An Msh2 Point Mutation Uncouples DNA Mismatch Repair and Apoptosis

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ABSTRACT

Mutations in the human DNA mismatch repair gene MSH2 are associated with hereditary nonpolyposis colorectal cancer as well as a significant proportion of sporadic colorectal cancer. The inactivation of MSH2 results in the accumulation of somatic mutations in the genome of tumor cells and resistance to the genotoxic effects of a variety of chemotherapeutic agents. Here we show that the DNA repair and DNA damage-induced apoptosis functions of Msh2 can be uncoupled using mice that carry the G674A missense mutation in the conserved ATPase domain. As a consequence, although Msh2G674A homozygous mutant mice are highly tumor prone, the onset of tumorigenesis is delayed as compared with Msh2-null mice. In addition, tumors that carry the mutant allele remain responsive to treatment with a chemotherapeutic agent. Our results indicate that Msh2-mediated apoptosis is an important component of tumor suppression and that certain MSH2 missense mutations can cause mismatch repair deficiency while retaining the signaling functions that confer sensitivity to chemotherapeutic agents.

INTRODUCTION

The DNA mismatch repair (MMR) system guards against genomic instability, and mutations in the human MMR genes MutS homolog 2 (MSH2) and MutL homolog 1 (MLH1) are the cause of the majority of hereditary nonpolyposis colorectal cancer [HNPCC (1)]. Recent studies indicate that MMR proteins not only protect mammalian genomes by repairing mismatched bases that result from erroneous DNA replication, but also by mediating DNA damage-induced apoptosis as part of the cellular response to endogenous and exogenous agents (2–4). These studies showed that cell lines derived from HNPCC and MMR-defective sporadic tumors or MMR-deficient mice displayed increased mutation rates in their genomes and also had increased resistance to the genotoxic effects of a variety of DNA damage-inducing agents, including cisplatin, temozolomide and N-methyl-N’-nitro-N-nitrosoguanidine [MNNG (5–10)]. In addition, as demonstrated initially in yeast and later in mammalian cells, MMR has been implicated in the removal of endogenous lesions such as mutagenic 8-oxoguanine that is incorporated from the oxidized deoxynucleotide triphosphate pool during DNA replication (11, 12). It has been suggested that the failure to clear DNA damage-bearing cells may be responsible in part for the increased mutation frequency in MMR mutant cells and also may confer a selective advantage in tumor cells (13–16). This hypothesis is consistent with the observation that MMR deficiency in mouse tissues leads to an elevation in mutation frequency after the mice are exposed to DNA-damaging agents (10, 17).

These studies were performed with MMR-deficient cell lines that completely lack particular MMR proteins and therefore lack all of the functions of those proteins. However, a significant proportion of HNPCC patients carry missense mutations in MMR genes (1), and it is unclear how these mutations affect individual MMR protein functions in DNA repair and damage responses.

We therefore decided to generate a mouse line carrying the Msh2G674A missense mutation to assess its impact on MMR and response to DNA damage and examine the consequences with respect to cancer susceptibility. The mutation results in a glycine to alanine change at amino acid residue 674 within the conserved ATPase domain at the COOH-terminal region. This domain is characterized by the Walker “type A” motif GXXXXXGKS/T (G denotes the modified G674 amino acid residue) known to coordinate the phosphate groups of ATP in many proteins that hydrolyze ATP (18–20). Mutations in this MutS domain in bacteria and yeast result in MMR defects, and overexpression of these mutant proteins was shown to cause dominant mutator phenotypes (21–24). The importance of ATP processing for MMR and tumorigenesis is underscored by the significant number of HNPPC missense mutations that are located in the ATP-binding domains of MSH2 (25).

Here we show that the Msh2G674A mutation has differential effects on the DNA repair and DNA damage response functions. Whereas it caused DNA repair deficiency that resulted in a strong cancer predisposition phenotype in the mice, it did not affect the DNA damage response function of Msh2. As a consequence, tumorigenesis in Msh2G674A/G674A mice was delayed as compared with that in Msh2−/− mice. In addition, unlike Msh2−/− cells, Msh2G674A/G674A mouse embryonic fibroblasts (MEFs) and teratocarcinomas remained sensitive to treatment with genotoxic agents.

MATERIALS AND METHODS

Generation of Msh2G674A Mice. A 3.6-kb HincII fragment containing Msh2 exon 13 was isolated from a 129SvEv bacterial artificial chromosome genomic library and subcloned. A mutation was introduced that changed codon 674 from glycine (GTT) to alanine (GCT) by site-directed mutagenesis (Stratagene Quick Change Kit). A 5.0-kb NotI fragment containing two LoxP sites flanking a neomycin-PGKhygromycin resistance cassette was subcloned into the single SpeI site. The modified HincII fragment was subsequently used to modify the Msh2 genomic locus in bacterial artificial chromosome clone mBT83K13 of the RPCT-22 129 mouse genomic library by RecET-mediated recombination (26). A 24-kb KpnI fragment containing the modified locus was excised from the bacterial artificial chromosome clone and used for gene targeting in WW6 embryonic stem (ES) cells (27). Three correctly targeted ES cell lines were injected into C57BL/J6 blastocysts. Male chimeras from all three lines were mated to C57BL/6 females and transmitted the mutant allele through their germ line. Subsequently, F1 males carrying the mutant allele were mated to Zpc3Cre transgenic females (C57BL/6j) to remove the resistance cassette by LoxP-mediated recombination. Male and female mice carrying the modified allele were intercrossed to generate Msh2−/−, Msh2G674A−/−, and Msh2G674A/G674A mutant mice.

Reverse Transcription-PCR Analysis. Total RNA was isolated from Msh2 mutant ES cell lines using Trizol (GibcoBRL). Reverse transcription-PCR was performed with forward primer 5'-CGTAAAGCCTAATCCGACGCCCT-3' and reverse primer 5'-GGATGGAAAGAAGTCCTCCAGC-3' using the one Taq reverse transcription-PCR reaction kit (Roche) according to the manufacturer's
instructions. The following cycling conditions were used: 30 min at 50°C (1 cycle); 2 min at 94°C, 30 s at 60°C, and 45 s at 68°C (35 cycles); and 7 min at 68°C (1 cycle). The resulting 280-bp fragment was digested with either MstII to detect the wild-type RNA transcript or Ahh to detect the mutant RNA transcript.

Western Blot Analysis. MEF cell extracts were prepared according to standard procedures, and 50 μg of protein of each cell lysate were separated on a 10% SDS-PAGE gel. Protein was transferred onto a PROTRAN membrane, and the membranes were subsequently incubated with mouse monoclonal antibodies directed against Msh2 (Ab-2; Oncogene), Msh6 (clone 44; BD Biosciences), and β-actin (C-2; Santa Cruz Biotechnology).

Gel Mobility Shift Assays. The in vitro sense oligonucleotide 5′-GGGAAAGTCGACC-GCCCCACGTGTCAGCCTGCCC-3′ was end-labeled with [γ-32P]ATP and annealed in 1× DNA binding buffer [12% glycerol, 20 mM HEPES (pH 7.9), 100 mM KCl, 1 mM DTT, and 5 mM MgCl2] with 3× molar ratios of antisense oligonucleotide 5′-GAGCATAGGAGGCTGACATTGGGGCCTGGCAGCTT-CCC-3′ to form a GC homoduplex probe or with 3× molar ratios of antisense oligonucleotide 5′-GAGCATAGGAGGCTGACATTGGGGCCTGGCAGCTT-CCC-3′ to form a G/T mismatch-containing homoduplex probe. Twenty μg of nuclear extract were preincubated in 1× DNA binding buffer, 1 μg of poly(dI-dC), and 20 ng of unlabeled homoduplex for 5 min on ice in a total volume of 19 μl. Ten ng of radiolabeled DNA probe was subsequently added, and the binding mixture was incubated on ice for 30 min. For reaction using cold probe competition, cold competitor was included in the preincubation mixture. For adenine nucleotide exchange experiments, ATP or ATP-γ-S was added 15 min after the addition of the DNA probe. The reaction mixture was then subjected to electrophoresis in a 1% polyacrylamide gel in 1× Tris-borate EDTA buffer. The gels were dried, and the percentage of relative binding of Msh2-Msh6 and Msh2G674A-Msh6 complexes to G/T oligonucleotide probe in the presence of increasing amounts of cold competitor or increasing ATP concentration was quantified using a STORM PhosphorImager with ImageQuant software (Molecular Dynamics) and calculated as [(Msh2-Msh6-probe complex/Msh2-Msh6-free probe)]

Cell-Free Extracts and MMR Assay. The MMR proficiency of MES cell line cytosolic extracts was measured using M13mp2 DNA substrates and subsequent transfection of bacterial cells as described previously (29). Repair efficiency is expressed (in percentage) as 100 × (1 − the ratio of the percentages of mixed bursts obtained from extract-treated and untreated samples). The substrates used are described in the Fig. 3 legend.

Microsatellite Instability (MSI) Analysis. Mutations in microsatellite sequences were assayed by PCR of single target molecules. Equal amounts of tail DNA isolated from 10 mice each of Msh2+/−, Msh2−/−, and Msh2G674A+/− mouse strain were pooled and diluted to 0.5–1 genome equivalents. Cycling reactions for the three markers analyzed, U12235, D7Mit91, and D7Mit123, were performed as described previously (30).

MEF Survival Analysis. MEF cells (2 × 105) of each Msh2 genotype were seeded onto a single well of a 24-well plate in 10% FCS/DMEM. On the following day, the cells were exposed to cisplatin, 6-thioguanine, or MNNG at different drug concentrations for 24 h or for different time periods. After drug exposure, the cells were washed once with PBS, washed once with PBS/methanol (1:1), fixed in 0.5 ml of 100% methanol, and air dried. The cells were subsequently stained with 0.1% crystal violet and washed extensively with PBS, and the dye was extracted with 1.5 genome equivalents. Cycling conditions for PCR were 1 cycle; 2 min at 94°C, 30 s at 60°C, and 45 s at 68°C (35 cycles); and 7 min at 68°C (1 cycle). The resulting 280-bp fragment was digested with either KpnI; BamHI; S, SpeI; and H, HpaI hybridization probe for Southern blot analysis. B. Southern blot analysis of representative animals carrying the different Msh2 alleles: wild-type (Msh2+/−), undeleted heterozygous Msh2G674A/G674A (Msh2G674A+/−), deleted heterozygous Msh2G674A/G674A (Msh2G674A+/−), and deleted homozygous Msh2G674A/G674A (Msh2G674A−/−). The sizes of the modified undeleted (15.7 kb) and modified deleted (11.0 kb) alleles are indicated. C, reverse transcription-PCR analysis of total RNA in mouse embryonic fibroblast cells. Top diagram, PCR primers located in exon 12 and exon 13 were used to amplify a 280-bp cDNA fragment. Codon 674 can be digested with MstII in wild-type cDNA and with Ahh in the mutant cDNA. The sizes of the expected restriction fragments are indicated for each restriction enzyme. Bottom panel, restriction digestion of cDNA generated from RNA isolated from wild-type (Msh2+/−) and homozygous mutant Msh2−/− (Msh2G674A−/−) cells verifies expression of the mutant allele. D. Western blot analysis of cell extracts isolated from wild-type (Msh2+/−), heterozygous Msh2G674A+/− (Msh2G674A+/−), and homozygous mutant Msh2G674A−/− (Msh2G674A−/−) mouse embryonic fibroblast cells. The analysis indicates normal expression of the Msh2G674A and Msh6 protein.

RESULTS

Generation of Msh2G674A Mutant Mice. The mutant mouse line was created by a knockin gene targeting strategy (Fig. 1A). Analysis of 22 litters of the F2 offspring showed that the Msh2G674A allele was transmitted in a normal Mendelian ratio with 40 Msh2+/−, 89 Msh2G674A+/−, and 37 Msh2G674A−/− mice. None of the heterozygous or homozygous animals displayed any developmental abnormalities. Molecular analysis showed that the Msh2G674A mutation allowed normal gene expression and did not interfere with the stability of the mutant protein (Fig. 1, C and D). In eukaryotes, Msh2 forms complexes with either Msh6 or Msh3 to initiate the repair of single base mutations (Msh2-Msh6) or larger insertion/deletion mutations (Msh2-Msh3) (31–37). The formation of these complexes is important for the stability of the MutS proteins, and immunohistochemical analysis in tumor cells showed that the loss of MSH2 is frequently associated with the loss of MSH6 (38). Western blot analysis of cell extracts derived from Msh2G674A−/− mice showed that the mutation did not alter the stability of either mutant Msh2G674A or Msh6 protein in the cells (Fig. 1D). In addition, immunohistochemical analysis indicated that the subcellular distribution of the mutant Msh2G674A or the Msh6 protein was not affected (data not shown).
Mismatch Binding Activity in Msh2<sup>G674A/G674A</sup> Cell Extracts.

We next studied the mismatch binding activities of nuclear extracts isolated from Msh2<sup>+/+</sup> Msh2<sup>−/−</sup>, and Msh2<sup>G674A/G674A</sup> ES cells. Using gel mobility shift assays, we did not detect any significant differences between Msh2<sup>+/+</sup> and Msh2<sup>G674A/G674A</sup> extracts in their DNA binding affinity using an oligonucleotide substrate containing a G/T mismatch. In addition, proteins in both extracts bound with similar, albeit lower, affinity to a homoduplex oligonucleotide substrate, whereas Msh2<sup>−/−</sup> extracts did not show any binding activity (Fig. 2A; data not shown). In contrast to the Msh2<sup>+/+</sup> extracts, the mutant Msh2<sup>G674A/G674A</sup> extracts were partially resistant to ATP-dependent mismatch release, even at concentrations that exceed normal physiological conditions (Fig. 2B).

The addition of the poorly hydrolyzable ATP-γ-S analog also resulted in mismatch release in Msh2<sup>+/+</sup> extracts but not in the Msh2<sup>G674A/G674A</sup> extracts (data not shown). These results are consistent with previous studies in yeast and suggest that the resistance of Msh2<sup>G674A/G674A</sup> cell extracts to ATP-dependent release from mismatched DNA is caused by defective or altered ATP binding resulting from the substitution of alanine for glycine in the P-loop (24).

MMR Deficiency in Msh2<sup>G674A/G674A</sup> Cells. To test the impact of the Msh2<sup>G674A</sup> mutation on DNA MMR, we measured the repair activity in ES cell extracts using substrates containing GG mismatches, single-base insertion/deletion mismatches, or 2-base insertion/deletion mismatches with a nick either 3′ or 5′ to the mismatched base (Fig. 3). Whereas both Msh2<sup>−/−</sup> and heterozygous Msh2<sup>G674A/−</sup> extracts repaired all of these substrates, extracts prepared from homozygous Msh2<sup>G674A/G674A</sup> cells did not. The repair defect in the Msh2<sup>G674A/G674A</sup> extracts was comparable with the defect that was observed in Msh2<sup>−/−</sup> extracts.

MSI in Msh2<sup>G674A/G674A</sup> Mice. We assessed the in vivo mutator phenotype in the Msh2<sup>G674A/G674A</sup> mice by analyzing MSI in tail genomic DNA. We found that at the dinucleotide marker D7Mit91, 28% (38 of 134) of alleles tested were unstable in Msh2<sup>G674A/G674A</sup> mice; in contrast, 9% (11 of 118) of alleles in Msh2<sup>−/−</sup> genomes were unstable. Similarly, 20% (24 of 123) of the alleles at the mononucleotide marker U12235 in Msh2<sup>G674A/G674A</sup> mice were unstable, compared with 3% (7 of 244) unstable alleles that were found previously in Msh2<sup>−/−</sup> animals (39). This analysis indicated that the genomes of Msh2<sup>G674A/G674A</sup> mice displayed a highly significant increase in mutation frequency at these two markers (P < 0.0001 for D7Mit91 and U12235, Msh2<sup>G674A/G674A</sup> compared with Msh2<sup>−/−</sup>). Furthermore, the MSI at these loci in the Msh2<sup>G674A/G674A</sup> animals was comparable with the MSI observed in Msh2<sup>−/−</sup> mice [D7Mit91, 27% (36 of 132); U12235, 21% (19 of 92)]. These results indicate that the Msh2<sup>G674A</sup> mutation impairs the repair function of the protein and significantly increases the mutator phenotype in the genomes of the mutant mice.

Survival and Cancer Susceptibility in Msh2<sup>G674A</sup> Mutant Mice. When cohorts of Msh2<sup>G674A/G674A</sup>, Msh2<sup>−/−</sup>, and Msh2<sup>+/+</sup> mice were followed for a period of 12 months, the overall survival and cancer
susceptibility of the Msh2^G674A/G674A_ mice were clearly affected. None of the Msh2^G674A/G674A_ mice died in the first 3 months of life, and by 6 months, >90% of Msh2^G674A/G674A_ mice were still alive (Fig. 4). By 9 months of age, >60% of Msh2^G674A/G674A_ mice were alive; however, the number of surviving animals declined rapidly in the next 3 months, and all of the remaining mice died by 12 months of age. Only one Msh2^G674A/G674A_ and none of the Msh2^+/+_ mice died during the same period of time. The reduced survival in the Msh2^G674A/G674A_ mutant mice was caused by an increase in cancer predisposition. Most of the animals that died and were available for analysis had developed non-Hodgkin’s lymphomas (10 of 16 animals, 63%) between the ages of 9 and 12 months (Supplementary Table 1). A smaller number of mice between the ages of 7 and 10 months (3 of 16 animals, 19%) developed gastrointestinal adenocarcinomas. One animal at 9 months of age developed a squamous basal cell carcinoma of the skin (1 of 16 animals, 6%). In 2 animals that were 10 months of age, no obvious tumors could be detected (2 of 16 animals, 12%). Although the tumor spectrum in the Msh2^G674A/G674A_ mice resembled that seen in previously studied Msh2^+/+_ mouse strains (40–42), we noted a striking difference in survival. Whereas the 50% survival of Msh2^+/+_ mice on various mixed genetic backgrounds was reported at approximately 6 months of age, it took between 9 and 10 months for 50% of the Msh2^G674A/G674A_ mice to die. To directly compare the survival between the Msh2^G674A/G674A_ and Msh2^+/+_ lines on a similar genetic background, we generated a cohort of Msh2^+/+_ and Msh2^G674A/G674A_ mice that were backcrossed several times onto the C57BL/6 background. This comparison confirmed that the difference in survival between the Msh2^+/+_ and Msh2^G674A/G674A_ mice was highly significant (Fig. 4; P = 0.001, log-rank test).

**DNA Damage Response in Msh2^G674A/G674A_ MEF Cells.** The difference in survival in the Msh2^+/+_ and Msh2^G674A/G674A_ mice suggested that the mutant protein retained some function important for tumor suppression. In recent years, several studies demonstrated that MSH2-deficient human colorectal cancer cell lines as well as mouse embryonic fibroblast lines have an increased resistance to treatment with a variety of DNA-damaging agents including cisplatin, MNNG, and 6-thioguanine. It was proposed that the resistance to DNA damage-induced apoptosis in MMR-deficient cancer cells might provide a selective advantage in the initial stages of tumorigenesis (16). We therefore analyzed the genotoxic response to treatment with DNA-damaging agents in Msh2^+/+_, Msh2^+/−_, and Msh2^G674A/G674A_ MEF lines. Consistent with previous results, Msh2^+/−_ MEF cells were largely resistant to treatment with cisplatin at the drug levels tested (Fig. 5, A and B). In contrast, both Msh2^+/+_ and Msh2^G674A/G674A_ MEF lines were sensitive to cisplatin exposure. The differences in sensitivity between the Msh2^+/+_ or Msh2^G674A/G674A_ cells and Msh2^+/−_ cells were highly significant (P ≤ 0.0003). The Msh2^+/−_ cells also displayed increased resistance to treatment with 6-thioguanine and, to a lesser extent, to treatment with MNNG, whereas Msh2^+/+_ and Msh2^G674A/G674A_ cells showed higher sensitivity at the same drug concentrations (Supplementary Fig. 1). The cisplatin sensitivity in Msh2^+/+_ and Msh2^G674A/G674A_ cells was associated with significant increases in apoptosis as assessed by terminal deoxynucleotidyl transferase-mediated nick end labeling assay (Fig. 5C; P < 0.0001 for both Msh2^+/+_ and Msh2^G674A/G674A_ compared with untreated cells). In contrast, no significant increase in the number of apoptotic cells was seen in Msh2^+/−_ cells when compared with untreated cells. Interestingly, there was also a significant increase in the number of terminal deoxynucleotidyl transferase-mediated nick end labeling-positive cells in the untreated Msh2^G674A/G674A_ MEF cultures compared with the untreated Msh2^+/+_ or Msh2^+/−_ cell cultures (Ps < 0.001).

**Cisplatin Sensitivity in Msh2^G674A/G674A_ Teratocarcinomas.** In the next set of experiments, we studied the in vivo cisplatin sensitivity of Msh2 mutant teratocarcinomas in athymic nude mice. ES cells have the capacity to develop into teratocarcinomas when injected into immunodeficient athymic nude mice and were shown to provide a suitable model system to study drug sensitivity. In addition, the use of Msh2^+/−_ ES cells as xenografts in athymic nude mice demonstrated a major impairment in the cisplatin responsiveness of the tumor in vivo (43, 44). Because the results in the Msh2^G674A/G674A_ MEF lines indicated that the Msh2^G674A_ mutation would behave differently in this model system, we studied tumor growth in nude mice injected with Msh2^+/+_, Msh2^+/−_, or Msh2^G674A/G674A_ ES cells and measured their responsiveness to cisplatin treatment. We found that without cisplatin treatment, ES cells of all three Msh2 genotypes rapidly developed into teratocarcinomas after implantation (Fig. 6, A–C). However, treatment with cisplatin had different...
effects on tumor growth in the three cell lines. Whereas Msh2<sup>−/−</sup> cells did not respond to cisplatin treatment, and tumor growth occurred at similar rates in cisplatin-treated and untreated mice (Fig. 6B), the growth of both Msh2<sup>−/−</sup> and Msh2<sup>G674A/G674A</sup> tumors was significantly suppressed after cisplatin treatment (Fig. 6, A and C). These results are consistent with the observations made in the MEF cells and demonstrate that although the Msh2<sup>G674A</sup> mutation has a severe impact on DNA mismatch repair, it still confers a wild-type-specific response to cisplatin-induced DNA damage. In this regard, Msh2<sup>G674A</sup> is a separation of function mutation.

**DISCUSSION**

We generated a mouse line that carries the Msh2<sup>G674A</sup> missense mutation and studied the consequences on individual MMR functions and cancer susceptibility. The analysis of Msh2<sup>G674A</sup> mice showed that this mutation results in MMR deficiency and increased cancer susceptibility in homozygous mutant mice. In contrast, DNA mismatch repair was not significantly impaired in heterozygous mutant mice, indicating that the mutation does not act in a dominant manner. Our analysis of this mouse line demonstrates that the roles of MMR in the prevention of DNA replication errors and DNA damage-induced apoptosis can be separated by Msh2 missense mutations, and both functions are important for tumor suppression. Although, the Msh2<sup>G674A/G674A</sup> mutant mice show a strong cancer predisposition phenotype with a tumor spectrum similar to that of Msh2<sup>−/−</sup> mice, the extended survival in these animals in the first 9 months of life is consistent with the idea that the loss of the DNA damage-induced apoptotic response could provide a selective advantage for tumor cells in the initial stages of tumorigenesis (45, 46). We also observed a rapid decline in survival of the Msh2<sup>G674A/G674A</sup> animals between 9 and 12 months of age, which might be explained by the eventual accumulation of genomic mutations in cells that are not cleared by apoptosis. These cells could then accelerate tumorigenesis in the older Msh2<sup>G674A/G674A</sup> mice, once the initial barrier to tumorigenesis is overcome. Our results indicate that the increased mutation rates caused by MMR deficiency are sufficient to drive tumorigenesis and that it is the combination of increase in mutation rates and defective apoptosis that cooperates to result in tumorigenesis. Our results also demonstrate that the DNA damage-induced apoptosis function of Msh2 can delay but not prevent tumorigenesis.

The DNA repair defect in the Msh2<sup>G674A/G674A</sup> mutant mice is consistent with previous studies in bacteria and yeast and indicates that the ATPase domain is essential for the activation of the repair processes that facilitate the removal of mismatched bases (21–24). However, in contrast to the Msh2-null allele, the Msh2<sup>G674A</sup> mutation did not significantly affect the cellular response to DNA damage-inducing agents, indicating that normal ATP processing with subsequent repair is not essential for the apoptosis signaling function of Msh2. Different models have been developed in the past to explain the resistance of MMR-deficient cell lines to DNA-damaging agents. One model suggested that DNA repair-competent cells engage in futile repair cycles after treatment with alkylating agents because MMR is a strand-specific mechanism and is always directed to the newly synthesized strand (3, 5). Because DNA adducts in the template strand cannot be removed, the MMR reaction is continuously initiated upon repair synthesis, leading ultimately to the formation of double strand breaks that provide a signal for apoptosis. Alternatively, it was proposed that the binding of MSH2-MSH6 and also MLH1-PMS2 complexes to damaged bases at the replication fork could block DNA replication or other processes such as transcription and repair, leading to cell cycle arrest and cell death (47). The molecular analysis of Msh2<sup>G674A/G674A</sup> cells shows that although the mutant Msh2<sup>G674A</sup> protein is capable of mismatch binding, it does not allow normal MMR to proceed and therefore supports the latter notion. Our results are consistent with the idea that MMR components can function as sensors for genetic damage (16, 48) and are also in agreement with a recent model by Brown et al. (49), which proposes that MSH2-MLH1 complexes act as molecular scaffolds that physically link downstream effectors involved in DNA damage response pathways such as the ATM (ataxia telangiectasia mutated) gene product and checkpoint kinase 2 (CHK2). In this model, MSH2-bound CHK2 and MLH1-bound ATM complexes interact at the sites of DNA damage, resulting in the phosphorylation of CHK2 by ATM and the subsequent activation of the S-phase checkpoint and apoptotic pathways. Furthermore, MMR-mediated apoptosis appears to be activated through p53-dependent and p53-independent pathways and also involves the activation of p73 (9, 50, 51). In contrast to MMR-deficient cell lines, which display variable defects in the induction of p53 and p73, Msh2<sup>G674A/G674A</sup> MEF cells have normal induction of both proteins, indicating that normal Msh2 ATPase activity is not required for this response (data not shown). The presence of mutant Msh2<sup>G674A</sup> protein in Msh2<sup>G674A/G674A</sup> ES cells might allow the formation of mutant Msh2<sup>−</sup>-MLh complexes that are capable of signaling cell cycle arrest and apoptosis and provide an explanation for the increased number of apoptotic cells that we observed in the untreated Msh2<sup>G674A/G674A</sup> MEF cultures. In these cells, the DNA repair defect caused by the Msh2<sup>G674A</sup> mutation prevents the removal of misincorporated bases or oxidized bases such as 8-oxoguanine; however, it does not interfere with the recognition and binding of such lesions. The persistence of these DNA lesions in the genome and their continuous recognition by the mutant MMR complexes may in turn result in checkpoint activation and increased apoptosis. This hypothesis is supported by preliminary studies that revealed decreased cell proliferation and alterations in the cell cycle in Msh2<sup>G674A/G674A</sup> MEF cells. The availability of Msh2<sup>G674A</sup> mutant mice will aid in future studies to investigate the role of Msh2 in these processes.

Our results also suggest that a subset of tumors that carry MSH2 missense mutations will remain responsive to treatment with chemotherapeutic agents, a finding that may have important implications for the treatment of HNPCC patients. Different MSH2 missense mutations will likely have varied effects on DNA repair and apoptosis. Therefore, determining the genotype/phenotype correlations of MMR point mutations in HNPCC patients may provide valuable information for treatment and prognosis.

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5 S. J. Scherer and W. Edelmann, unpublished observations.
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