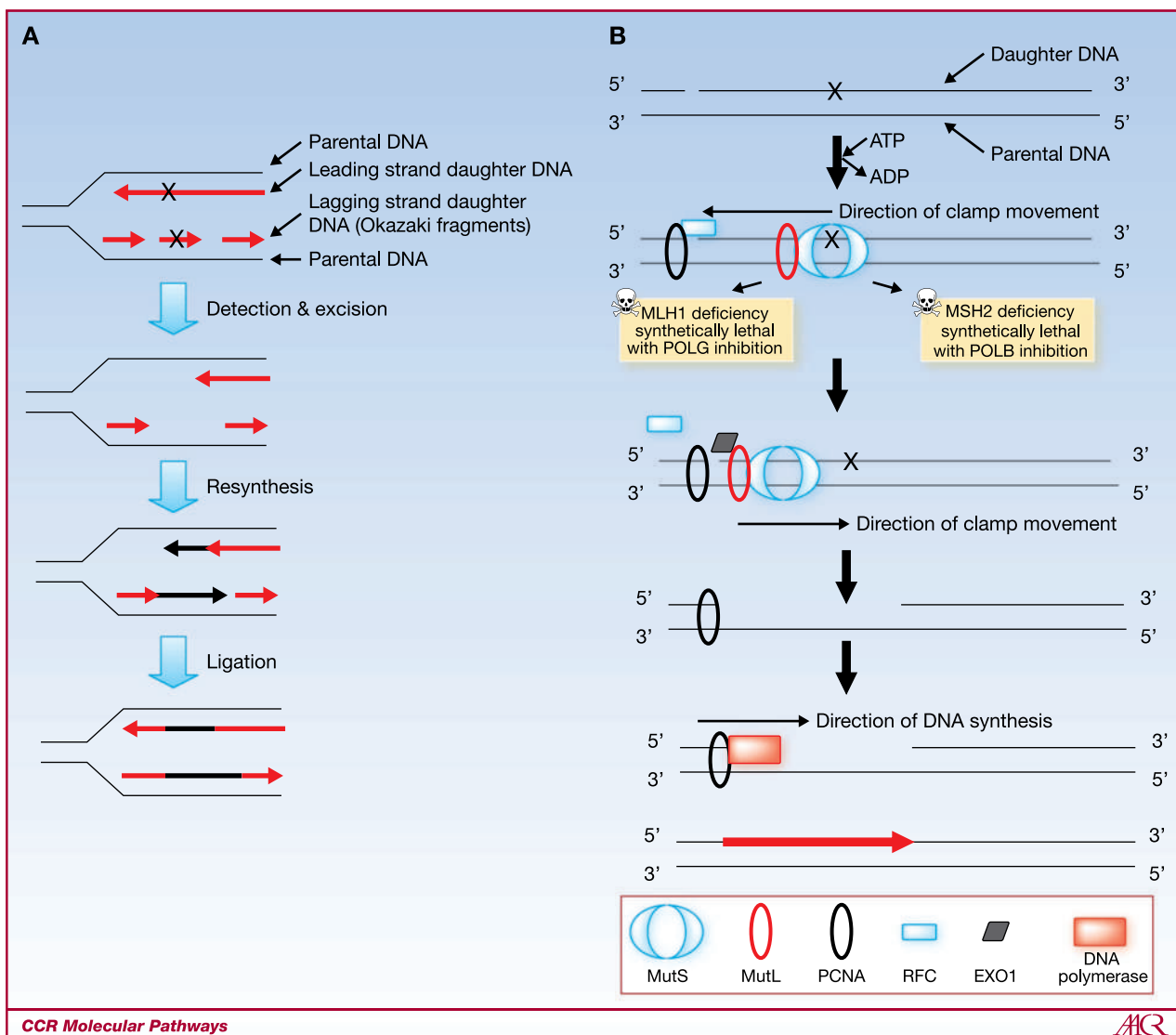


Fig. 2. A, schematic of mammalian MMR as a postreplicative repair process. Mismatches (X) caused by errors in replication are shown in the daughter strands of replicated DNA (mismatches are shown on both the leading and lagging strands of replicated DNA). MMR involves (i) detection of the mismatch and excision of daughter strand DNA around the mismatch, (ii) resynthesis of DNA across the mismatch site using parental DNA as a template, and (iii) ligation of resynthesized DNA onto existing daughter strand DNA. B, a simplified mechanistic model of human MMR. MutS detects a mismatch (X) and recruits MutL. In a sliding clamp conformation, the MutS/MutL complex leaves the site of mismatch and slides up and down the DNA double helix. For simplicity, the movement of MutS/MutL in one (upstream) direction is shown. Eventually the MutS/MutL complex encounters a single-strand DNA gap on the daughter strand that is also bound by PCNA and RFC. This encounter displaces RFC and allows EXO1 to access the daughter strand DNA. Guided by MutS/MutL, EXO1 degrades DNA across the site of the mismatch, after which EXO1 is inactivated by MutL. During this excision process, the parental strand of DNA is stabilized by RPA (not shown). Once the mismatch DNA is excised, a DNA polymerase synthesizes new DNA, which is eventually ligated onto the existing daughter strand. Synthetic lethal interactions are indicated, whereby upon MSH2 deficiency and silencing of POLB or upon MLH1 deficiency and silencing of POLG, there is an accumulation of oxidative DNA lesions, which results in cellular lethality. Adapted with permission from Jiricny (56).

machinery recognize these mismatch lesions, but it also mediates the cell-cycle checkpoint and apoptotic responses that normally occur after exposure to MNNG. When cells are MMR deficient, the cell-cycle and apoptotic responses to these agents are suppressed and cells are chemoresistant. A number of differing models attempt to explain the chemosensitivity of MMR-proficient cells and the chemoresistance of MMR-deficient cells. The first model, known as

futile cycling (18), is based on MMR replacing DNA on the newly synthesized DNA strand after replication. In this model, replication of DNA adducts such as O^6 meG induces the misincorporation of a base; this in turn triggers a MMR reaction that removes DNA on the newly synthesized DNA strand but not the O^6 meG lesion itself. The persistence of the O^6 meG lesion provokes subsequent cycles of MMR, which ultimately activate ATR- and/or ATM-dependent

cell-cycle arrest and/or apoptotic pathways, perhaps by causing persistent single-strand breaks that ultimately stall replication forks. In the absence of MMR, adducts such as O⁶meG are not recognized, futile cycles are not invoked, and cells, therefore, tolerate and exhibit resistance to DNA-damaging agents that induce such lesions. An alternative explanation for the chemoresistance of MMR-proficient cells is the direct signaling model in which MutS and MutL, activated by mismatches containing DNA lesions, directly or indirectly interact with ATM or ATR, eliciting cell-cycle arrest and apoptosis (19).

Noncanonical Functions of Mismatch Repair Proteins

A growing body of evidence suggests that MMR proteins have distinct roles out of the canonical MMR pathway. An example of this is a suggested role for MMR in the transition to hormone independence in breast and prostate cancer progression (20). Specifically, MSH2 has been shown to be a potent coactivator of estrogen receptor (ER) alpha (21), and MSI status (a surrogate of MMR deficiency) has been associated with negative expression of ER and progesterone receptor (PR), although the MSI-ER-PR correlation requires further clarification (22, 23). Additionally, MMR proteins have proposed roles in mitotic and meiotic recombination and antibody class-switch recombination in which they have proposed roles in the conversion of single-strand breaks to double-strand breaks (24, 25).

MMR proteins may also have a function in the suppression of homologous recombination (HR), a DNA repair process associated with the repair of double-strand DNA breaks and stalled replication forks (26). Whereas HR serves to repair potentially lethal DNA breaks, if uncontrolled, it can cause inappropriate DNA recombination, which itself is deleterious to the integrity of the genome. MMR may, therefore, further ensure genome integrity by suppressing the deleterious effects of other DNA repair processes.

Clinical-Translational Advances

The critical role of the MMR pathway in carcinogenesis is exemplified by the fact that germline mutations in MMR genes are responsible for the mutator phenotype and predisposition to cancer (27). MMR deficiency can be inherited, as in the case of Lynch syndrome [also known as hereditary non-polyposis colorectal carcinoma (HNPCC)], or can occur via biallelic *MLH1* promoter hypermethylation, as is observed in ovarian, endometrial, gastric, and colorectal carcinoma, among others (28). In the case of colorectal cancer, MMR deficiency is estimated to be present in 15 to 17% of all primary cancers (29, 30). Interestingly, different MMR gene deficiencies lead to distinct phenotypic and clinical manifestations. For example, *MLH1* mutations are particularly associated with an increased risk of

colon cancers, whereas *MSH2* mutations have a higher incidence of extracolonic tumors (31, 32). Patients carrying heterozygous *MSH6* or *PMS2* mutations usually display a decreased frequency and a later onset of HNPCC (27).

MMR deficiency can be identified using a number of different markers. For example, DNA sequencing can be used to identify mutations in MMR genes. More routinely, tumor biopsies are used to assess the presence or absence of MMR proteins (by immunohistochemistry) or MSI status. Usually, five different microsatellites are assessed for stability, and tumors are defined as MSI-high (MSI-H) if two or more microsatellite markers show instability, MSI-low (MSI-L) in which one of the five markers are unstable, and microsatellite stable (MSS) in which none of the five markers show instability (33). MSI-low tumors have also been classified as MSS, and the difference between MSI-L and MSS seems to be largely quantitative.

The standard adjuvant treatments for colorectal cancer involve 5-FU-based chemotherapy regimens (34). Although the evidence is somewhat contradictory, studies have suggested that MMR deficiency is associated with resistance to treatment with 5-FU (35, 36). MMR deficiency has also been associated with resistance to platinum compounds such as cisplatin and carboplatin treatment, but sensitive to oxaliplatin treatment (37, 38). These differences in response to differing DNA-damaging agents are most likely due to the differential recognition of specific drug-induced adducts. The efficacy of topoisomerase inhibitors, such as etoposide and irinotecan, in MMR-deficient cancers has also been debatable (39–41).

Synthetic lethal targeting of mismatch repair deficiency

The exploitation of synthetic lethal interactions has been suggested as a means to target the loss of tumor suppressor gene function, such as MMR gene deficiency. Two genes are synthetically lethal if loss of one is compatible with cellular viability, but loss of both leads to cell death. Therefore, in principle, identification of synthetic lethal interactions with MMR deficiency could potentially result in the identification of specific therapeutic targets for this disease area. The promising efficacy of a synthetic lethal strategy using poly(ADP-ribose) polymerase (PARP) inhibitors in *BRCA1* or *BRCA2* mutation carriers with breast or ovarian cancer (42–44) suggests that these genetic approaches could have genuine clinical utility.

We have applied this approach to MMR-deficient cancers by identifying a synthetic lethal interaction between MSH2 deficiency and treatment with the chemotherapeutic agent methotrexate (45). Treatment with methotrexate results in an accumulation of DNA oxidative lesions, such as 8-oxoG in MSH2-deficient cells but not in MSH2-proficient cells, and we propose that this accumulation of DNA damage is incompatible with cellular viability in MSH2-deficient cells. A phase II, nonrandomized clinical trial of methotrexate in MSH2-deficient metastatic colorectal cancer (MESH, NCT00952016) is currently underway, incorporating measurement of 8-oxoG DNA lesions as a biomarker.

Interestingly, there is growing evidence that different MMR gene deficiencies seemingly mediate different phenotypes. For example, MSH2-deficient cells are profoundly sensitive to methotrexate in *in vitro* culture, whereas MLH1 deficiency does not mediate the same sensitivity (45). Similarly, MSH2-deficient cells are sensitive to psoralen, an agent that induces DNA interstrand crosslinks, but MLH1-deficient cells tend to be more resistant (46, 47). MSH2 and MLH1 also differ in other synthetic lethal relationships. MSH2 deficiency is synthetically lethal with inhibition of DNA polymerase β in a series of cellular models (e.g., inhibition of DNA polymerase β kills MSH2-deficient cells), but DNA polymerase β /MLH1 synthetic lethality is not observed. Conversely, inhibition of DNA polymerase γ is synthetically lethal with MLH1 deficiency but not MSH2 deficiency (48). The MSH2-DNA polymerase β synthetic lethality correlates with the nuclear accumulation of 8-oxoG DNA lesions, whereas the MLH1/DNA polymerase γ synthetic lethality is characterized by accumulation of 8-oxoG lesions in mitochondrial DNA. Although the components of the human mitochondrial MMR system are not absolutely clear, mismatch-binding activity has been observed in mitochondrial extracts from cells lacking MSH2, suggesting a distinctive pathway for nuclear and mitochondrial MMR (49). The repair protein Y-box binding protein 1 (YB-1) has been implicated in mitochondrial MMR, as it localizes to mitochondria and significantly contributes to MMR activity detected in mitochondrial extracts from a human cell line (49). Moreover, we and others have shown that MLH1 is also localized to the mitochondria (48, 50).

Recently, we have shown that silencing of the PTEN-induced putative kinase 1 (*PINK1*) is synthetically lethal in MMR-deficient cell lines originating from *MSH2*, *MLH1*, or *MSH6* dysfunction.³ Inhibition of *PINK1* in an MMR-deficient background results in an elevation of reactive oxygen species and the accumulation of both nuclear and mitochondrial oxidative DNA lesions. Taken together, these data highlight the potential for focusing upon oxidative damage as a therapeutic target in MMR deficiency. Furthermore, mechanism-based biomarkers could be used

to identify patients likely to respond favorably to therapy. For example, combined MSH2/POLB, MLH1/POLG, or MMR/PINK1 inhibition is characterized by a sustained increase in the levels of 8-oxoG, suggesting that 8-oxoG measurement may provide a mechanism-based marker of drug efficacy, to be used alongside more standard measures of tumor response.

In targeting MMR deficiency, the presence of secondary mutations needs to be taken into consideration. One consequence of MMR is MSI, which can potentially lead to mutations in critical genes, which contain coding repeat sequences. Mutations can accumulate in tumor suppressor genes and oncogenes such as *MRE11A*, *ATR*, *RAD50*, *TGFBR2*, *IGF2R*, and *PTEN* (51–53). Among these are mutations in a number of double-strand break repair genes (*ATR*, *MRE11A*, and *RAD50*), which have particular significance as loss of double-strand break repair gene function can cause PARP inhibitor sensitivity (54). Similarly, loss of *PTEN* function can also confer sensitivity to PARP inhibitors (55). Therefore, combination therapies including methotrexate and PARP inhibitor may provide the potential of targeting both the primary mutation and the secondary mutation, in parallel.

Summary

Our existing understanding of the DNA MMR pathway suggests that MMR has important roles in both the predisposition to cancer and also the response to therapy. It is hoped that a further dissection of both the canonical and noncanonical functions of MMR proteins will allow the further refinement of cancer treatment and our understanding of how DNA repair defects foster tumorigenesis. It also seems likely that synthetic lethal strategies that target MMR-deficient tumors could eventually be used clinically. These novel strategies will, in turn, be refined by applying a mechanistic understanding of this DNA repair pathway to the clinical scenario. Ultimately, this may enable tumors to be treated specifically by targeting their underlying MMR defect, resulting in greater therapeutic windows and reduced normal tissue toxicity.

Disclosure of Potential Conflicts of Interest

S. Martin, C. Lord, A. Ashworth: methotrexate patent pending; S. J. Martin, C. J. Lord, A. Ashworth: synthetic lethal approaches in MMR patent pending.

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³ Martin SA, Hewish M, Lord CJ, Ashworth A. Parallel high throughput RNA interference screens identify *PINK1* as a potential therapeutic target for the treatment of DNA mismatch repair deficient cancer, manuscript in preparation.

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