An *Msh2* Conditional Knockout Mouse for Studying Intestinal Cancer and Testing Anticancer Agents

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BACKGROUND & AIMS: Mutations in the DNA mismatch repair (MMR) gene *MSH2* cause Lynch syndromes I and II and sporadic colorectal cancers. $Msh2^{null}$ mice predominantly develop lymphoma and do not accurately recapitulate the colorectal cancer phenotype. **METHODS:** We generated and examined mice with a conditional *Msh2* disruption ($Msh2^{LoxP}$), permitting tissue-specific gene inactivation. $ECMsh2^{LoxP/LoxP}$ mice carried an *EIIa-Cre* transgene, and $VCMsh2^{LoxP/LoxP}$ mice carried a *Villin-Cre* transgene. We combined the $VCMsh2^{LoxP}$ allele with either $Msh2^{\Delta 7null}$ ($VCMsh2^{LoxP/null}$) or $Msh2^{G674D}$ mutations ($VCMsh2^{LoxP/G674D}$) to create allelic phase mutants. These mice were given cisplatin or 5-fluorouracil/leucovorin and oxaliplatin (FOLFOX), and their tumors were measured by magnetic resonance imaging. **RESULTS:** Embryonic fibroblasts from $ECMsh2^{LoxP/LoxP}$ mice do not express MSH2 and are MMR deficient. Reverse transcription, polymerase chain reaction, and immunohistochemistry from $VCMsh2^{LoxP/LoxP}$ mice demonstrated specific loss of *Msh2* messenger RNA and protein from epithelial cells of the intestinal tract. Microsatellite instability was observed in all *VCMsh2* strains and limited to the intestinal mucosa. Resulting adenomas and adenocarcinomas had somatic truncation mutations to the *adenomatous polyposis coli* (*Apc*) gene. $VCMsh2^{LoxP/LoxP}$ mice did not develop lymphoma. Comparison of allelic phase tumors revealed significant differences in multiplicity and size. When treated with cisplatin or FOLFOX, tumor size was reduced in $VCMsh2^{LoxP/G674D}$ but not $VCMsh2^{LoxP/null}$ tumors. The apoptotic response to FOLFOX was partially sustained in the intestinal mucosa of $VCMsh2^{LoxP/G674D}$ animals. **CONCLUSIONS: $Msh2^{LoxP/LoxP}$ mice in combination with appropriate Cre recombinase transgenes have excellent potential for preclinical modeling of Lynch syndrome, MMR-deficient tumors of other tissue types, and use in drug development.**

Keywords: Mismatch Repair; *Msh2*; Tumorigenesis; Chemotherapy.

Approximately 150,000 new cases of colorectal cancer (CRC) are diagnosed per year in the United States. More than 50,000 patients die from it yearly. Generally classified into familial predisposition syndromes and sporadic cancers, several critical genes involved in both have been identified. Familial adenomatous polyposis is caused by mutations in the *APC* gene. Lynch syndromes I and II are caused by mutations in the mismatch repair (MMR) genes. *MSH2* was found to be one of the most commonly mutated MMR genes.[1–3] *Msh2* is necessary for repair of base-base as well as insertional deletion mismatches, and its absence results in increased mutation levels. Mice lacking MSH2 have a tumor predisposition phenotype.

To develop mouse models for Lynch syndrome, 3 $Msh2^{null}$ knockout mouse lines have been generated: 2 by targeted disruption of *Msh2* exon 12[4,5] and 1 by disruption of exon 7.[6] Homozygous mutant mice of all 3 $Msh2^{null}$ knockouts are MMR-deficient and display a highly increased predisposition to lymphoma. A proportion of older animals also develop intestinal neoplasms that are associated with *Apc* inactivation.[7] However, the predominance of the lymphoma phenotype has limited the use of these animals as preclinical models.

We report a novel conditional knockout mouse model for the tissue-specific inactivation of *Msh2* ($Msh2^{LoxP}$). In this model, MMR can be inactivated by *Cre-LoxP*-mediated inactivation of *Msh2* in different tissues by the expression of various *Cre-recombinase* transgenes. To constitutively inactivate MMR similar to $Msh2^{null}$ knockout mice, we mated $Msh2^{LoxP}$ mice with *EIIa-Cre* recombinase transgenic mice (termed $ECMsh2^{LoxP}$). To specifically inactivate MMR in the intestinal mucosa, we combined the $Msh2^{LoxP}$ allele with the *Villin-Cre* transgene ($VCMsh2^{LoxP}$).

Abbreviations used in this paper: 5-FU, 5-fluorouracil; FOLFOX, 5-fluorouracil/leucovorin and oxaliplatin; MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stable; PCR, polymerase chain reaction.

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ECMsh2$^{LoxP/LoxP}$ mice display complete MMR deficiency and have a cancer phenotype similar to *Msh2*^{null} knockout mice. In contrast, in *VCMsh2*$^{LoxP/LoxP}$ mice, MMR deficiency is limited to the intestinal epithelium, and the mice develop exclusively intestinal neoplasms. These data show that *Msh2*^{LoxP} mice in combination with specific *Cre* recombinase transgenes allow the tissue-specific inactivation of MMR and the development of suitable mouse models for Lynch syndrome.

We also demonstrate that it is possible to study allelic effects of different *Msh2* mutations on intestinal tumorigenesis in *VCMsh2*^{LoxP} mice by combining the *Msh2*^{LoxP} allele with either a Lynch syndrome-related missense mutation (*Msh2*^{G674D}) or an *Msh2*$^{\Delta 7null}$ mutation (*Msh2*^{null}). Tumors from these allelic phase mutants have also been tested for their response to 2 chemotherapeutic regimens, cisplatin and 5-fluorouracil/leucovorin and oxaliplatin (FOLFOX), and their growth recorded by magnetic resonance imaging (MRI). Although some tumors in *VCMsh2*$^{LoxP/null}$ mice were responsive to the 2 drugs, the majority was resistant to both chemotherapies. In contrast, almost all *VCMsh2*$^{LoxP/G674D}$ tumors were found to generally respond well to cisplatin and FOLFOX. The differences in responsiveness of tumors correlated with the absence of a significant DNA damage response in *VCMsh2*$^{LoxP/null}$ mice and partial retention of this response in *VCMsh2*$^{LoxP/G674D}$ mice.

Materials and Methods

Generation of Msh2^{LoxP} *Mice*

The targeting vector for the *Msh2*^{LoxP} mouse was made by recombinogenic methods.[8,9] An *Msh2* genomic fragment spanning exon 10 through intron 18 was amplified by polymerase chain reaction (PCR) from BAC clone 183K13 (RP-22 library), and subcloned into pBR322. A *LoxP* site was introduced into *Msh2* intron 12-13 followed by introduction of a *LoxP-FRT-PGKneo*^{r}*-FRT* selection cassette into *Msh2* intron 11-12. The vector was linearized and transfected into WW6 embryonic cells.[10] Male chimeric mice were generated and bred to C57Bl/6J females to generate *Msh2neo*$^{LoxP\text{-}FRTneo/+}$ F1 offspring. The *PGKneo*^{r} cassette was subsequently deleted in vivo by crossing *Msh2neo*$^{LoxP\text{-}FRTneo/+}$ heterozygotes to *FLP deleter* mice.[11] Offspring from these crosses were genotyped by PCR, Southern blot, and sequence analyses (data not shown) to confirm the integrity of the *Msh2*^{LoxP} allele. All procedures were in accordance with Institutional Animal Care and Use Committee Protocols.

Generation of Msh2^{LoxP} Cre Recombinase *Transgenic Mouse Lines*

Msh2$^{LoxP/+}$ mice were crossed with *EIIa-Cre recombinase* transgenic animals to generate *ECMsh2*$^{LoxP/+}$.[12] Heterozygotes were intercrossed to generate *ECMsh2*$^{LoxP/LoxP}$ mice.

Msh2$^{Lox/P/+}$ mice were mated with *B6;D2-Tg(Vil-Cre)* to create *VCMsh2*$^{LoxP/+}$ mice then intercrossed to create *VCMsh2*$^{LoxP/LoxP}$ mice.[13] *VCMsh2*$^{LoxP/+}$ mice were also mated to animals carrying the *Msh2*$^{\Delta 7}$ knockout allele (termed *Msh2*^{null})[6] and the *Msh2*^{G674D} knock-in allele. Offspring with 1 floxed *Msh2* allele and 1 mutant allele, *VCMsh2*$^{LoxP/null}$ or *VCMsh2*$^{LoxP/G674D}$, respectively, were obtained.

PCR Genotyping Msh2^{LoxP} *Mice*

Tail DNA was isolated using the DNAeasy kit (Qiagen, Valencia, CA) from 10-day-old mice. PCR primers used for genotyping were 184F (TACTGATGCGGGTTGAAGG), 184R (AACCAGAGCCTCAACTAGC), and 165R (GGCAAACTCCTCAAATCACG). Cycling conditions will be given upon request.

MMR Analysis in ECMsh2$^{LoxP/LoxP}$ *Mouse Embryonic Fibroblast Cell Lines*

Cytosolic extracts were prepared from mouse embryonic fibroblasts (MEF) cells as described in Thomas et al.[14] A heteroduplex G-G substrate was prepared, and DNA repair reactions were performed as previously described.[14,15]

Western Blotting and Immunohistochemistry

MEF cell extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto polyvinylidene fluoride membranes and probed with rabbit anti-mouse MSH2 polyclonal antibody (MSH2 N-20:sc494; Santa Cruz Biotechnology, Santa Cruz, CA), an *Msh6* monoclonal antibody (BD Biosciences, Franklin Lakes, NJ), or a glyceraldehyde-3-phosphate dehydrogenase monoclonal antibody (Ambion, Austin, TX). For immunohistochemical analysis, monoclonal antibodies directed against *Msh2* (N-20:sc494; Santa Cruz Biotechnology); *Apc* (GTX15270; GeneTex, Inc, Irvine, CA); and E-cadherin (24E10; Cell Signaling Technology, Danvers, MA) were used.

Generation of Kaplan–Meier Survival Plots

Prism 3.0 software (Graphpad Software, Inc, San Jose, CA) was used to calculate percent survival of animals.

Histopathologic Analysis

Mice were killed, and the gastrointestinal (GI) tract was removed, opened longitudinally, and fixed in 10% neutral-buffered formalin or Bouins solution. The number of tumors and their location were recorded under a dissecting microscope. For histologic analysis, tumors were embedded in paraffin, sectioned to 5 μm, and stained with H&E. Relative tumor size was measured using a Vernier Caliper with fine adjustment.

MSI Analysis

Genomic DNAs from tail, spleen, and flat mucosa were subjected to PCR amplification using a dilution