**MSH2** missense mutations alter cisplatin cytotoxicity and promote cisplatin-induced genome instability

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**ABSTRACT**

Defects in the mismatch repair protein MSH2 cause tolerance to DNA damage. We report how cancer-derived and polymorphic **MSH2** missense mutations affect cisplatin cytotoxicity. The chemotolerance phenotype was compared with the mutator phenotype in a yeast model system. **MSH2** missense mutations display a strikingly different effect on cell death and genome instability. A mutator phenotype does not predict chemotolerance or vice versa. **MSH2** mutations that were identified in tumors (Y109C) or as genetic variations (L402F) promote tolerance to cisplatin, but leave the initial mutation rate of cells unaltered. A secondary increase in the mutation rate is observed after cisplatin exposure in these strains. The mutation spectrum of cisplatin-resistant mutants identifies persistent cisplatin adduction as the cause for this acquired genome instability. Our results demonstrate that **MSH2** missense mutations that were identified in tumors or as polymorphic variations can cause increased cisplatin tolerance independent of an initial mutator phenotype. Cisplatin exposure promotes drug-induced genome instability. From a mechanistical standpoint, these data demonstrate functional separation between **MSH2**-dependent cisplatin cytotoxicity and repair. From a clinical standpoint, these data provide valuable information on the consequences of point mutations for the success of chemotherapy and the risk for secondary carcinogenesis.

**INTRODUCTION**

Defects in mismatch repair (MMR) proteins promote hereditary [hereditary nonpolyposis colorectal cancer (HNPCC)] and several sporadic forms of cancer (1). The mismatch recognition protein MSH2 and the molecular matchmaker MLH1 are most commonly mutated in MMR-defective tumors. While many of these mutations result in a truncation and loss of the proteins, several single point mutations have been identified that are believed to equally promote carcinogenesis by establishing a mutator phenotype.

In addition to the repair of replication errors, MMR proteins are involved in several other cellular responses. Among these is the response to DNA damage, such as inflicted by most chemotherapeutic agents. A MMR-dependent induction of cell cycle arrest and/or apoptosis is observed. Defects in MMR proteins, therefore, not only result in genome instability, but can also promote tolerance to chemotherapy (2). MMR-deficient cells show increased survival and resistance to treatment with methylating agents and 6-thioguanine (6-TG). In addition, an increased tolerance to the common chemotherapeutic agent cisplatin observed, which manifests itself in a 2- to 3-fold increase in cell survival after cisplatin exposure (2). Though MMR proteins recognize cisplatinated DNA adducts, they are not involved in the repair of this damage (3). We have recently demonstrated that the repair function of MSH2 is not required for the MMR-dependent induction of cell death after cisplatin exposure, suggesting an uncoupling of both events and a direct role for the MMR protein in damage signaling (4) (F. Salsbury, J. Clodfelter, M. Gentry, T. Hollis and K. Drotschmann, manuscript in preparation).

Though a complete knockout in MMR results in increased cisplatin tolerance, it is unknown how single point mutations in MMR genes affect the response to a chemotherapeutic agent. This is of particular significance, if the missense mutation is identified in tumor cells.

A drug tolerance phenotype can be associated with enhanced susceptibility to DNA damage-induced mutations. As a consequence, MMR-defective cells show a high spontaneous mutation rate after drug exposure (5). This acquired genome instability has been attributed to the growth advantage of MMR-deficient cells after chemotherapeutic treatment, which results in the expansion of cultures of repair-defective cells and the accumulation of mutations in downstream genes. This acquired mutator phenotype may contribute to the development of therapy-related, secondary
tumors, as was suggested for the development of acute myeloid leukemia (AML). Fifty percent of therapy-related AML cases show microsatellite instability, a hallmark of MMR defects. Such cases are predominantly observed after chemotherapeutic treatment of primary malignancies with methylating agents, or the treatment of non-malignant cells with immunosuppressiva after organ transplantation (5). Even moderate alkylation tolerance can predispose to AML via MMR defects. It is currently unknown whether exposure to other agents contributes to increased mutagenesis in a similar way. Several single MSH2 mutations were identified in tumor cells. It is unknown how these point mutations affect the development of a supermutator phenotype.

Here, we analyzed single point mutations in MSH2 that are homologous to those identified in tumors, or described as polymorphisms, for their effect on cytotoxicity in response to cisplatin and the development of acquired genome instability. We demonstrate that MSH2 missense mutations decrease cytotoxicity and confer increased tolerance. This tolerance phenotype is independent of a mutator phenotype. Cisplatin exposure of cells harboring MSH2 mutations promotes the development of acquired genome instability, independent of an initial cisplatin-independent mutator phenotype. This drug-induced mutator phenotype may be caused by unprocessed cisplatin adducts in DNA. Given the fact that the mutations analyzed here were identified in tumor cells or described as polymorphisms, these data have important implications for the choice and efficacy of cancer treatment.

MATERIALS AND METHODS

Strains

All yeast strains used in this study are isogenic and have been described previously (4,6). Expression plasmids have been described previously (4,6). Mutations were introduced by site-directed mutagenesis (Stratagene).

Immunoblot analysis

Cells were grown in synthetic media lacking uracil overnight at 30°C and harvested by centrifugation. Cell lysis was achieved by the addition of glass beads, and debris was pelleted by centrifugation. The protein concentration of the supernatant was determined and equal amounts separated by gel electrophoresis. Blot analysis was performed using a polyclonal anti-Msh2 antibody (6).

Genetic assays

Treatment with cisplatin and cell survival assays were performed as described previously (4). The IC$_{50}$ (concentration resulting in 50% cell death) values and confidence limits were obtained as described previously (4). Briefly, overnight cultures of *Saccharomyces cerevisiae* strains grown in selective media (lacking the respective amino acid for the selection of the ARS-CEN plasmid) were split in half and diluted. At early log phase, one half of the cultures obtained indicated concentrations of cisplatin in selective media. After incubation with (and without) cisplatin for 16–24 h, appropriate dilutions were plated onto selective media without drug and cells allowed to grow at 30°C for 4 days. Wild-type strains were grown in complete media containing all essential amino acids. Colonies were counted, and the number of colonies after treatment was compared with the total number of colonies without drug and expressed as percent survival. The IC$_{50}$ was determined by fitting parametric response curves using standard least-squares linear models with both linear and quadratic terms for cisplatin dose. For each clone, the following model was fitted:

$$Y_i = \beta_0 + \beta_1 x + \beta_2 x^2 + \varepsilon_i,$$

where $Y_i$ is the % cell death; $\beta_0$, $\beta_1$ and $\beta_2$ are standard least-squares regression coefficients; $x$ is cisplatin dose in $\mu$M; and $\varepsilon_i$ is the usual normal error term for the linear regression model. After fitting a model for each cell type, the quadratic formula and estimated regression coefficients were used to solve for $x$ such that $Y = 50\%$. Mutation rate determinations based on fluctuation tests have been described previously (4,6). For the determination of the *CAN1* gene mutations after cisplatin exposure, cells were exposed to the indicated cisplatin concentration overnight and plated onto canavanine containing media. Individual colonies were isolated and the *CAN1* gene PCR-amplified using primers 5$'$-CAG ACT TCT TAA CTC CTG-3$'$ and 5$'$-GGA ATG TGA TTA AAG GTA ATA AAA CG-3$'$. The PCR product was sequenced using primers Can-SF-1: 5$'$-ATT CTG TCA CGC AGT CCT; Can-SF-2: GAA CTA GTT GGT ATC ACT; Can-SR-1: TGT CTC CAT GTA AGC CAA; Can-SR-2: ATA TTA TAC CTG GAC CCC; Can-SR-3: ATG AAA AGA CCT GTA CCA. Sequences were compared with the wild-type *CAN1* sequence.

RESULTS

Cisplatin cytotoxicity in strains harboring *MSH2* missense mutations

We determined the effect of single *MSH2* point mutations on cisplatin cytotoxicity and addressed its correlation with a mutator phenotype in a yeast model system (6). Numerous *MSH2* missense mutations have been identified in sporadic and hereditary tumors of different origin. In addition, an increasing number of amino acid alterations in *MSH2* have been described as genetic variants (6–8) (http://www.insight-group.org; http://www.nfdht.nl). Based on a sequence alignment of MutS proteins (Figure 1A) (9), homologous mutations were introduced into the yeast *MSH2* gene (6). All mutants produce protein levels indistinguishable from wild-type MSH2 (Figure 1B). Figure 1C shows the location of mutations on the MutS crystal structure. *MSH2* missense mutations are randomly distributed over the entire protein. Mutations Y109C (Figure 1D, blue) and R542P are located in the DNA-binding domains, while G770R affects the ATPase domain (red). Other mutations affect regions that are involved in intra- and interdomain contacts (e.g. L402F, Figure 1D).

The effects of *MSH2* mutations on cisplatin cytotoxicity were analyzed in a yeast model system (4). Dose-dependent cell survival determines the effect of individual mutations (4). The IC$_{50}$ was used as an indicator for the effect of a particular mutation on cisplatin cytotoxicity. A complete knockout in *MSH2* results in the previously described, typically 2- to 3-fold increase in tolerance of cells to cisplatin treatment (Table 1, 2.4-fold). The effect on cytotoxicity was compared with altered repair activity, as determined in the *CAN1* reporter system (4). This reporter monitors any mutations that inactivate...
Table 1. Effect of MSH2 missense mutations on DNA damage response and repair

<table>
<thead>
<tr>
<th>MSH2 allele</th>
<th>IC₅₀ [µM cisplatin]</th>
<th>CL</th>
<th>RR</th>
<th>Mutation rate in CAN1 [10⁻⁶]</th>
<th>MR</th>
<th>CL</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>msh2Δ</td>
<td>360</td>
<td>330–390</td>
<td>2.4</td>
<td>15</td>
<td>11–19</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>MSH2 wt</td>
<td>150</td>
<td>130–170</td>
<td>1</td>
<td>0.47</td>
<td>0.37–0.66</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Separation-of-function: increased cisplatin tolerance without a mutator phenotype Y109C</td>
<td>290</td>
<td>240–340</td>
<td>1.9</td>
<td>1.2</td>
<td>0.25–4.1</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>L402F</td>
<td>250</td>
<td>190–310</td>
<td>1.7</td>
<td>3.6</td>
<td>1.4–5.5</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>Separation-of-function: increased mutator phenotype with unaltered cisplatin cytotoxicity G770R</td>
<td>130</td>
<td>120–140</td>
<td>0.87</td>
<td>19</td>
<td>14–25</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>R371S</td>
<td>180</td>
<td>170–190</td>
<td>1.2</td>
<td>39</td>
<td>20–54</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>P640L</td>
<td>200</td>
<td>130–260</td>
<td>1.3</td>
<td>24</td>
<td>20–34</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Increased cisplatin tolerance and a mutator phenotype R542P</td>
<td>300</td>
<td>250–340</td>
<td>2</td>
<td>19</td>
<td>13–26</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>No/weak effect on cisplatin cytotoxicity and mutation rate E194A</td>
<td>130</td>
<td>101–150</td>
<td>0.87</td>
<td>4.4</td>
<td>2.4–7.2</td>
<td>9.4</td>
<td></td>
</tr>
</tbody>
</table>

CL, 95% confidence limits; RR, relative rate (bold); compared with wild type (underlined); MR, mutation rate.

Figure 1. Missense mutations in MSH2. (A) Alignment of human (hMSH2) and yeast (yMsh2) amino acid sequences indicating the location of point mutations. Stars above the alignment indicate the side of mutation (bold); underneath the diagram single point mutations in the yeast MSH2 protein that were analyzed here are indicated. (B) Western blot analysis demonstrating the expression of mutant MSH2 genes. The corresponding MSH2 mutation is indicated. (C) Structure of the MSH2-homologous subunit B of E.coli MutS in complex with mismatched DNA (36) with the amino acid alterations indicated at homologous sides. Coloring depicts the domain structure of the protein, with blue: mismatch binding (I), green: connector (II), yellow: core (III), orange: clamp (IV) and red: ATPase/dimerization (V) domains. Amino acid designations are yeast numbers. (D) Close-up view of the location of Y109 in the mismatch-binding domain I, and L402 in the core domain III, respectively.
the arginine permease gene; inactive MMR results in a 32-fold increase in the mutation rate (Table 1). Non-overlapping, 95% confidence limits between different strains indicate statistical significance.

Most prominent changes in cisplatin cytotoxicity are observed with mutations Y109C and L402F (Table 1). Mutation Y109C shows a significant increase in the tolerance to cisplatin exposure. The IC₅₀ of 290 μM has overlapping confidence limits with the knockout strain, suggesting that the response in both strains is indistinguishable (Table 1). In contrast, this mutation does not result in a mutator phenotype, and the mutation rate is not significantly different from the wild-type strain (2.6-fold increase, with overlapping confidence limits to wild type). The Y109C mutation is homologous to the Y103C mutation in human MSH2, which was identified in association with HNPCC (http://www.insight-group.org; http://www.nfdht.nl).

The L402F mutation results in an increase in cisplatin tolerance, which is significantly different from the wild-type response (Table 1, IC₅₀ of 250 μM). In contrast, the mutant strain shows a weak mutator phenotype (7.7-fold elevated over wild type; compared with 32-fold for the knockout strain). The corresponding mutation L390F in human MSH2 was described as a genetic variation in the general population with an allele frequency of 0.005 (8).

These effects are in contrast to consequences observed for MSH2-G770R. The cisplatin cytotoxicity in this strain is unaltered when compared with the wild type (130 μM compared with 150 μM for the wild-type strain). The G770R mutation confers a strong mutator phenotype (40-fold) that is indistinguishable from a complete knockout strain (Table 1, 32-fold). G770R affects a residue in the ATPase domain of MSH2 (Figure 1C). The corresponding mutation R371S and P640L do not significantly alter cisplatin cytotoxicity (1.2- and 1.3-fold, respectively, with overlapping confidence limits with wild type). The mutation rate is considerably elevated in both strains (83- and 51-fold, respectively, Table 1). The homologous human mutations are found in association with HNPCC [(10,11), http://www.insight-group.org; http://www.nfdht.nl].

Mutation R542P significantly increases both cisplatin resistance (IC₅₀ of 300 μM with confidence limits overlapping with the msh2Δ strain) and the mutation rate of the cell (40-fold; Table 1). The homologous mutation was identified in a patient with ovarian cancer suggestive of HNPCC (12). Mutation E194A does not significantly alter cisplatin cytotoxicity and displays a weak mutator phenotype.

These data demonstrate that single point mutations in MSH2 can alter cisplatin cytotoxicity. Individual mutations affect the cytotoxicity differently. No obvious association with the location of the mutation in the protein is observed. The chemotolerant phenotype is not correlated with a mutator phenotype.

### A cisplatin-induced mutator phenotype in strains harboring Y109C and L402F

We next determined whether the presence of a single point mutation in MSH2 promotes the development of acquired genome instability after cisplatin exposure. The mutation rate of cisplatin-tolerant clones was analyzed in the CAN1 reporter system. The mutator phenotype of cisplatin-tolerant clones (Table 2) was compared with the mutation rate without exposure to identify a cisplatin-induced change (Table 1). Exposure of the wild type strain to cisplatin does not alter the mutation rate (mutation rates 0.47 and 0.37 × 10⁻⁶, respectively, Table 2). Mutations MSH2-Y109C and L402F, respectively, which confer cisplatin tolerance and no or a weak mutator phenotype in the absence of cisplatin exposure (Table 1), show a significant cisplatin-induced mutator phenotype. After drug exposure, cisplatin-tolerant clones harboring MSH2-Y109C show a significant mutator phenotype that is 5.2-fold elevated when compared with the initial mutation rate (compare 6.2 with 1.2 × 10⁻⁶; Table 2). This acquired genome instability results in an overall 13-fold elevation in the mutation rate when compared with the wild-type rate (without exposure). MSH2-L402F weakly elevates the mutation rate in the absence of cisplatin (3.6 × 10⁻⁶; 7.7-fold above wild-type levels; Tables 1 and 2). Cisplatin-resistant clones of this strain display a significant 2.5-fold increase in the mutation rate when compared with this initial rate. This causes an overall 19-fold elevation, resulting in a mutation rate that is indistinguishable from a complete knockout strain (compare 9 × 10⁻⁶ for L402F with 15 × 10⁻⁶ for msh2Δ, with overlapping confidence limits, Table 2). Both strains harboring Y109C and L402F, respectively, promote acquired genome instability.

The already strong mutator phenotype of cells harboring G770R (Table 1) is further elevated by exposure to cisplatin and reaches a mutation rate that goes beyond the knockout strain.

<table>
<thead>
<tr>
<th>Table 2. Drug-induced supermutator phenotype</th>
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<tbody>
<tr>
<td><strong>Without exposure</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>msh2Δ</td>
</tr>
<tr>
<td>MSH2 wt</td>
</tr>
<tr>
<td>Y109C</td>
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<tr>
<td>L402F</td>
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<tr>
<td>G770R</td>
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<tr>
<td>R371S</td>
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<tr>
<td>P640L</td>
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<tr>
<td>R542P</td>
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<td>E194A</td>
</tr>
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</table>

MR, mutation rate; CL, 95% confidence limits; RR, relative rate (bold); compared with wild type (underlined).
The mutational spectrum of acquired genome instability

To analyze whether the cisplatin-induced genome instability observed for mutations Y109C and L402F is due to an accumulation of unprocessed, cisplatin-induced mutations, the mutation spectrum was determined. Individual, cisplatin-tolerant mutator clones from strains harboring either mutation (Table 2) were isolated. The reporter gene CAN1 was sequenced to determine the nature of inactivating mutations. The sequence was compared with the wild-type sequence for the CAN1 gene. In addition, the mutation spectrum was compared with the published mutation spectrum of an msh2Δ strain without drug exposure (13). The overall distribution of insertion/deletion and base substitution mutations is unaltered when compared with the described spectrum for the msh2Δ strain (13). A total of 83 and 75% of cisplatin-resistant clones of cells harboring MSH2-Y109C and L402F, respectively, are insertion/deletions [as compared with 85% for the msh2Δ strain (13)]. The spectrum of mutations prevalent in Y109C or L402F expressing clones without cisplatin exposure is indistinguishable from the MSH2-defective strain. One nucleotide deletions in a stretch of six adenines, and a 1 nt addition in a run of six thymines are observed (data not shown). These mutations are commonly found in MSH2-deficient strains (13). The mutation spectrum of cisplatin-tolerant cells harboring either one of the point mutations shows considerable differences (Table 3). Mutations in previously undescribed sequence contexts are observed. The spectrum of cisplatin-resistant clones of Y109C primarily shows a 1 nt deletion in two adjacent guanines. In addition, the deletion of 4 nt (AAAGT) is observed. Both sequence contexts are targets for the addition of cisplatin, which crosslinks two adjacent purines, primarily GpG, but also ApG. Similarly, mutation L402F generates a 1 nt deletion within two adjacent guanines, though at a different site. An additional thymine deletion is observed directly downstream of a guanine dinucleotide repeat (Table 3). The only base substitution observed in the L402F mutant strain is a T to T mutation that alters the cisplatin target GA to a TA, consistent with errors occurring at the site of the cisplatin-intrastrand crosslink. These data demonstrate that cisplatin-resistant clones of MSH2-Y109C and L402F accumulate mutations at sites of potential cisplatin adduction.

Table 3. Mutation spectrum in CAN1 of cisplatin-resistant clones

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Insertion/deletion</th>
<th>Base substitution</th>
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<tbody>
<tr>
<td>Y109C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔG</td>
<td>GG → G</td>
<td>C → T</td>
</tr>
<tr>
<td>ΔA</td>
<td>A6 → A5</td>
<td></td>
</tr>
<tr>
<td>+TT</td>
<td>T3 → T5</td>
<td></td>
</tr>
<tr>
<td>AAAGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L402F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔG</td>
<td>GG → G</td>
<td>TGA → TTA</td>
</tr>
<tr>
<td>ΔT</td>
<td>GGTTA → GGTA</td>
<td></td>
</tr>
<tr>
<td>+T</td>
<td>T6 → T7</td>
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mutator phenotype is comparable with the one we describe here (22). The mutation spectrum for MMS-induced genome instability identified primarily transversions. In contrast to MMS, cisplatin adducts are not subject to BER and are not expected to generate abasic sites. In addition, our data here identify primarily frameshift mutations at target sequences for cisplatin adduction (Table 3). Both, our study and the one by Glaab et al. (20) demonstrate that MMR-dependent cytoxicity and mutagenicity can function independent from each other.

The MSH2 mutations that promote induced genome instability are randomly distributed over the protein with no obvious ‘clustering’. Hence, no predictions of their phenotypes can be made based on the nature of the mutation. We cannot exclude that the point mutations confer a weak or ‘silent’ mutator phenotype that will become more important under conditions when the mutational burden of the cell is increased. Recent studies suggested that the amount of DNA damage may be crucial in the response of cells harboring mutated MMR proteins (23). Mutant proteins may retain different capacities for the processing of different substrates and a functional ‘dose response’ may be observed. However, MMR proteins are not suggested to be involved in the direct repair of cisplatin adducts (3). If the induced mutator phenotype of MSH2-Y109C and MSH2-L402F, respectively, was due to a saturation of the (reduced) repair capacity of these mutants after cisplatin exposure, the mutation spectrum should represent more random changes in the DNA. Though we observe some mutations in sequences outside of cisplatin targets, the overall mutation spectrum is shifted toward cisplatin target sequences (Table 3). This would suggest that the induced mutation rate is due to alterations specific for these sequences. Together with the cell survival studies (Table 1), our data suggest that the mutant proteins are unable to eliminate damaged cells via a cell death pathway, cisplatin adducts are retained in the DNA and can be subject for aberrant or translesion bypass. In addition, mutant proteins may have a ‘hidden’ defect in the antirecombination function, which under massive DNA damage would result in uncontrolled recombinational events (see below).

Owing to their ability to recognize DNA damage, MMR proteins may contribute to replication blockage and thereby initiate a cell death pathway (2). If the damage-sensing and signaling function is disrupted, increased translesion bypass by error-prone polymerases might occur. It was previously shown that the net bypass of cisplatin adducts is increased in MMR-deficient cell lines, and that this increased bypass is correlated with increased drug resistance in these cultures (24). This observation supports the hypothesis that mutations that alter the DNA damage-sensing ability of the protein will prevent blockage and promote increased lesion bypass. This switch would then result in an increase in the mutational burden in the cell rather than cell death. Importantly, we demonstrate here that single point mutations in MSH2, which are either derived from tumor tissue or were identified as polymorphisms, can influence and promote this effect. The functional implications of the missense mutations for which a cisplatin-induced mutator phenotype is observed support this hypothesis. These mutations affect regions involved in DNA binding or ATPase, or affect structurally important areas which may have consequences for DNA–ATP interactions.

In addition to translesion synthesis, recombinational bypass of DNA damage can be initiated. It was previously shown that low doses of MNNG can induce intrachromosomal recombination. Zhang et al. (28) demonstrated that MMR proteins are required for the recombinational event. The recombinational event was found to be stimulated by futile repair cycles and hence requires functional repair activity (28). Our data demonstrate that the repair function of the proteins is not required for MMR-mediated cisplatin cytotoxicity. The induction of recombinational bypass by futile repair cycles of cisplatin-containing DNA is hence unlikely.

In response to cisplatin, MMR proteins exhibit an antirecombination effect; a defect in this mechanism contributes to increased damage tolerance in MMR-defective tumors (29). Increased recombinational bypass of cisplatin lesions results in elevated sister chromatid exchange. This was observed in MMR-deficient ovarian cancer cell lines (29). However, another study demonstrated that MMR proteins are not involved in the generation of chromosomal aberrations induced by cisplatin (30). No gross rearrangements are observed after the treatment of cells with cisplatin in our study (Table 3). In addition, cisplatin-induced recombination in a mutS strain is indistinguishable from the wild-type response (31). Until recently, no mutants had been identified that would separate the functional requirements for the MMR-dependent antirecombination effect from those in repair (32). However, recent studies investigating the MutS antirecombination effect on cisplatinated DNA in Escherichia coli (23) identified a MutS mutant that lost this antirecombination effect. The loss of this effect was associated with increased cisplatin resistance, but did not affect MNNG sensitivity or the repair function. Similarly, Durant et al. (29) demonstrated that MMR proteins in yeast inhibit the recombinational bypass of cisplatin adducts. Mutants in either rad52 or rad1 reverse the increased resistance found in the MMR-deficient strains. Loss of MMR proteins hence abolishes the antirecombination effect in yeast and results in increased RAD52/RAD1-dependent recombinational bypass of damage.

In the light of our results, mutations L402F and Y109C may exhibit a (partial) defect in the antirecombination function of the protein. With increasing concentrations of cisplatin damage, increased, aberrant recombinational bypass may be observed. If this was the case, an additional mutation in recombinational would be predicted to reverse the effects observed for these point mutations. To address this question and elucidate the precise mechanism(s) behind the results described here, further biochemical and genetic analyses of the mutants have been initiated in the laboratory.
Different hypotheses have been put forward to address the mechanism of MMR-dependent damage response. Futil cycles of repair have primarily been suggested for the response to alkylation damage. This hypothesis is based on the observation that MMR-dependent cell cycle arrest to alkylation damage occurs after the second S phase. However, this observation is largely dependent on the dose of the chemotherapeutic agent (2,5). This mechanism requires the repair function of these proteins.

The concentration-dependent response to MNNG suggested the different participation of MMR proteins in response pathways to different levels of damage. At low dosage, O6meG/T mismatches are generated, resulting in delayed response. Processing of these mismatches is absolutely dependent on MMR, which presumably initiates futile cycles of repair. At high doses of MNNG, DNA damage signaling becomes too rapid for replication-association, and cell cycle arrest is largely independent of MMR; however, cell killing remains to be dependent on MMR (33). This observation can be explained by either (i) MMR function as DNA damage sensors of high density O6meG/C in DNA and direct signaling or (ii) processing of O6meG/C initiated, unprocessed AP sites.

The hypothesis of direct signaling suggests a more direct involvement of MMR proteins in the induction and recruitment of proapoptotic proteins in response to DNA damage and the initiation of apoptosis. MMR proteins can contribute to replication fork blockage, or the block of transcription or repair. A prediction of this model is that the repair function of the proteins would not be required for the damage-induced cell death. Several pieces of evidence support this hypothesis for the processing of cisplatin. A repair-deficient mutation in MSH6 was shown to retain wild-type response to several chemotherapeutic agents (15,34). R. Fischel suggested that the ‘sliding clamp model’ supports an ATP-dependent, direct signaling mechanism (35).

We [(4), F. Salsbury, J. Clodfelter, M. Gentry, T. Hollis and K. Drotschmann, manuscript in preparation] and others (14) were able to identify separation-of-function mutations in MSH2 that distinguish damage response from repair. The data presented here support this earlier observation. Here, we demonstrate that single point mutations in MSH2 can affect repair or cisplatin sensitivity, but not necessarily both. This separation-of-function suggests a repair-independent mechanism of cell death signaling by MMR proteins. Whether this is true for different types of damage remains to be determined.

The MSH2 missense mutations are homologous to mutations identified in tumors with suggestions for a causative effect on carcinogenesis. Other mutations were described as genetic variations in the general population. Our data demonstrate that such single point mutations can contribute to genome instability, a prerequisite for carcinogenesis, or increased cell survival after cisplatin exposure, a prerequisite for failure of chemotherapy and clonal expansion of mutant cells. The lack of a correlation between both phenotypes requires knowledge of the individual effects for applicability in the clinical setting, with the goal to improve the efficacy of chemotherapy. Clonal selection of clones that are not initial mutators will be observed after cisplatin treatment. If these clones promote a drug-induced mutator phenotype, even primary cancers that are not suggestive of MMR defects will be at risk for secondary carcinogenesis. The association of chemotolerance with cisplatin-induced genome instability that is promoted by these point mutations caution about the treatment of tumors of certain genetic backgrounds with chemotherapeutic agents. Whether the effects are transferable to the treatment with chemotherapeutics other than cisplatin remains to be determined. Current literature suggests differential MMR-dependent cell death in dependence of the nature and dose of damage. A comparison to the response to other drugs is underway.

The observation that mutations that were described as genetic variations in the general population can promote increased cell survival after cisplatin exposure, suggests that the presence of such polymorphisms will modulate the individual’s capacity to process exposure to DNA damage. This may be potentially important for the exposure to therapeutic, as well as environmental agents.

Taken together, we demonstrate here that tumor-derived, as well as polymorphic missense mutations in MSH2 can alter cisplatin cytotoxicity and result in a tolerance phenotype that is independent of an effect on genome stability. This chemotolerance can be associated with a drug-induced mutator phenotype that significantly increases the initial mutation rate. Data presented here have important implications for the treatment of cancers with single point mutations in a MMR protein and the prevention of tolerance and secondary carcinogenesis. In addition, our results caution that the presence of polymorphic mutations with unknown functional consequences may significantly alter the response to clinical and environmental mutagens.

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