Minireview

The Role of DNA Mismatch Repair in Drug Resistance

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Abstract

Loss of DNA mismatch repair (MMR) has been observed in a variety of human cancers. In addition to predisposing to oncogenesis, loss of MMR activity is of concern with respect to the use of chemotherapeutic agents to treat established tumors. Loss of MMR results in drug resistance directly by impairing the ability of the cell to detect DNA damage and activate apoptosis and indirectly by increasing the mutation rate throughout the genome. The MMR proteins are involved in mediating the activation of cell cycle checkpoints and apoptosis in response to DNA damage. MMR-deficient cells have been reported to be resistant to the methylating agents procarbazine and temozolomide, the alkylating agent busulfan, the platinum-containing drugs cisplatin and carboplatin, the antimitabolite 6-thioguanine, and the topoisomerase II inhibitors etoposide and doxorubicin. In the case of cisplatin, busulfan, temozolomide, and procarbazine, the degree of resistance has been shown to be sufficient to produce a large difference in clinical responsiveness in vivo in tumor model systems. The available preclinical data suggest that tumors that contain a significant fraction of cells deficient in MMR will demonstrate reduced responsiveness to specific drugs. The challenge now is to assess the clinical significance of the presence of deficient cells in tumors and to discover drugs that retain activity against MMR-deficient cells.

Introduction

Maintenance of genomic stability requires the proper functioning of DNA replication, repair, and recombination processes. Among these, MMR plays a prominent role in the correction of replicative mismatches that escape DNA polymerase proofreading. MMR was originally described in bacteria that are able to repair mispair-containing bacteriophages (1). In the bacterial system, the important components are MutS, MutL, and MutH, named after their corresponding bacterial mutator strains. The MutS protein initially recognizes and binds to mismatched DNA (2). After this, MutH and MutL form a complex with MutS to carry out excision repair; MutH has endonuclease activity, but the specific activity of MutL has not been identified. The important role played by the MMR proteins is emphasized by the fact that they are highly conserved from bacteria to yeast to mammals. Biochemical and genetic studies in human cells have defined five genes whose products play key roles in MMR including hMSH2 (3, 4), hMSH3 (5, 6), and hMSH6 (also called GTBP (7) or p160 (8)), which are homologues of MutS, and hMLH1 (9, 10) and hPMS2 (11), which are homologues of MutL (Table 1). A sixth human gene, hPMS1, has also been suggested to be important for MMR, although biochemical studies supporting its involvement are not yet available (12). Both the bacterial and the eukaryotic systems direct repair to the newly replicated DNA strand, require multiple components, and can cut the strand to be repaired either upstream or downstream of the mismatch. hMSH2, either by itself (13) or when dimerized with either hMSH6 (7) or hMSH3 (14), binds small DNA mismatches (Fig. 1). The MutL homologues hMLH1 and hPMS2 form a heterodimer (15) and join the complex after the initial binding by either hMSH2-hMSH6 or hMSH2-hMSH3. Analysis of the mismatched nucleotide-binding specificity of the hMSH2-hMSH3 and hMSH2-hMSH6 protein complexes showed that they have overlapping but not identical binding specificity (14).

Loss of MMR causes destabilization of the genome and results in high mutation rates, particularly in microsatellite sequences in both noncoding (16, 17) and coding portions of the genome. Such sequences are found in the coding regions of the HPRT (18), APRT (19), APC (20), type II TGF-β (21), and BAX (22) genes, and mutation rates are increased at these loci in deficient cells. The majority of hereditary nonpolyposis colon cancer cases are due to underlying defects in either MLH1 or MSH2 (9, 10, 23, 24). Defects in PMS1 or PMS2 are less frequent (11, 24). Although the MMR system seems to be normal in the heterozygote cells containing a single functional gene copy, during carcinogenesis, the remaining wild-type allele is somatically mutated, resulting in the complete loss of MMR function in the tumor (4). In addition to hereditary nonpolyposis colon cancer, loss of MMR occurs frequently in many types of sporadic cancers as well, including endometrial, small and nonsmall cell lung, pancreatic, gastric, ovarian, cervix, and breast cancer (25–29). Mice that are deficient in either MLH1, MSH2, or PMS2 have microsatellite instability in many tissues and a predisposition to form tumors, especially lymphomas (30–33).

Recent studies have documented that loss of MMR is an important mechanism of resistance to a variety of clinically important drugs (Table 2), due in part to the fact that the MMR system can recognize and bind to various types of adducts in DNA as well as to mismatches. Rather than being a major effector of the removal of such adducts, the main role of the MMR seems to be as a detector of specific types of DNA damage. A schematic diagram of the proposed mechanism is presented in Fig. 1. This review will focus on how loss of MMR results in drug resistance.

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3 The abbreviations used are: MMR, DNA mismatch repair; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; MNU, N-methyl-N-nitrosourea; AGT, O'-alkylguanine-DNA alkyltransferase.
Alkylation Agents

There exist a number of cell lines with known inactivating mutations in the MMR genes. The human colorectal adenocarcinoma cell line HCT116, which is hMLH1 deficient due to a hemizygous mutation in hMLH1 resulting in a truncated non-functional protein, exhibits microsatellite instability and does not correct mismatches in cell-free extracts (34, 35). Transfer of chromosome 3, on which the hMLH1 gene is located, into HCT116 cells corrects the MMR defect, reverses the mutator phenotype, and sensitizes the cells to the methylating agent MNNG, indicating that restoration of functional MMR abolishes methylation tolerance (36). Similarly, the human endometrial adenocarcinoma cell line HEC59, which is hMSH2 deficient due to different mutations in each of the two hMSH2 alleles (35), is resistant to MNNG when compared to a subline into which chromosome 2 containing a wild-type copy of hMSH2 has been transferred (37). Furthermore, the human endometrial adenocarcinoma cell line HEC-1-A, which carries mutations in hPMS2, exhibits microsatellite instability and resistance to MNNG when compared to the MMR-proficient KLE cells (38).

Methylating agents such as MNU, MNNG, procarbazine, and temozolomide (an activated form of procarbazine) form a variety of adducts in DNA, among which O6-methylguanine is the most cytotoxic. Although the MMR system does not seem to be able to recognize the alkylated guanine directly (39), it does recognize the O6-methylguanine-thymine mispair that occurs after erroneous incorporation of a thymine rather than a cytosine opposite the O6-methylguanine during the next cycle of DNA replication (40). One hypothesis is that having recognized the mismatch, the MMR system incises the thymine-containing strand; excises the thymine and surrounding bases, creating a gap; and then fills in the gap via repair synthesis. However, because a thymine is again incorporated opposite the persisting O6-methylguanine, the site is once again recognized by the MMR system, and a new round of attempted repair is triggered. This futile cycling is envisioned as increasing the risk of a double-strand break at the time of the next S phase that could then trigger apoptosis (41). This model predicts that loss of MMR confers tolerance to methylating agents such as MNU and MNNG by virtue of the fact that the cell does not attempt repair. In addition to the experiments in which MMR was restored by chromosome transfer, this hypothesis is supported by the fact that selection of cells for resistance to MNU or MNNG frequently results in loss of MMR (42) and loss of guanine-thymine mismatch binding activity (40, 43).

Loss of MMR seems to be important as a mechanism of resistance to two drugs currently used in the clinic, both of which produce large numbers of O6-methylguanine adducts, namely temozolomide, a monofunctional methylating imidazotetrazinone, and procarbazine, a methylhydrazine derivative (44–46). There is an interesting interplay between AGT and the MMR system that controls sensitivity to these agents. AGT is capable of transferring the alkyl group from the O6 position of guanine in DNA to its active site cysteine residue, and in cells that express this enzyme, this is the major route by which these types of adducts are removed from the DNA. However, these alkylated guanines are only toxic to the cell if they are detected by the MMR system. For cells with normal MMR, high levels of AGT prevent the cytotoxic effect by removing the O6-alkylguanine DNA adducts, and inactivation of AGT by a potent inhibitor, O6-benzylguanine (47), sensitizes cells to killing by temozolomide. In MMR-deficient cell lines, inactivation of AGT fails to sensitize cells to temozolomide, suggesting that even large numbers of DNA adducts are not cytotoxic in the absence of the MMR detector, although they may be very mutagenic. Thus, MMR mutations seem to override the AGT mechanism of resistance (44); if the cell cannot detect the presence of the adducts in DNA, then it matters less how many such adducts are present. Human glioblastoma multiforme xenografts treated repeatedly with procarbazine during growth in nude mice developed drug resistance concurrently with a deficiency in MMR associated with loss of detectable hMSH2 expression (46). Although MMR-deficient cells are resistant to MNU, MNNG, temozolomide, and procarbazine, it is not yet known whether they are resistant to other chemotherapeutic methylating agents.

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Table 1

<table>
<thead>
<tr>
<th>Bacterial MMR</th>
<th>Human homologue</th>
<th>Chromosomal localization</th>
<th>Size (kDa)</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MutS</td>
<td>hMSH2</td>
<td>2p22-21</td>
<td>105</td>
<td>3, 4</td>
</tr>
<tr>
<td></td>
<td>hMSH3</td>
<td>5q11-13</td>
<td>127</td>
<td>5, 6</td>
</tr>
<tr>
<td></td>
<td>hMSH6</td>
<td>2p16-15</td>
<td>153</td>
<td>7, 8</td>
</tr>
<tr>
<td>MutL</td>
<td>hMLH1</td>
<td>3p23-21</td>
<td>85</td>
<td>9, 10</td>
</tr>
<tr>
<td></td>
<td>hPMS1</td>
<td>2q31-33</td>
<td>106</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>hPMS2</td>
<td>7p22</td>
<td>96</td>
<td>11</td>
</tr>
</tbody>
</table>

Fig. 1 Basic sequence of events that seem to be the basis for cytotoxicity of carboplatin and cisplatin: A, formation of the adduct; B, misincorporation of a base opposite the adduct at the next round of replication; C, recognition of the adduct/mispair by the MMR system; D, attempted futile repair; E, generation of a signal that triggers apoptosis. Pt, an intrastrand 1,2 d(GpG) adduct.
Table 2  The effect of loss of MMR on sensitivity to cytotoxic agents

<table>
<thead>
<tr>
<th>Resistance (Ref. no.)</th>
<th>No effect (Ref. no.)</th>
<th>Hypersensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Busulfan (46)</td>
<td>BCNU* (44, 48)</td>
<td>Unknown</td>
</tr>
<tr>
<td>Carboplatin (62)</td>
<td>Melphalan (48, 49)</td>
<td></td>
</tr>
<tr>
<td>Cisplatin (59–62)</td>
<td>Oxaliplatin (62)</td>
<td></td>
</tr>
<tr>
<td>Doxorubicin (61)</td>
<td>Paclitaxel (49)</td>
<td></td>
</tr>
<tr>
<td>Etoposide (49)</td>
<td>Perfosfamide (49)</td>
<td></td>
</tr>
<tr>
<td>Mercaptopurine (54)</td>
<td>Transplatin (62, 65)</td>
<td></td>
</tr>
<tr>
<td>MNNG (36, 42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNU (46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procabazine (46)</td>
<td>Temozolomide (44–46)</td>
<td></td>
</tr>
<tr>
<td>6-Thioguanine (53, 54)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.

Dacarbazine, for example, produces N\(^7\)-methylguanine and N\(^\alpha\)-methyladenine adducts that are repaired by the base excision repair system as well as O\(^\alpha\)-methylguanine adducts.

Very recently a MMR-proficient human glioblastoma multiforme xenograft was reported to be responsive to busulfan when treated with an LD\(_{10}\) dose, whereas a procarbazine-selected MMR-deficient subline was not (46). This suggests that the adducts formed by busulfan might also be recognized as mismatches by the MMR proteins, either directly or after a round of replication and misincorporation of a base opposite the adduct. The adducts produced by busulfan are well characterized than those for the other alkylating agents because of its lower reactivity and the relatively rapid depurination of the adducted bases. Additional studies are needed to document that the components of the MMR system responsible for the difference in sensitivity are quite specific in their ability to recognize the adducts produced by these agents that are not recognized by the MMR protein complex.

6-Thioguanine and Mercaptopurine

The cytotoxicity of 6-thioguanine and mercaptopurine results primarily from their conversion to 2′-deoxy-6-thioguanosine triphosphate and subsequent incorporation into DNA (50). They characteristically produce delayed cytotoxicity (51) and a variety of chromosomal abnormalities including an increased rate of sister chromatid exchange (52). MMR-deficient cells are 5- to 10-fold more resistant to 6-thioguanine (49, 53). After incorporation into DNA, 6-thioguanine can be chemically methylated by S-adenosylmethionine to form S\(^\alpha\)-methylthioguanine. This adduct is not a good substrate for AGT and therefore would be expected to persist in the DNA (54). Particularly when preceded by a 5′ cytosine, S\(^\alpha\)-methylthioguanine paired with cytosine is recognized by hMUTS (40, 55). During DNA replication, S\(^\alpha\)-methylthioguanine can pair with thymine as well as its normal partner, cytosine, and the resultant S\(^\alpha\)-methylthioguanine-thymine pairs are also identified by the MMR system as replication errors (54). Studies of the replication of DNA containing 6-thioguanine suggest that it does not miscode with sufficient frequency for the toxicity to be ascribed to thioguanine-thymine bp alone (56), consistent with the hypothesis that attempted repair of either S\(^\alpha\)-methylthioguanine-cytosine or S\(^\alpha\)-methylthioguanine-thymine can result in cytotoxicity. The currently available data support the argument that it is the ability of the MMR system to recognize the abnormalities in the DNA produced by the incorporation of 6-thioguanine and attempt repair that triggers cytotoxicity. If the detector function of the MMR system is disabled, despite the fact that the adducts may persist in the DNA, cytotoxicity is markedly reduced.

Platinum Compounds

DNA is the primary intracellular target of cisplatin, and eukaryotic cells respond to the presence of cisplatin adducts in DNA by activating signal transduction pathways that result in cell cycle arrest and apoptosis (57). The first indication that MMR might be a determinant of sensitivity to cisplatin was the observation that introduction of mutS or mutL mutations into Escherichia coli already hypersensitive to cisplatin due to the presence of a dam mutation caused them to become resistant (58). Subsequently, it was demonstrated that in two unrelated cell systems, loss of either hMLH1 (HCT116 cells) or hMSH2 (HEC59 cells) function resulted in low-level cisplatin (59–61) and carboplatin resistance (62), and that some human tumor cell lines selected for resistance to cisplatin [e.g., 2008/A (63)] exhibit microsatellite instability (59, 60) and are defective in strand-specific MMR (61). The fact that loss of MMR results in resistance to both cisplatin and carboplatin was not unexpected, because although carboplatin contains a 1,1-cyclobutane-carboxylato-leaving group and undergoes aquation more slowly, the structures of the aquired forms of cisplatin and carboplatin are the same, as are the types of adducts.

Pure hMSH2 has been reported to bind to platinated DNA in mobility shift assays (64). Human MutS\(a\), a heterodimer of hMSH2 and hMSH6, has been shown to bind to DNA containing adducts produced by cisplatin (65) and has greatest affinity for lesions in which a thymine has been misincorporated opposite the 3′ guanine (66). Adducts formed by oxaliplatin or transplatin are not recognized by the MMR system (62, 65). Oxaliplatin and transplatin do not differ in their cytotoxicity to MMR-proficient and -deficient tumor cell lines (62), suggesting that the components of the MMR system responsible for the difference in sensitivity are quite specific in their ability to discriminate between different types of closely related DNA adducts.

It is not yet clear how the platinum adducts are recognized by the MMR proteins. It has been demonstrated biochemically that hMSH2 can bind to a 1,2 d(GpG) adduct, but it is also possible that the MMR system recognizes monoadducts, monoadducts modified by reaction with glutathione, or interstrand adducts as well. It is possible that cisplatin adducts distort the DNA in a manner that mimics the presence of either a single-base mismatch or an insertion/deletion mispair. The current paradigm is that, as for the methylating agents, the MMR system serves as a detector for cisplatin-damaged DNA; resistance is thought to result from failure of the cell to recognize the adducts and to activate signaling pathways that trigger apoptosis (Fig.

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1). If this paradigm is correct, then the detector must be able to initiate activation of signaling pathways. Indeed, it has recently been reported that cisplatin activates c-jun NH2-terminal kinase 1 by a p21-activated kinase protein 65 and mitogen-activated protein kinase kinase 4-independent mechanism more efficiently in MMR-proficient cells than in MMR-deficient cells, and that cisplatin activates c-Abl kinase in the MMR-proficient cells, whereas this response is completely absent in MMR-deficient cells (67). This reveals that activation of c-jun NH2-terminal kinase 1 and c-Abl by cisplatin is in part dependent on the integrity of the MMR function, suggesting that these kinases are part of the signal transduction pathway activated when MMR proteins recognize cisplatin adducts in DNA.

Other Chemotherapeutic Agents

Loss of MMR has been shown to be associated with low-level resistance to etoposide (49) and doxorubicin (61). How loss of MMR produces low-level resistance to these topoisomerase II inhibitors is less clear than for those agents that react directly with DNA to produce adducts that distort its structure in a manner similar to that of true DNA mismatches. It may be that the MMR proteins serve as a detector of the cleavable complex (68) produced by the binding of etoposide or doxorubicin to topoisomerase II or that the MMR proteins normally act to stabilize the drug-induced cleavable complex on the DNA and thus serve to augment the DNA damage. Additional studies are required to document the interactions between the MMR proteins and topoisomerase II. The observation that there is no difference in the cytotoxicity of paclitaxel between MMR-proficient and -deficient cells (49) is consistent with the fact that this agent is not known to interact with DNA at all.

MMR and the G2-M Phase Cell Cycle Checkpoint

It has been suggested that the MMR system is involved in promoting G2 cell cycle arrest and cell death after treatment with MNNG in cells that are MMR proficient (69). Similarly, a G2 arrest has been reported in MMR-proficient cells after treatment with 6-thioguanine (53). In contrast, the same exposure to 6-thioguanine did not induce a G2 arrest but rather just a G1 delay in MMR-deficient cells. The arrest at the G2 cell cycle checkpoint may permit the cell to attempt repair of DNA mismatches and prevent the replication of mutated DNA, similar to the arrest at the G1-S phase checkpoint mediated by p53 and p21 in response to DNA damage. The signal generated by the detection of MNNG or 6-thioguanine damage by the MMR system is unknown, although there is some evidence that it works via regulation of the p34cdc2-cyclin B complex (70). Very recently, it has been reported that MLH1-deficient human tumor cell lines also fail to engage G2 cell cycle arrest after cisplatin damage (71). The link between the MMR system and G2 arrest suggests that the MMR system is involved not only in the repair of true mismatches but also in processes that limit the replication of cells when DNA damage is detected.

Clinical Significance

Loss of MMR can result in drug resistance directly by impairing the ability of the cells to detect damage and indirectly by increasing the mutation rate at loci that mediate resistance to other classes of drugs. Although, by the direct route, loss of MMR results only in relatively small degrees of resistance to the platinum-containing drugs, several lines of evidence suggest that this resistance is nevertheless of substantial biological and clinical significance: (a) this low-level resistance to cisplatin has been reported to be sufficient to produce progressive enrichment for MMR-deficient tumor cells during treatment in vitro (72); (b) MSH2+/− embryonic stem cells (31) grown as xenografts have been shown to be responsive to treatment with a single LD10 dose of cisplatin, whereas isogenic MSH2−/− tumors were not, suggesting that the degree of cisplatin resistance conferred by loss of MMR is sufficient to produce a large difference in clinical responsiveness in vivo (72). Similarly, a MMR-proficient glioblastoma multiforme xenograft has been shown to be much more responsive to treatment with procarbazine, temozolomide, and busulfan than a procarbazine-selected MMR-deficient subtype (46); (c) loss of MMR has been reported in tumor cell lines selected for resistance to cisplatin (60) or doxorubicin (61); and (d) the frequency of positive immunoblot analysis for hMLH1 protein in ovarian carcinomas obtained after chemotherapy with a cisplatin or carboplatin-containing regimen was shown to be substantially lower than the frequency among tumors sampled before treatment (71). Although the samples were not paired, and therefore two different groups of patients were compared, this loss of hMLH1 expression is consistent with the concept that treatment with platinum drugs can enrich for MMR-deficient cells.

The issue of when loss of MMR occurs during oncogenesis remains controversial even for hereditary nonpolyposis colon cancer, which represents the best-defined clinical situation (73). However, once such cells are present in the tumor, their genomic instability may result in the accumulation of additional mutations that contribute to the phenomenon of tumor progression. Enrichment of these cells as a result of chemotherapy would be expected to accelerate this process. Indeed, microsatellite instability, a hallmark of the genomic instability due to loss of MMR (74), has been reported to be present in up to 94% of the patients with therapy-related leukemia or myelodysplastic syndromes, consistent with drug-induced enrichment for genetically unstable cells.

Overview

A coherent picture of how MMR mediates cytotoxicity has emerged from studies of the mechanism of action of the methylating agents, 6-thioguanine, and cisplatin in particular. In each case, the MMR system recognizes the damaged bases or the mismatch that results from attempted replication across the damaged base. Recognition is followed by events, not yet well defined, that generate a signal capable of activating apoptosis. Operationally, the MMR system is thus functioning as a detector; when the detector is disabled, the cell cannot sense the damage present in its DNA, the apoptotic cascade is not activated, and the cell is phenotypically drug-resistant. Exactly how the MMR system generates a proapoptotic signal is not yet clear. The DNA lesions produced by methylating agents, 6-thioguanine, and cisplatin are all quite mutagenic, so that it is reasonable to expect that cells that fail to die because the MMR detector is disabled will have very high mutation rates not only...
because of the failure to repair DNA polymerase errors, but also because of the persistence of the adducts in the DNA. Clinical studies of the significance of MMR-deficient cells in tumors with respect to the rate of development of drug resistance are now urgently needed.

References


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