Introduction

HNPCC (hereditary non-polyposis colon cancer) is a highly penetrant, autosomal dominant cancer syndrome, characterized by early onset of colorectal cancers. It is estimated that HNPCC accounts for 5% of all colorectal cancers. In addition to cancers of the colon, a subset of patients also develops cancers at other organ sites, including the stomach, small intestine, ovary and endometrium [1]. The vast majority of HNPCC patients carry heterozygous mutations in one of the MMR (DNA mismatch repair) genes [2], and, currently, more than 400 MMR mutations have been reported ([3], and International Collaborative Group on HNPCC). The majority of these mutations were found in MLH1 (MutL homologue 1) (approx. 50%), MSH2 (MutS homologue 2) (approx. 40%) and MSH6 (approx. 10%), and mutations in other MMR genes are rare [4]. Mutations in the MSH2 and MLH1 genes are typically found in HNPCC families that fulfil the Amsterdam criteria, which include the onset of colorectal cancer before the age of 50 and three or more affected family members in at least two generations [5]. At the molecular level, tumours with MSH2 or MLH1 mutations display increased rates of replication errors at short repeat sequences, termed MSI (microsatellite instability) [6–8]. In contrast, mutations occurring in MSH6 can result in atypical HNPCC, which is often characterized by a later cancer onset and low or absent MSI in the tumours.

Abstract

Mutations in MMR (DNA mismatch repair) genes underlie HNPCC (hereditary non-polyposis colon cancer) and also a significant proportion of sporadic colorectal cancers. MMR maintains genome stability and suppresses tumour formation by correcting DNA replication errors and by mediating an apoptotic response to DNA damage. Analysis of mouse lines with MMR missense mutations demonstrates that these MMR functions can be separated and allows the assessment of their individual roles in tumour suppression. These studies in mice indicate that, although the increased mutation rates caused by MMR defects are sufficient to drive tumorigenesis, both functions co-operate in tumour suppression.

Molecular characteristics of the MMR system

The mammalian MMR system functions in the post-replicative repair of base substitution mutations as well as small insertion/deletion mutations. The characteristic repair steps of MMR include the recognition of mismatched bases by MutS proteins and the recruitment of MutL proteins to initiate the subsequent repair steps resulting in the removal and resynthesis of the DNA strand carrying the mutated base(s) [9]. Although the principle repair mechanism is conserved among bacteria, yeast and mammals, the eukaryotic MMR system is more complex, and several MutS and MutL homologues have been identified in yeast and mammals [10]. In eukaryotes, the initiation of the repair process is mediated by three different MutS homologues: MSH2, MSH3 and MSH6. These three MutS homologues form heterodimeric complexes consisting of MSH2–MSH6 (MutSα) and MSH2–MSH3 (MutSβ). The MSH2–MSH6 complex is responsible for the repair of base-base mispairs, while the MSH2–MSH3 complex is responsible for the repair of larger insertion/deletion mispairs of two to four bases [11–13]. In addition, both MSH2–MSH6 and MSH2–MSH3 complexes are redundant in the repair of some single-base insertions/deletions. Two other MutS homologues, MSH4 and MSH5, are not involved in MMR, but instead have specialized roles in the control of meiotic recombination [14,15].

Four different MutL homologues have been described in mammals: MLH1, PMS1 (post-meiotic segregation 1), PMS2 and MLH3 (reviewed in [16,17]). Similarly to MutS homologues, the MutL homologues interact to form three heterodimeric complexes: a complex between MLH1 and PMS2 (MutLα), a complex between MLH1 and PMS1 (MutLβ) and a complex between MLH1 and MLH3 (MutLγ). The primary MutL activity for mitotic MMR is provided by MutLα. It interacts with MutSα and MutSβ, much like the interaction
MMR proteins in DNA-repair and DNA-damage-response pathways

Figure 1 | MMR proteins in DNA-repair and DNA-damage-response pathways
(a) The MSH2-MSH6 heterodimer binds to base mismatch replication errors and recruits the MLH1–PMS2 heterodimer to initiate downstream repair events in an ATP-dependent process. (b) After exposure to genotoxic agents, the MSH2–MSH6 heterodimer recognizes damaged sites, and interacts with the MLH1–PMS2 heterodimer to signal cell cycle arrest. DNA damage signalling does not require ATP processing and could result in either the repair of damaged sites or apoptosis.

between bacterial MutS and MutL. This interaction is essential for the activation of the later MMR steps, which include the excision of the DNA strand carrying the mismatched base(s) and its resynthesis [18–21].

DNA-damage-response function

In recent years, important roles for several MMR proteins, in addition to the correction of biosynthetic errors, have been recognized, including the signalling of an apoptotic response triggered by exposure to DNA damage (Figure 1). The first evidence for a role of MMR in the processing of damaged DNA came from the observation that MMR-deficient bacteria were resistant to killing by low doses of alkylating agents, such as MNU (N-methyl-N-nitrosourea) and MNNG (N-methyl-N-nitro-N-nitrosoguanidine) [22,23]. Similarly, mammalian cells deficient in MSH2, MSH6, MLH1 and PMS2 showed resistance to DNA-damaging agents such as 6-TG (6-thioguanine), cisplatin and temozolomide [16, 24,25]. Complementation by chromosome transfer of MSH2, MSH6 and MLH1 reversed the resistance phenotype in mammalian cells and clearly implicated the MMR system in this response [26,27]. The role of the other MMR proteins, such as MSH3, in this process is less clear, as Msh3-deficient mouse embryonic stem cells do not display resistance to alkylating agents or to 6-TG [28]. Because some of these agents are currently being used as chemotherapeutic agents, it is of interest to understand the mechanism by which MMR-defective cancer cells acquire resistance to these drugs.

In bacteria, it was proposed that the presence of DNA damage on the template strand would lead to MMR-initiated repetitive rounds of futile repair cycles on the nascent unmodified strand, which would eventually lead to lethal double-strand breaks. In the absence of MMR-initiated repair, the lesions on the template strand are tolerated, allowing the bacterial cells to survive [29]. MMR-deficient human cells display similar tolerance to DNA damage; however, the mechanism by which it occurs may be different or more complex. It was previously recognized that the cytotoxic effect of alkylating agents on mammalian cells involves an apoptotic signal. After treatment of MutSα-deficient MT1 and wild-type TK6 cells with temozolomide or alkylating agents, only the wild-type TK6 cells underwent programmed cell death [30]. Similarly, HCT116 colorectal cancer cells that are deficient in MutLβ do not show an apoptotic response to treatment with chemical carcinogens, indicating that the MMR-induced apoptotic response is dependent not only on MutSα but also on MutLβ [26]. The MutSα-dependent apoptotic response to alkylating agents has also been demonstrated in animal studies. Msh2-deficiency reduced the apoptotic response to MNNG, temozolomide and cisplatin in the murine small intestine as compared with wild-type mice [31]. Similarly, Msh2-deficiency increased resistance to DMH (1,2-dimethylhydrazine)-induced apoptosis in the crypt cells of the large and small intestine [32].

The treatment of mammalian cells with low doses of methylating agents, such as MNNG, temozolomide or the base analogue 6-TG, induces a cell cycle arrest at G2/M that is dependent on a functional MMR system. This checkpoint activation is accompanied by the activation of the ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia-related) kinases and the phosphorylation of their respective downstream targets CHK1 (checkpoint kinase 1) and CHK2, although only ATR/CHK1 appear to be required for G2/M arrest [33–35].

At present, it is not entirely clear what apoptotic pathways are activated by MMR in response to the different types of DNA-damaging agents. MMR-mediated apoptosis appears to require both p53-dependent and -independent pathways and can also involve the activation of p73 [36–38].

Mouse lines with missense mutations in MMR genes

Mouse lines with inactivating mutations in all known Msh and Mlh genes have been generated (reviewed in [16,39]). The systematic analysis of these MMR-knockout mouse lines provided detailed information about the biological functions of each MMR protein and also significant insights into their importance for tumour suppression. However, the phenotypes seen in these mice reflect the loss of all cellular MMR functions. To define further the importance of each
MMR function in genome maintenance and tumour suppression, it is necessary to generate knock-in mouse lines with missense mutations in the individual MMR genes. The analysis of such mice is also of importance because a significant number of MMR mutations in HNPCC patients comprise missense mutations (>25% of MSH2 or MLH1 mutations and >45% of MSH6 mutations) [3]. The impact of these missense mutations on the individual MMR functions and their significance for cancer predisposition often remains uncertain. Therefore the generation of mouse lines with MMR knock-in mutations that resemble HNPCC mutations provides a unique opportunity to assess their impact on DNA repair and DNA damage response at the organismal level, and also to correlate the molecular defects in each MMR function with the observed cancer phenotype.

The first knock-in mouse line that was created carried a mutation in the Msh2-coding region corresponding to amino acid 674 [42]. The mutation resulted in a glycine-to-alanine change within the conserved ATPase domain of the Msh2 protein that is critical for the initiation of the repair process by MutS homologues [41–43]. The analysis of Msh2GA/GA mice (termed Msh2GA) demonstrated that missense mutations in Msh2 can have differential effects on the DNA repair and DNA-damage-response function. The biochemical analysis of Msh2GA/GA mutant extracts showed that the mutant Msh2GA–Msh6 complex retained normal mismatch recognition. However, it was resistant to ATP-mediated mismatch release and had lost its ability to signal mismatch repair, resulting in MMR-deficiency that was similar to Msh2−/− mutant extracts. As a consequence, homozygous mutant Msh2GA/GA embryonic stem cells displayed increased mutation rates. In contrast, the genotoxic response of Msh2GA/GA mutant MEF (mouse embryonic fibroblast) cells to different DNA-damaging agents, including cisplatin, 6-TG and MNNG, was not affected, indicating that the DNA-damage-response function was normal in Msh2GA/GA mutant cells. Indeed, the Msh2GA/GA mutant cells displayed an apoptotic response that was similar to that of wild-type cells after exposure to these agents, while an apoptotic response was absent in Msh2-null mutant cells. These data also indicate that the damage-response function can occur without normal ATP processing (Figure 1).

The repair defect in the Msh2GA/GA homozygous mutant mice resulted in a strong cancer-predisposition phenotype in the mice. Like the Msh2−/− mutant mice, all Msh2GA/GA mutant mice succumbed to lymphoid tumours and/or gastrointestinal tumours by 12 months of age. However, the 50% survival rate differed between Msh2−/− (6 months) and Msh2GA/GA (9–10 months) mutant mice, indicating a delayed onset of tumorigenesis in the Msh2GA/GA mice. The extended survival of these mice is consistent with the idea that the DNA-damage-induced apoptosis function can provide a barrier in the initial stages of tumorigenesis [44]. These results also indicate that the increased mutation rate in MMR-deficient cells is sufficient to drive tumorigenesis, and that the combination of increased mutation rates and defective apoptosis can accelerate tumorigenesis.

To dissect further the effect of missense mutations on the individual functions of the Msh2–Msh6 complex, another mouse line with a missense mutation in the Msh6 gene was created [45]. This mutation results in a threonine-to-aspartate change at amino acid 1217 in the Msh6 coding region (termed Msh6TD). Unlike the Msh2GA mutation, the Msh6TD mutation does not directly affect the ATPase domain of Msh6. However, structural modelling suggests that the Msh6TD mutation is located opposite the Msh2 ATP-binding domain in the Msh2–Msh6 complex and thus might interfere with ATP binding or processing [46]. A similar mutation at the corresponding site in Saccharomyces cerevisiae caused high rates of both base substitution and frameshift mutations, while msh6-deletion mutations are characterized by increased rates of base substitution mutations, suggesting that the Msh6TD mutation might act in a dominant manner [47].

The biochemical analysis of Msh6TD/mice mutant cell extract showed that the mutant Msh2–Msh6TD complex had mismatch-binding capacity, but was resistant to ATP-induced mismatch release. However, Msh6TD extracts were deficient in the repair of both base substitutions and dinucleotide insertion/deletion loops. This is in contrast with the Msh6−/− extracts, which are defective in the repair of base substitutions but not of insertion/deletion loops. Similarly, the analysis of MSI in genomic DNA isolated from tail, spleen and intestinal mucosa showed that Msh6TD mutant mice displayed increased instability at both mononucleotide and dinucleotide repeat markers, whereas no instability was seen in Msh6−/− mutant mice. These results are consistent with the idea that the lack of Msh6 in Msh6−/− cells does not interfere with the functions of the Msh2–Msh3 complex, whereas the presence of the mutant Msh6TD protein interferes with the Msh2–Msh3-mediated repair of dinucleotide insertion/deletion mutations. Similar to Msh2GA mutant cell lines, the presence of the Msh6TD protein did not affect the DNA-damage-induced apoptosis function of MEF cells.

The cancer phenotype in Msh6TD mice was comparable to Msh6−/− mice. However, there was a delayed cancer onset in Msh6TD mice in the first 10 months of life as compared with Msh6−/− mice, suggesting that the normal DNA with damage-response function in Msh6TD mice did delay tumorigenesis. Most interestingly, the Msh6TD/+ heterozygous mice also displayed reduced survival in the second year of life, which was caused by a strong predisposition to lymphoma. The tumours that developed in both heterozygous and homozygous Msh6TD mice displayed MSI, although the levels of MSI differed between the genotypes, with Msh6TD homozygous tumours displaying an MSI-high phenotype and Msh6TD/+ heterozygous tumours displaying a moderate MSI phenotype. In contrast, Msh6−/− tumours did not display an MSI phenotype. Furthermore, immunohistochemical analysis of homozygous or heterozygous Msh6TD tumours revealed that the MSI phenotype was not associated with the loss of MMR proteins, including Msh2 and Msh3, indicating further that the Msh6TD mutation acted in a dominant manner. A model to explain the dominant mechanism by which the Msh6TD protein interferes with the Msh2–Msh3 directed
repair of insertion/deletion mutations is depicted in Figure 2. Interestingly, an MSH6 mutation at the corresponding amino acid position (Thr1219) was found in an HNPCC patient and resulted in a threonine-to-isoleucine change [48]. The mutation was associated with early-onset colorectal cancer in the patient, and the tumours displayed a high MSI level and also stained positive for several MMR proteins. The analysis of Msh6TD mutant mice provides a possible explanation for the observed cancer phenotype and is an example of the complementary insights that can be gained from the analysis of MMR point mutations in yeast, mice, and humans.

The analysis of Msh2GD and Msh6TD mutant cell lines showed that, although both mutations caused MMR deficiency, they retained their ability to signal an apoptotic response to DNA damage. This separation of function phenotype might also provide some clues about the mechanism of resistance to genotoxic agents in MMR-deficient tumour cell lines. The apparent repair deficiency caused by these mutations makes it less likely that a futile repair cycle is responsible for the observed resistance phenotype. Moreover, recent studies suggest the MMR proteins may act as DNA damage sensors that are linked via signal cascades to cell cycle checkpoints and downstream mediators of apoptosis. In one scenario, it was proposed that MutSα and MutLα function as molecular scaffolds that physically link downstream effectors such as ATM and CHK2 at the site of DNA damage, resulting in the phosphorylation of CHK2 and the subsequent activation of S-phase arrest and apoptosis [49]. The observation that the mutant Msh2GD–Msh6 and Msh2–Msh6TD complexes retain their ability to bind to mismatched DNA substrates is consistent with this proposal. In addition, another mutant mouse line that carries a glycine-to-alanine mutation at codon 67 in one of the ATPase domains of Mlh1 also resulted in MMR deficiency, but displayed normal DNA damage signalling (S.J. Scherer, E. Avdievich, B. Kneitz and W. Edelmann, unpublished work).

Conclusions

The generation and analysis of MMR point mutant mouse lines allows an assessment of the importance of individual MMR functions for genome stability and cancer susceptibility. The studies described here demonstrate that MMR missense mutations can effectively separate two MMR functions that are important in tumour suppression, the repair of replication errors and the DNA damage response. They also demonstrate that, although both functions cooperate in tumour suppression, the increased mutator phenotype caused by these mutations is sufficient to drive tumorigenesis. In contrast, the DNA-damage-response function of MMR appears to be important in the initial stages of tumorigenesis, but cannot prevent tumour formation in association with a strong repair defect. The analysis of Msh6TD mice demonstrates further that MMR mutations can cause an increased mutator phenotype in the tissue of heterozygous carriers. This observation suggests that certain alterations in MMR genes can also cause similar repair defects in humans, with important implications for cancer susceptibility. The results obtained from these studies suggest that phenotype–genotype correlations could provide valuable information for a more individualized diagnosis and therapy of patients with MMR mutations.
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References

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