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Mouse models of DNA mismatch repair in cancer research

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Abstract

Germline mutations in DNA mismatch repair (MMR) genes are the cause of hereditary non-polyposis colorectal cancer/Lynch syndrome (HNPCC/LS) one of the most common cancer predisposition syndromes, and defects in MMR are also prevalent in sporadic colorectal cancers. In the past, the generation and analysis of mouse lines with knockout mutations in all of the known MMR genes has provided insight into how loss of individual MMR genes affects genome stability and contributes to cancer susceptibility. These studies also revealed essential functions for some of the MMR genes in B cell maturation and fertility. In this review, we will provide a brief overview of the cancer predisposition phenotypes of recently developed mouse models with targeted mutations in *MutS* and *MutL* homologs (*Msh* and *Mlh*, respectively) and their utility as preclinical models. The focus will be on mouse lines with conditional MMR mutations that have allowed more accurate modeling of human cancer syndromes in mice and that together with new technologies in gene targeting, hold great promise for the analysis of MMR-deficient intestinal tumors and other cancers which will drive the development of preventive and therapeutic treatment strategies.

Keywords

Mouse models; DNA mismatch repair; Msh2; Mlh1; Hereditary non-polyposis colon cancer; Lynch syndrome

1. Introduction

Since the identification of the mammalian *MutS* and *MutL* homolog (*MSH* and *MLH*, respectively) gene family and the discovery that mutations in some of these genes underlie Hereditary nonpolyposis colorectal cancer/Lynch syndrome (HNPCC/LS), mouse models with mutations in all known *MSH* and *MLH* genes have been generated to study their roles in genome maintenance and tumor suppression. The analysis of these mouse lines has revealed that specific mammalian *MSH* and *MLH* family members have different significance for these processes largely consistent with their known roles in the repair of DNA replication errors (RERs) and the cellular DNA damage response (DDR). For example, mouse lines with knockout mutations in *Msh2*, *Msh6*, *Mlh1* and *Pms2* that form

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.dnarep.2015.11.015>.

the MutS α (Msh2-Msh6) and MutL α (Mlh1-Pms2) complexes and play major roles in the repair of RERs in eukaryotic cells and the MMR-dependent DDR, display strong mutator and cancer predisposition phenotypes [1–8]. In contrast, mouse lines with mutations in *Msh3* or *Mlh3* that participate in other MMR complexes such as MutS β (Msh2-Msh3) and MutL γ (Mlh1-Mlh3) display milder cancer phenotypes [9–11]. While mouse lines deficient in Msh4 and Msh5 that form the Msh4-Msh5 complex, together with some of the *MutL* proteins, play essential roles in meiotic recombination but do not display any obvious cancer phenotypes [12–15] [16] this issue. Overall, the cancer predisposition phenotypes of MMR mutant mouse lines show concordance with the incidence of *MutS* and *MutL* gene mutations in HNPCC/LS patients in which *MSH2* and *MLH1* are the most frequently mutated genes, while mutations in *MSH6*, *PMS2* and *MLH3* occur less frequently [17].

The analysis of *MutS* and *MutL* mutant mouse models also revealed essential roles for some of the MMR proteins in a number of different biological processes in mammals, including somatic hypermutation and class switch recombination during B cell maturation [18,19] this issue. In addition, the analysis of *Msh2* and *Msh3* knockout mouse lines revealed that the MutS β complex facilitates triplet repeat expansion, which is associated with a number of neurological diseases [20] this issue. The roles of MMR proteins in these latter processes are beyond the scope of this article, but are described in more detail in other chapters of this review issue.

In addition to constitutive knockout MMR mouse models, several separation-of-function MutS α and MutL α knock-in mice have been generated (termed *Msh2*^{G674A}, *Msh2*^{G674D}, *Msh6*^{T1217D}, *Mlh1*^{G67R} and *Pms2*^{E702K}) [21–25]. These knock-in mutations selectively impaired mismatch repair of base substitutions and 1-2 base insertion/deletions but largely left the MMR-dependent DDR intact allowing dissection of the significance of the two MMR functions for tumor suppression. All of the above mentioned knock-in mice displayed increased cancer susceptibility phenotypes highlighting the critical role of mismatch repair of RERs in tumor suppression. Nevertheless, the onset of tumorigenesis was delayed in these mouse models, suggesting that the MMR-dependent-DDR function is important for suppressing the initial stages of tumorigenesis. The analysis of MMR knockout and knock-in mutant mice was described in previous review articles and their phenotypes are summarized in Table 1 [26,27].

The loss of MMR in *Msh 2* and *Mlh1* homozygous mutant mice results in strong cancer predisposition phenotypes that are, however, predominantly characterized by T-cell lymphomas and to a lesser extent by skin tumors and small intestinal adenomas and adenocarcinomas. Therefore, in recent years, a major focus in the development of MMR mutant mouse lines was the establishment of mouse models that more accurately model the cancer phenotype of HNPCC/LS patients and to create preclinical models for the assessment of potential preventative and therapeutic interventions. In this review, we will provide an overview of these efforts with an initial description of the generation of conventional MMR knockout mouse lines that carried mutations in MMR genes and tumor suppressor or oncogenes genes to study the impact on intestinal and other HNPCC/LS-associated tumorigenesis. The main emphasis of this review will be an overview of more recently

generated mouse lines with conditional MMR knockout mutations and their use as preclinical models of HNPCC/LS.

2. Mouse lines carrying constitutive MMR and tumor suppressor gene knockout mutations

To develop mouse models that more closely mimic the tumor spectrum seen in HNPCC/LS patients, MMR-deficient mouse lines were intercrossed with mouse lines carrying knockout mutations in some of the tumor suppressor or oncogenes frequently mutated in human colorectal cancers such as *APC*, *TRP53* and *KRAS* (summarized in Supplemental Table 1). Initially, MMR mutant mice were crossed with mouse lines carrying heterozygous germline mutations in the *Apc* tumor suppressor gene. The large majority of MSI-positive colorectal cancers in human patients carry somatic mutations in the *APC* tumor suppressor gene indicating that loss of APC function is critical for the initiation and/or progression of MMR-deficient intestinal tumorigenesis. The combination of homozygous mutations in *Msh2*, *Msh6*, *Mlh1* and *Pms2* with heterozygous *Apc* mutations limited the tumor development to the intestinal tract, and the tumor incidence correlated with the severity of the MMR defects in the different mice. For example, loss of *Msh2* or *Mlh1* caused dramatic increases in tumor numbers, while loss of *Msh6* or *Pms2* caused more moderate increases in intestinal tumor numbers and loss of *Msh3* did not increase intestinal tumor numbers [6,28–30]. Similar to human MSI-positive colorectal cancers, MMR-deficiency in these mice was associated with somatic mutations in the remaining wild type *Apc* allele in the intestinal tumors [3,30]. MMR deficient mice have also been crossed with *p53* mutant mice since *p53* mutations occur frequently in human MMR-deficient colorectal cancers. Although *p53*-deficiency resulted in greatly accelerated tumorigenesis, the tumor spectrum in *Msh2*^{-/-}; *p53*^{-/-} and *Msh6*^{-/-}; *p53*^{-/-} mice was characterized mainly by T-cell lymphoma [31,32]. Interestingly, loss of *Msh3* did not accelerate tumorigenesis in *Msh3*^{-/-}; *p53*^{-/-} mice; however, it changed the tumor spectrum from lymphoma to sarcoma, indicating that *Msh3* is a potent modulator of *p53*-driven tumorigenesis [33]. The analysis of *Msh3*^{-/-} cells further revealed increased chromatid breaks and persistence of γ H2AX foci after ionizing radiation indicating a defect in DNA double strand break repair (DSBR). As a consequence, *Msh3*; *p53* mutant tumors displayed increased loss of heterozygosity, elevated genome-wide copy number variation in addition to a moderate MSI phenotype. Overall, these studies indicated that the MutS β complex is important for tumor suppression due to its roles in both DSBR and MMR, especially in late onset tumors or on predisposing genetic backgrounds with mutations in the *p53* gene or other tumor suppressor genes.

Another study tested the effect of oncogenic K-ras mutations on MMR-deficient intestinal tumorigenesis in mice. The conditional expression of a *K-ras*^{V12} transgene in *K-ras*^{V12}/*Ah-Cre*; *Msh2*^{-/-} mice showed that oncogenic K-ras cooperates with MMR-deficiency and accelerates tumorigenesis in both the small and large intestine. However, the underlying molecular mechanisms remain unclear [34].

In addition to colorectal cancers HNPCC/LS patients develop endometrial cancers at high frequency. To study the effect of MMR-deficiency on endometrial tumorigenesis *Msh2* knockout mice were intercrossed with mice carrying a heterozygous knockout mutation in

the E-cadherin gene which is frequently inactivated in human endometrial cancers (*Msh2*^{-/-}; *Cdh1*^{+/-} mice) [35]. A proportion of *Msh2*^{-/-}; *Cdh1*^{+/-} mice developed endometroid-like tumors that displayed loss or reduced expression of E-cadherin. Interestingly, the GC-box, a cis-acting regulatory transcription element in the promoter region of the *E-cadherin* gene was mutated in several tumors in *Msh2*^{-/-}; *Cdh1*^{+/-} mice, suggesting that a reduction in the promoter activity of the remaining E-cadherin wild type allele accounted for the loss of E-cadherin expression. Similarly, *Mlh1*^{-/-} mice that carried a heterozygous *Pten*^{+/-} knockout allele displayed accelerated endometrial tumorigenesis compared to *Pten*^{+/-} mice that was also associated with the loss of the wild type *Pten* allele [36]. MMR knockout mouse lines have also been intercrossed with mouse lines carrying mutations in other DNA repair genes to study their interaction in genome maintenance. Although not a topic of this review, these mouse lines are also summarized in Supplemental Table 1.

3. Mouse lines carrying conditional MMR mutations and their use as preclinical models

Most of the MMR mutant mouse lines that were previously developed carried homozygous knockout or knock-in mutations and as a result developed predominantly T and B cell lymphomas. Genetically, these mouse lines are more akin to human patients with constitutional mismatch repair deficiency (CMMR-D) syndrome that carry biallelic germline mutations in the *MSH2*, *MSH6*, *MLH1* and *PMS2* genes [37]. Similar to MMR knockout mouse models, these patients display MMR-deficiency in all tissues and develop early onset cancers mainly including haematological malignancies and/or brain tumors, as well as early-onset colorectal cancers. In addition, although the combination of MMR-deficiency with tumor suppressor or oncogene gene mutations in the compound mutant mice described above led to accelerated tumorigenesis in the target tissues, these mouse models display complete MMR-deficiency in all of their tissues. In contrast, HNPCC/LS patients normally carry heterozygous germline mutations in *MSH2* or *MLH1* and tumorigenesis is associated with a second mutagenic event in the remaining wildtype MMR allele in intestinal epithelial cells later in life.

In an attempt to create a more faithful mouse model for HNPCC/LS, a conditional knockout mouse model was created in which an *Msh2*^{loxP} allele can be tissue-specifically inactivated by Cre-recombinase mediated deletion of a floxed exon 12 [25]. The combination of this *Msh2*^{loxP} allele with an intestine specific Villin-Cre recombinase transgene [38] (*Villin-Cre*; *Msh2*^{loxP/loxP}, termed *VCMsh2*^{loxP}) led to the loss of Msh2 and MMR deficiency exclusively in the intestinal epithelium. As a consequence, *VCMsh2*^{loxP} mice developed 1–2 intestinal adenocarcinomas and/or carcinomas that were pathologically identical to HNPCC/LS tumors at very high penetrance within the first year of life. The *VCMsh2*^{loxP} model also provided the possibility to create compound mouse models by combining the *Msh2*^{loxP} allele with either a complete loss-of-function *Msh2* knockout allele (*Msh2*⁻) or with an *Msh2* separation-of-function allele (*Msh2*^{G674D}) to mimic mutations previously found in HNPCC/LS tumors and to study the effects of allelic phasing on intestinal tumorigenesis and drug response *in vivo*. The combination of these alleles with the *Msh2*^{loxP} allele resulted in the development of intestinal tumors in both *VCMsh2*^{loxP/-} and

VCMsh2^{loxP/G674D} mice similar to the original *VCMsh2^{loxP}* mouse line. However, the response of intestinal tumors to the commonly used anticancer agent FOLFOX (5-Fluorouracil, Leucovorin, Oxaliplatin) differed between the two mouse lines. MRI imaging revealed that in *VCMsh2^{loxP/-}* mice, the response of the intestinal tumors to FOLFOX treatment was impaired due to the complete loss of the MMR-dependent DDR function in the intestinal tumor cells. In contrast, in *VCMsh2^{loxP/G674D}* mice the presence of the *Msh2^{G674D}* allele mediated a significant FOLFOX response that resulted in tumor regression. These results indicate that the MMR-dependent DDR plays an important role in the *in vivo* response of intestinal tumors to FOLFOX treatment and suggests that the precise evaluation of MMR gene mutations could be helpful in determining more adequate chemotherapeutic treatment strategies for HNPCC/LS patients.

The *VCMsh2^{loxP}* mouse line was also employed as an *in vivo* model to determine the potential of long-term treatment with nonsteroidal anti-inflammatory drugs (NSAIDs), such as acetylsalicylic acid (ASA or aspirin) and nitric oxide-donating ASA (NO-ASA), as a chemopreventive approach in HNPCC/LS patients [39]. Previous epidemiological studies and studies in rodents indicated that ASA use may be effective in reducing the incidence of colorectal cancers [40,41] and that the NO moiety in NO-ASA may reduce NSAIDs-associated side effects such as gastropathy [42,43]. Furthermore, studies in cell lines previously showed that both ASA and NO-ASA suppressed the MSI mutator phenotype in MMR-deficient colon cancer cell lines through genetic selection that appears to enhance apoptosis in critically unstable cells [44,45]. The exposure of *VCMsh2^{loxP}* mice to ASA delayed but did not prevent intestinal tumorigenesis and moderately extended the life span of the animals. The analysis of ASA-treated *VCMsh2^{loxP}* mice further suggested that the positive effect of ASA on survival is caused by suppression of MSI in the intestinal epithelia, although further studies are required to support this notion. In contrast, while exposure to low doses of NO-ASA also extended life span, exposure to high doses of NO-ASA had a negative effect on survival and actually increased the intestinal tumor incidence and MSI in intestinal epithelial cells. These studies indicate that long-term treatment with ASA may provide a chemopreventive option for HNPCC/LS patients and that treatment with low doses of NO-ASA may help reduce the gastropathy associated with long-term ASA exposure (see also [46] this issue).

The potential of NSAIDs in the chemopreventive treatment of HNPCC/LS patients was also shown in another study employing the *VCMsh2^{loxP}* model [47]. In this study, the effect of mesalazine (5-aminosalicylic acid, 5-ASA), a drug that is structurally related to aspirin and used in the treatment of ulcerative colitis, was studied in *VCMsh2^{loxP}* mice. Different to ASA treatment, mesalazine reduced both the incidence and multiplicity of intestinal tumors in *VCMsh2^{loxP}* mice. Mesalazine also reduced MSI in the intestinal epithelial cells, but had no effect on cell viability or induction of apoptosis at the concentrations used. These results are consistent with the idea that mesalazine improves replication fidelity in MMR-deficient cancer cells and decelerates carcinogenesis. In the same study thymoquinone, a natural compound from *Nigella sativa*, was studied and had similar effects on replication fidelity and intestinal tumorigenesis in *VCMsh2^{loxP}* mice providing another candidate compound for chemoprevention in HNPCC/LS patients.

While in *VCMsh2^{loxP}* mice the *Villin-Cre* recombinase transgene is expressed during development and adulthood resulting in constitutive MMR-deficiency in the intestinal epithelium, the *Msh2^{loxP}* allele can also be employed to model MMR-deficient sporadic colon cancer [48]. To achieve this, the *Msh2^{loxP}* allele was combined with an *Apc^{loxP}* allele to generate *Msh2^{loxP/loxP}; Apc^{loxP/loxP}* mice and tumorigenesis was initiated by viral infusion of *Adenoviral-Cre* into the colon by laparotomy. This approach led to the development of multiple colonic adenomas and a small number of advanced adenocarcinomas. This model was then used to study the effect of NVP-BEZ235, a dual PI3K/mTOR inhibitor, on small and large intestinal tumorigenesis. Dysregulation of the PI3K/AKT/mTOR pathway is frequently observed in many human tumor types [49], and this mouse model together with advanced imaging techniques offered the possibility of closely monitoring the histologic and genetic changes in MMR-deficient intestinal tumors before and after drug treatment. The tumors that developed in these mice displayed variable dysregulation of a number of genes relevant to intestinal tumorigenesis, including down-regulation of *Pten*, upregulation of *Akt* and *pMek* among others. Treatment of colonic tumors with NVP-BEZ235 alone or in combination with the MEK inhibitor ADZ4266 resulted in partial tumor regression, however, the response was not seen in highly advanced tumors. Overall, these studies suggested that gene directed treatment of MMR-deficient intestinal tumors with NVP-BEZ235 alone or in combination with ADZ4266 may be effective in a subset of MMR-deficient colorectal cancers and that other genes or pathways that are dysregulated in drug resistant tumors such as PDPK1 (phosphoinositide dependent protein kinase 1) may be useful targets in treating these tumors.

In a subsequent study, another Lynch-like mouse model was created. In this model an *Msh2^{loxP}* allele was created in which exons 12 and 13 are flanked by two inverted *loxP* sites. This *loxP* configuration leads to the inversion of both exons upon *Cre-loxP* mediated recombination [50]. This allele (*Msh2^{fllox}*) was combined with a constitutive *Msh2* knockout allele (*Msh2^{-/-}*) and the *Lgr5-EGFP-IRES-CreERT2* transgene [51] to generate *Lgr5-CreERT2; Msh2^{fllox/-}* mice. In this mouse line the *Lgr5* promoter drives the expression of a tamoxifen-responsive *Cre*-recombinase (*creERT2*) in self-renewing intestinal stem cells that are located at the crypt base making it possible to induce mosaic *Msh2* inactivation in a small number of intestinal crypts in adult mice after tamoxifen injection. As expected, a proportion of tamoxifen treated mice developed *Msh2*-deficient intestinal adenocarcinomas late in life. In addition, this model was used to study the response to temozolomide (TMZ), a methylating chemotherapeutic agent that is being used in the treatment of gliomas and melanomas. Exposure to TMZ causes the formation of O⁶-methylguanine lesions that if left unrepaired can cause the formation of aberrant base pairs upon replication. These aberrant base pairs are subsequently recognized by MMR complexes, which mediate cell cycle arrest and apoptosis [52,53] this issue. In MMR-deficient cells, this genotoxic response is impaired resulting in the survival of MMR-deficient cells that also display increased mutation rates. The exposure to high doses of TMZ caused a significant expansion of *Msh2*-deficient crypts in *Lgr5-CreERT2; Msh2^{fllox/-}* mice consistent with the idea that *Msh2*-deficient crypts acquire a growth advantage over wildtype crypts. In addition, TMZ exposure accelerated intestinal tumor development that most likely was caused by an increased mutational load.

Therefore, the authors concluded that HNPCC/LS patients should not be exposed to TMZ to avoid expansion and selection of highly tumorigenic Msh2-deficient cells.

Besides the intestinal epithelium, other tissues have been targeted using conditional MMR mouse models. For example, the *Msh2^{loxP}* mouse line [25] was used to investigate the role of Msh2 in the development of Huntington's disease. Huntington's disease gene (*HTT*) CAG (*HdhQ111*) knock-in mice were crossed with *Msh2^{loxP}* mice and *D9-Cre* recombinase transgenic mice to study the role of Msh2 in CAG-triplet repeat expansion specifically in medium-spiny GABA-ergic projection neurons (MSNs) in the striatum that are known to be extremely susceptible to the negative effects of the mutant huntingtin protein [54]. This study demonstrated that Msh2 acts within MSNs as a genetic enhancer of somatic *HTT* CAG expansions and as a modifier of *HTT* CAG-dependent phenotypes in mice. Based on these findings, it was suggested that intervening in the expansion process in these neurons could have therapeutic benefit.

Besides *Msh2^{loxP}* mice, a mouse line for the conditional inactivation of *Mlh1* has been generated in which exon 4 can be deleted by *Cre-loxP* recombination [55]. Deletion of exon 4 mediated by an *Ella-Cre* recombinase transgene [56] in *Ella-Cre; Mlh1^{loxP/loxP}* mice led to MMR-deficiency from the zygote stage onward, resulting in a cancer and infertility phenotype indistinguishable from constitutive *Mlh1^{-/-}* knockout mice. To investigate the tumor suppressor function of Mlh1 specifically during T-cell development, the *Mlh1^{loxP}* mice were mated with *Lck-Cre* recombinase transgenic mice [57] to generate *Lck-Cre; Mlh1^{loxP/loxP}* mice (*Mlh1^{T ex4/T ex4}*). In *Mlh1^{T ex4/T ex4}* mice *Mlh1* is lost in thymocytes at the first stages of maturation resulting in a reduced T-cell lymphoma incidence when compared to *Mlh1^{-/-}* mice. These results indicated a critical role for Mlh1 in suppressing lymphomagenesis at the very early stages of T-cell development and that loss of MMR at later stages causes only moderate increases in T-cell lymphomagenesis.

4. Future directions

Although constitutive knockout and knock-in mouse lines have been invaluable in determining the functions of individual *Msh* and *Mlh* genes in diverse biological processes and evaluating their roles in tumor suppression, the availability of *Msh2^{loxP}* and *Mlh1^{loxP}* conditional knockout mouse lines enables the analysis of MMR in specific cells or tissues. A summary of previously generated conditional mutant MMR mouse lines is shown in Table 2. The availability of different *Cre*-recombinase transgenic mouse lines, active in different tissues and/or inducible at different times, has greatly expanded new avenues for studying MMR functions *in vivo*. Moreover, the use of *Cre*-recombinase alleles targeting MMR in the stem cell compartment in the intestine or other tissues, similar to the *Lgr5-EGFP-IRES-CreERT2* allele, will provide novel insights into the role of MMR in tumor suppression. The *Lgr5-EGFP-IRES-CreERT2* transgene allows the identification and isolation of MMR-deficient *Lgr5⁺* stem cells not only within the normal intestinal mucosa but also in intestinal adenocarcinomas. The analysis of intestinal tumor cells expressing *Lgr5* or other stem cell markers will likely be important to determine the roles these cells play in the response of MMR-deficient cancers to anticancer drugs. In addition, these mouse lines will provide

important preclinical models to study the interaction of diet or environmental carcinogens with MMR-deficient stem cells in the development of intestinal and other cancers.

An important future goal will be to engineer mouse models that develop MSI-positive colonic tumors similar to those that develop in HNPCC/LS patients. However, similar to other mouse models of intestinal cancer, and as mentioned above, all currently available MMR-deficient mouse models develop tumors predominantly in the small intestine. Although the reasons for this are not well understood, it is likely that specific genetic differences between humans and mice are responsible. For example, colonic tumors in HNPCC/LS patients frequently carry mutations in coding repeat sequences of genes thought to be important for tumorigenesis and the vast majority of these tumors with microsatellite instability carry frameshift mutations in a 10 bp polyadenine repeat tract in the coding region of the *TGF β R2* gene [58,59]. This repeat is disrupted by a single guanine in the mouse *Tgf β R2* gene making it resistant to RERs that result in frameshift mutations. The “humanization” of such repeats in the mouse genome might present a possible solution to this challenge. CRISPR/Cas9 technology is a relatively new and powerful approach for the manipulation of the mouse genome and allows the rapid introduction of changes into relevant genes in new or already existing mouse models [60]. Another possible genetic approach could be to induce MMR-deficiency specifically in large intestinal stem cells by combining the *Msh2*^{loxP} or *Mlh1*^{loxP} alleles with *Lrig1-CreERT2* transgenic mice, that were recently shown to induce colon cancer in an *Apc*^{loxP} mouse model [61].

The development of mouse models that develop HNPCC/LS-like colon cancers will also be essential in analyzing differences that exist in the microbiome between the large and small intestine that likely affect tumorigenesis [62]. For example, a recent study revealed a significant role of the gut microbiota in colonic polyp formation in *Msh2*^{-/-}; *Apc*^{min} mice [63]. The gut microbes did not induce colon tumorigenesis by the production of mutagens or inflammatory responses, but rather by producing carbohydrate-derived metabolites such as butyrate that was proposed to induce hyper-proliferation of Msh2-deficient colon epithelial cells and increased colon polyp formation in these mice [63].

Finally, another approach to establish “humanized” HNPCC/LS mouse models might be the development of patient-derived tumor xenografts (PDX) mouse models in which patient-derived tumor tissues are directly transplanted into immunodeficient mice. In contrast to cell line-derived xenografts, PDX models can retain the histopathologic features and molecular characteristics of the original tumor even after extensive passaging in mice [64]. These PDX models in combination with the genetic models promise to be highly useful for drug development and biomarker discovery and also provide an important tool for personalized medicine for HNPCC/LS tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Reitmaier AH, Schmits R, Ewel A, Bapat B, Redston M, Mitri A, Waterhouse P, Mittrucker HW, Wakeham A, Liu B, et al. MSH2 deficient mice are viable and susceptible to lymphoid tumours. *Nat. Genet.* 1995; 11:64–70. [PubMed: 7550317]
2. de Wind N, Dekker M, Berns A, Radman M, te Riele H. Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell.* 1995; 82:321–330. [PubMed: 7628020]
3. Smits R, Hofland N, Edelmann W, Geugien M, Jagmohan-Changur S, Albuquerque C, Breukel C, Kucherlapati R, Kielman MF, Fodde R. Somatic Apc mutations are selected upon their capacity to inactivate the beta-catenin downregulating activity. *Genes Chromosom. Cancer.* 2000; 29:229–239. [PubMed: 10992298]
4. Edelmann W, Yang K, Umar A, Heyer J, Lau K, Fan K, Liedtke W, Cohen PE, Kane MF, Lipford JR, Yu N, Crouse GF, Pollard JW, Kunkel T, Lipkin M, Kolodner R, Kucherlapati R. Mutation in the mismatch repair gene Msh6 causes cancer susceptibility. *Cell.* 1997; 91:467–477. [PubMed: 9390556]
5. Prolla TA, Baker SM, Harris AC, Tsao JL, Yao X, Bronner CE, Zheng B, Gordon M, Reneker J, Arnheim N, Shibata D, Bradley A, Liskay RM. Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. *Nat. Genet.* 1998; 18:276–279. [PubMed: 9500552]
6. Edelmann W, Yang K, Kuraguchi M, Heyer J, Lia M, Kneitz B, Fan K, Brown AM, Lipkin M, Kucherlapati R. Tumorigenesis in Mlh1 and Mlh1/Apc1638N mutant mice. *Cancer.* 1999; 59:1301–1307.
7. Egashira A, Yamauchi K, Yoshiyama K, Kawate H, Katsuki M, Sekiguchi M, Sugimachi K, Maki H, Tsuzuki T. Mutational specificity of mice defective in the MTH1 and/or the MSH2 genes. *DNA Repair (Amst.).* 2002; 1:881–893. [PubMed: 12531017]
8. Kawate H, Sakumi K, Tsuzuki T, Nakatsuru Y, Ishikawa T, Takahashi S, Takano H, Noda T, Sekiguchi M. Separation of killing and tumorigenic effects of an alkylating agent in mice defective in two of the DNA repair genes. *Proc. Natl. Acad. Sci. U. S. A.* 1998; 95:5116–5120. [PubMed: 9560238]
9. de Wind N, Dekker M, Claij N, Jansen L, van Klink Y, Radman M, Riggins G, van der Valk M, van't Wout K, te Riele H. HNPCC-like cancer predisposition in mice through simultaneous loss of Msh3 and Msh6 mismatch-repair protein functions. *Nat. Genet.* 1999; 23:359–362. [PubMed: 10545954]
10. Edelmann W, Umar A, Yang K, Heyer J, Kucherlapati M, Lia M, Kneitz B, Avdievich E, Fan K, Wong E, Crouse G, Kunkel T, Lipkin M, Kolodner RD, Kucherlapati R. The DNA mismatch repair genes Msh3 and Msh6 cooperate in intestinal tumor suppression. *Cancer Res.* 2000; 60:803–807. [PubMed: 10706084]
11. Chen PC, Dudley S, Hagen W, Dizon D, Paxton L, Reichow D, Yoon SR, Yang K, Arnheim N, Liskay RM, Lipkin SM. Contributions by MutL homologues Mlh3 and Pms2 to DNA mismatch repair and tumor suppression in the mouse. *Cancer Res.* 2005; 65:8662–8670. [PubMed: 16204034]
12. Kneitz B, Cohen PE, Avdievich E, Zhu L, Kane MF, Hou H Jr. Kolodner RD, Kucherlapati R, Pollard JW, Edelmann W. MutS homolog 4 localization to meiotic chromosomes is required for chromosome pairing during meiosis in male and female mice. *Genes Dev.* 2000; 14:1085–1097. [PubMed: 10809667]
13. Edelmann W, Cohen PE, Kneitz B, Winand N, Lia M, Heyer J, Kolodner R, Pollard JW, Kucherlapati R. Mammalian MutS homologue 5 is required for chromosome pairing in meiosis. *Nat. Genet.* 1999; 21:123–127. [PubMed: 9916805]

14. de Vries SS, Baart EB, Dekker M, Siezen A, de Rooij DG, de Boer P, te Riele H. Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. *Genes Dev.* 1999; 13:523–531. [PubMed: 10072381]
15. Kolas NK, Cohen PE. Novel and diverse functions of the DNA mismatch repair family in mammalian meiosis and recombination. *Cytogene. Genome Res.* 2004; 107:216–231.
16. Manhart CM, Alani E. Roles for MMR proteins in promoting meiotic crossing over. *DNA Repair (Amst.)*. 2016 in this issue.
17. Peltomaki P, Vasen H. Mutations associated with HNPCC predisposition—update of ICG-HNPCC/INSiGHT mutation database. *Dis. Markers.* 2004; 20:269–276. [PubMed: 15528792]
18. Chahwan R, Edelmann W, Scharff MD, Roa S. AIDing antibody diversity by error-prone mismatch repair. *Sem. Immunol.* 2012; 24:293–300.
19. Zanotti KJ, Gearhart PJ. Antibody diversification caused by abortive MMR and promiscuous DNA polymerases. 2016 in this issue.
20. Schmidt M, Pearson CE. MMR and trinucleotide repeat instability. 2016 in this issue.
21. Lin DP, Wang Y, Scherer SJ, Clark AB, Yang K, Avdievich E, Jin B, Werling U, Parris T, Kurihara N, Umar A, Kucherlapati R, Lipkin M, Kunkel TA, Edelmann W. An Msh2 point mutation uncouples DNA mismatch repair and apoptosis. *Cancer Res.* 2004; 64:517–522. [PubMed: 14744764]
22. Yang G, Scherer SJ, Shell SS, Yang K, Kim M, Lipkin M, Kucherlapati R, Kolodner RD, Edelmann W. Dominant effects of an Msh6 missense mutation on DNA repair and cancer susceptibility. *Cancer Cell.* 2004; 6:139–150. [PubMed: 15324697]
23. Avdievich E, Reiss C, Scherer SJ, Zhang Y, Maier SM, Jin B, Hou H Jr. Rosenwald A, Riedmiller H, Kucherlapati R, Cohen PE, Edelmann W, Kneitz B. Distinct effects of the recurrent Mlh1G67R mutation on MMR functions, cancer, and meiosis. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:4247–4252. [PubMed: 18337503]
24. van Oers JM, Roa S, Werling U, Liu Y, Genschel J, Hou H Jr. Sellers RS, Modrich P, Scharff MD, Edelmann W. PMS2 endonuclease activity has distinct biological functions and is essential for genome maintenance. *Proc. Natl. Acad. Sci. U. S. A.* 2010; 107:13384–13389. [PubMed: 20624957]
25. Kucherlapati MH, Lee K, Nguyen AA, Clark AB, Hou H Jr. Rosulek A, Li H, Yang K, Fan K, Lipkin M, Bronson RT, Jelicks L, Kunkel TA, Kucherlapati R, Edelmann W. An Msh2 conditional knockout mouse for studying intestinal cancer and testing anticancer agents. *Gastroenterology.* 2010; 138:993–1002. e1001. [PubMed: 19931261]
26. Taketo MM, Edelmann W. Mouse models of colon cancer. *Gastroenterology.* 2009; 136:780–798. [PubMed: 19263594]
27. Edelmann L, Edelmann W. Loss of DNA mismatch repair function and cancer predisposition in the mouse: animal models for human hereditary nonpolyposis colorectal cancer. *Am. J. Med. Genet. Part C.* 2004; 129C:91–99. [PubMed: 15264277]
28. Reitmaier AH, Cai JC, Bjerknes M, Redston M, Cheng H, Pind MT, Hay K, Mitri A, Bapat BV, Mak TW, Gallinger S. MSH2 deficiency contributes to accelerated APC-mediated intestinal tumorigenesis. *Cancer Res.* 1996; 56:2922–2926. [PubMed: 8674041]
29. Baker SM, Harris AC, Tsao JL, Flath TJ, Bronner CE, Gordon M, Shibata D, Liskay RM. Enhanced intestinal adenomatous polyp formation in Pms2^{-/-};Min mice. *Cancer Res.* 1998; 58:1087–1089. [PubMed: 9515784]
30. Kuraguchi M, Yang K, Wong E, Avdievich E, Fan K, Kolodner RD, Lipkin M, Brown AM, Kucherlapati R, Edelmann W. The distinct spectra of tumor-associated Apc mutations in mismatch repair-deficient Apc1638N mice define the roles of MSH3 and MSH6 in DNA repair and intestinal tumorigenesis. *Cancer Res.* 2001; 61:7934–7942. [PubMed: 11691815]
31. Toft NJ, Curtis LJ, Sansom OJ, Leitch AL, Wyllie AH, te Riele H, Arends MJ, Clarke AR. Heterozygosity for p53 promotes microsatellite instability and tumorigenesis on a Msh2 deficient background. *Oncogene.* 2002; 21:6299–6306. [PubMed: 12214270]
32. Young LC, Keuling AM, Lai R, Nation PN, Tron VA, Andrew SE. The associated contributions of p53 and the DNA mismatch repair protein Msh6 to spontaneous tumorigenesis. *Carcinogenesis.* 2007; 28:2131–2138. [PubMed: 17615258]

33. van Oers JM, Edwards Y, Chahwan R, Zhang W, Smith C, Pechuan X, Schaetzlein S, Jin B, Wang Y, Bergman A, Scharff MD, Edelmann W. The MutSbeta complex is a modulator of p53-driven tumorigenesis through its functions in both DNA double-strand break repair and mismatch repair. *Oncogene*. 2014; 33:3939–3946. [PubMed: 24013230]
34. Luo F, Brooks DG, Ye H, Hamoudi R, Poulgiannis G, Patek CE, Winton DJ, Arends MJ. Conditional expression of mutated K-ras accelerates intestinal tumorigenesis in Msh2-deficient mice. *Oncogene*. 2007; 26:4415–4427. [PubMed: 17297472]
35. Kovtun IV, Harris KJ, Jatoi A, Jevremovic D. Increased incidence of endometrioid tumors caused by aberrations in E-cadherin promoter of mismatch repair-deficient mice. *Carcinogenesis*. 2011; 32:1085–1092. [PubMed: 21551128]
36. Wang H, Douglas W, Lia M, Edelmann W, Kucherlapati R, Podsypanina K, Parsons R, Ellenson LH. DNA mismatch repair deficiency accelerates endometrial tumorigenesis in Pten heterozygous mice. *Am. J. Pathol.* 2002; 160:1481–1486. [PubMed: 11943731]
37. Wimmer K, Etzler J. Constitutional mismatch repair-deficiency syndrome: have we so far seen only the tip of an iceberg? *Hum. Genet.* 2008; 124:105–122. [PubMed: 18709565]
38. el Marjou F, Janssen KP, Chang BH, Li M, Hindie V, Chan L, Louvard D, Chambon P, Metzger D, Robine S. Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. *Genesis*. 2004; 39:186–193. [PubMed: 15282745]
39. McIlhatton MA, Tyler J, Kerepesi LA, Bocker-Edmonston T, Kucherlapati MH, Edelmann W, Kucherlapati R, Kopelovich L, Fishel R. Aspirin and low-dose nitric oxide-donating aspirin increase life span in a Lynch syndrome mouse model. *Cancer Prev. Res.* 2011; 4:684–693.
40. Rao CV, Reddy BS. NSAIDs and chemoprevention. *Curr. Cancer Drug Tar.* 2004; 4:29–42.
41. Corpet DE, Tache S. Most effective colon cancer chemopreventive agents in rats: a systematic review of aberrant crypt foci and tumor data, ranked by potency. *Nutr. Cancer*. 2002; 43:1–21. [PubMed: 12467130]
42. Rigas B, Kashfi K. Nitric-oxide-donating NSAIDs as agents for cancer prevention. *Trends Mol. Med.* 2004; 10:324–330. [PubMed: 15242680]
43. Rigas B. The use of nitric oxide-donating nonsteroidal anti-inflammatory drugs in the chemoprevention of colorectal neoplasia. *Curr. Opin. Gastroenterol.* 2007; 23:55–59. [PubMed: 17133086]
44. Ruschoff J, Wallinger S, Dietmaier W, Bocker T, Brockhoff G, Hofstadter F, Fishel R. Aspirin suppresses the mutator phenotype associated with hereditary nonpolyposis colorectal cancer by genetic selection. *Proc. Natl. Acad. Sci. U. S. A.* 1998; 95:11301–11306. [PubMed: 9736731]
45. McIlhatton MA, Tyler J, Burkholder S, Ruschoff J, Rigas B, Kopelovich L, Fishel R. Nitric oxide-donating aspirin derivatives suppress microsatellite instability in mismatch repair-deficient and hereditary nonpolyposis colorectal cancer cells. *Cancer Res.* 2007; 67:10966–10975. [PubMed: 18006842]
46. Sijmons RH, Hofstra RMW. Clinical aspects of hereditary MMR gene mutations. 2016 in this issue.
47. Kortum B, Campreggher C, Lang M, Khare V, Pinter M, Evstatiev R, Schmid G, Mittlböck M, Scharl T, Kucherlapati MH, Edelmann W, Gasche C. Mesalazine and thymoquinone attenuate intestinal tumour development in Msh2loxP/loxP Villin-Cre mice. *Gut*. 2014
48. Kucherlapati MH, Esfahani S, Habibollahi P, Wang J, Still ER, Bronson RT, Mahmood U, Kucherlapati RS. Genotype directed therapy in murine mismatch repair deficient tumors. *PLoS One*. 2013; 8:e68817. [PubMed: 23935891]
49. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene*. 2008; 27:5497–5510. [PubMed: 18794884]
50. Wojciechowicz K, Cantelli E, Van Gerwen B, Plug M, Van Der Wal A, Delzenne-Goette E, Song JY, De Vries S, Dekker M, Te Riele H. Temozolomide increases the number of mismatch repair-deficient intestinal crypts and accelerates tumorigenesis in a mouse model of Lynch syndrome. *Gastroenterology*. 2014; 147:1064–1072. e1065. [PubMed: 25088490]
51. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H. Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature*. 2007; 449:1003–1007. [PubMed: 17934449]

52. Stojic L, Mojas N, Cejka P, Di Pietro M, Ferrari S, Marra G, Jiricny J. Mismatch repair-dependent G2 checkpoint induced by low doses of SN1 type methylating agents requires the ATR kinase. *Genes Dev.* 2004; 18:1331–1344. [PubMed: 15175264]
53. Li Z, Pearlman AH, Hsieh P. MMR and the DNA Damage Response. 2016 in this issue.
54. Kovalenko M, Dragileva E, St. Claire J, Gillis T, Guide JR, New J, Dong H, Kucherlapati R, Kucherlapati MH, Ehrlich ME, Lee JM, Wheeler VC. Msh2 acts in medium-spiny striatal neurons as an enhancer of CAG instability and mutant huntingtin phenotypes in Huntington's disease knock-in mice. *PLoS One.* 2012; 7:e44273. [PubMed: 22970194]
55. Reiss C, Haneke T, Volker HU, Spahn M, Rosenwald A, Edelmann W, Kneitz B. Conditional inactivation of MLH1 in thymic and naïve T-cells in mice leads to a limited incidence of lymphoblastic T-cell lymphomas. *Leuk. Lymphoma.* 2010; 51:1875–1886. [PubMed: 20858091]
56. Lakso M, Pichel JG, Gorman JR, Sauer B, Okamoto Y, Lee E, Alt FW, Westphal H. Efficient in vivo manipulation of mouse genomic sequences at the zygote stage. *Proc. Natl. Acad. Sci. U. S. A.* 1996; 93:5860–5865. [PubMed: 8650183]
57. Orban PC, Chui D, Marth JD. Tissue- and site-specific DNA recombination in transgenic mice. *Proc. Natl. Acad. Sci. U. S. A.* 1992; 89:6861–6865. [PubMed: 1495975]
58. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B, et al. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science.* 1995; 268:1336–1338. [PubMed: 7761852]
59. Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. *Cancer Res.* 1995; 55:5548–5550. [PubMed: 7585632]
60. Mou H, Kennedy Z, Anderson DG, Yin H, Xue W. Precision cancer mouse models through genome editing with CRISPR-Cas9. *Genome Med.* 2015; 7:53. [PubMed: 26060510]
61. Powell AE, Wang Y, Li Y, Poulin EJ, Means AL, Washington MK, Higginbotham JN, Juchheim A, Prasad N, Levy SE, Guo Y, Shyr Y, Aronow BJ, Haigis KM, Franklin JL, Coffey RJ. The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. *Cell.* 2012; 149:146–158. [PubMed: 22464327]
62. Gu S, Chen D, Zhang JN, Lv X, Wang K, Duan LP, Nie Y, Wu XL. Bacterial community mapping of the mouse gastrointestinal tract. *PLoS One.* 2013; 8:e74957. [PubMed: 24116019]
63. Belcheva A, Irrazabal T, Robertson SJ, Streutker C, Maughan H, Rubino S, Moriyama EH, Copeland JK, Kumar S, Green B, Geddes K, Pezo RC, Navarre WW, Milosevic M, Wilson BC, Girardin SE, Wolever TM, Edelmann W, Guttman DS, Philpott DJ, Martin A. Gut microbial metabolism drives transformation of MSH2-deficient colon epithelial cells. *Cell.* 2014; 158:288–299. [PubMed: 25036629]
64. Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, Arcaroli JJ, Messersmith WA, Eckhardt SG. Patient-derived tumour xenografts as models for oncology drug development. *Nature Rev. Clin. Oncol.* 2012; 9:338–350. [PubMed: 22508028]

Table 1

MMR knockout and knock-in mouse lines.

Gene		Molecular Defects				Phenotypic Defects		Ref.
Knock-out		MSI	DDR	TRI	SHM/CSR	Cancer	Fertility	
<i>MutS</i>	<i>Msh2</i> ^{-/-}	+++	+++	+++	+++	+++	-	1,2,3,7
	<i>Msh3</i> ^{-/-}	+	-	+++	-	+	-	9,10
	<i>Msh6</i> ^{-/-}	+	+++	-	++	++	-	4
	<i>Msh4</i> ^{-/-}	-	NA	NA	NA	-	+++	12
	<i>Msh5</i> ^{-/-}	-	NA	NA	NA/-	-	+++	13,14
<i>MutL</i>	<i>Mlh1</i> ^{-/-}	+++	+++	+++	+++	+++	+++	5,6,8
	<i>Mlh3</i> ^{-/-}	++	+++	+++	-	+++	++	11
	<i>Pms1</i> ^{-/-}	+	NA	NA	NA	-	++	5
	<i>Pms2</i> ^{-/-}	+++	+++	+++	-/+++	+++	+++	5

Knock-in		MSI	DDR	TRI	SHM/CSR	Cancer	Fertility	
<i>MutS</i>	<i>Msh2</i> ^{G674A}	+++	-	+++	+++/-	+++	-	21
	<i>Msh2</i> ^{G674D}	+++	-	NA	NA	+++	-	25
	<i>Msh6</i> ^{T1217D}	+++	-	NA	++/-	+++	-	22
<i>MutL</i>	<i>Mlh1</i> ^{G67R}	+++	-	NA	-/+++	+++	+++	23
	<i>Pms2</i> ^{E702K}	+++	-	NA	-/+++	+++	-	24

Abbreviations: MSI, microsatellite instability; DDR, DNA damage response; TRI, triplet repeat instability; SHM, somatic hypermutation; CSR, class switch recombination.

Table 2

Conditional MMR knockout mouse lines.

Gene	Cre-Transgene	Target Tissue	Observed Phenotype	MSI	Ref.
Msh2 ^{loxP}	<i>Villin-Cre</i>	Small and large intestine	Small intestinal tumors	+++	25
Msh2 ^{loxP}	<i>Ella-Cre</i>	All tissues	T-cell lymphomas Small intestinal tumors	na	25
Msh2 ^{loxP}	<i>Lgr5-CreERT2</i>	Lgr5 expressing stem cells	Small intestinal tumors	na	50
Msh2 ^{loxP}	<i>Adenoviral-Cre</i>	Cre encoding adenovirus	Large intestinal tumors in combination with Apc ^{loxP} allele	na	48
Msh2 ^{loxP}	<i>D9-Cre</i>	Medium-spiny GABA-ergic projection neurons (MSNs)	Reduced HTT-CAG expansions and modified huntingtin phenotypes	na	54
Mlh1 ^{loxP}	<i>Ella-Cre</i>	All tissues	T-cell lymphomas Gastrointestinal tumors	+++	55
Mlh1 ^{loxP}	<i>Lck-Cre</i>	Lck expressing early stage thymocytes	T-cell lymphomas	++	55