Genetic Manipulation of Homologous Recombination In Vivo Attenuates Intestinal Tumorigenesis

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Abstract

Although disruption of DNA repair capacity is unquestionably associated with cancer susceptibility in humans and model organisms, it remains unclear if the inherent tumor phenotypes of DNA repair deficiency syndromes can be regulated by manipulating DNA repair pathways. Loss-of-function mutations in BLM, a member of the RecQ helicase family, cause Bloom’s syndrome (BS), a rare, recessive genetic disorder that predisposes to many types of cancer. BLM functions in many aspects of DNA homeostasis, including the suppression of homologous recombination (HR) in somatic cells. We investigated whether BLM overexpression, in contrast with loss-of-function mutations, attenuated the intestinal tumor phenotype in model organisms. By crossing it onto both backgrounds. BLM-Tg decreased adenoma incidence in a dose-dependent manner in our ApcMin+ model of FAP, although levels of GIN were unaffected and concomitantly increased animal survival over 50%. It did not reduce intestinal tumorigenesis in ApcMin+/Msh2−/− mice. We used the pink-eyed unstable (p53+) mouse model to demonstrate that increasing BLM dosage in vivo lowered endogenous levels of HR by 2-fold. Our data suggest that attenuation of the Min phenotype is achieved through a direct effect of BLM-Tg on the HR repair pathway. These findings demonstrate that HR can be manipulated in vivo to modulate tumor formation at the organismal level. Our data suggest that lowering HR frequencies may have positive therapeutic outcomes in the context of specific hereditary cancer predisposition syndromes, exemplified by FAP. Cancer Prev Res; 8(7); 650–6. ©2015 AACR.

Introduction

The RecQ-like helicase family members WRN, BLM, and RECQL4 are linked to human genetic diseases characterized by genome instability, premature aging, and cancer predisposition (1, 2). BLM is a structure-specific helicase with 3′–5′ directionality which is involved in DNA double-strand break (DSB) repair (3). BLM functions in many aspects of DNA homeostasis, including the restart/repair of stalled and collapsed replication forks during DNA replication, repair of interstrand cross-links, and resolution of Holliday junctions (4–6). While it is accepted that BLM promotes resolution of Holliday intermediates by dissolution, thus suppressing crossovers, the role of BLM in homologous recombination (HR) is more complex than merely this late stage role. BLM also disrupts formation of RAD51-ssDNA filaments, leading to disruption of D-loops and thus suppression of HR at earlier stages (7). BLM-deficient cells have an approximate 10-fold increase in the number of sister chromatid exchanges (SCE) caused by inappropriate HR between sister chromatids at the S or G2 phases of the cell cycle (8). Bloom’s syndrome (BS) is a rare, recessive genetic disorder that is caused by loss-of-function mutations in the BLM gene (9). BS patients have a predisposition to develop many types of cancer, presenting with a mean age of 24 years at diagnosis.

Several lines of evidence indicate that BLM dosage is critical for controlling the onset of tumorigenesis in mice. Mouse models demonstrate that chromosomal instability directly correlates with the levels of BLM; as BLM decreases, genomic instability and tumor burden increase (10–12). In addition, haploinsufficiency for BLM on the C57Bl-6 J ApcMin/+ background increases spontaneous adenoma formation and dysplasia (11). Genomic analyses of ApcMin+/Blm−/− mice indicate that increased adenoma formation is a direct consequence of reduced BLM levels and an increase in somatic recombination. This, in turn, facilitates loss of the wild-type Apc allele by interchromosomal recombination and leads to increased loss-of-heterozygosity (LOH). In humans, similar conclusions have been reached about carriers of specific BLM mutations and their resulting susceptibilities to colorectal cancer (13).

Familial adenomatous polyposis coli (FAP) is a hereditary human cancer predisposition syndrome characterized by the growth of hundreds to thousands of small adenomatous polyps throughout the colon (reviewed in ref. 14). FAP requires the inheritance of a mutated allele of the adenomatous polyposis coli (APC) gene (15). Depending on the nature of the inherited germline allele, second-hit inactivation of the wild-type allele is achieved either by LOH of the APC locus or intragenic mutation of...
the APC gene (16). APC is also inactivated by intragenic mutation in 70% to 80% of individuals with sporadic colorectal cancer (14).

Given the demonstrated relationship between low or absent expression levels of BLM and cancer, we investigated whether constitutive overexpression of BLM modulated adenoma formation in the Apc<sup>Cmin</sup>/ mouse model of FAP (17). We hypothesized that if halving Blm gene dosage increased predisposition to tumorigenesis, overexpression would conversely decrease tumor susceptibility. Understanding the mechanism by which BLM attenuates tumor susceptibility will aid our fundamental understanding of its roles in maintaining genomic stability and suggest new strategies for cancer prevention involving direct regulation of DNA repair pathways. Our data suggest that levels of specific repair proteins may be titrated to achieve positive therapeutic outcomes in the context of specific hereditary cancer syndromes, exemplified by FAP.

Materials and Methods

Generation of the transgenic mouse line expressing BLM

The procedure is outlined below. The human BLM cDNA was amplified from plasmid pHK1 and cloned into the TA-vector (Promega). The construct was sequenced and verified. A 0.44 kb fragment, corresponding to the phosphoglycerate kinase (PGK) promoter, was cloned in to the 5′ end of the BLM cDNA. The PGK-BLM cDNA fragment was then cloned into the vector pOPRSVICat, containing a synthetic intron and the HSV thymidine kinase (TK) polyadenylation signal. The PGK-BLM cDNA-p(A) fragment was removed by restriction digestion from the vector, purified, and introduced into C57Bl-6J oocytes by pronuclear injection. Founder lines were generated and initially screened for the presence of the transgene by Southern blotting. A probe corresponding to the 3′ end of the BLM cDNA was used. Once germline transmission had been established, transgenic animals were routinely identified using PCR.

Generation of mice lines and genotyping

Apc<sup>Minc</sup>/ mice were originally obtained from The Jackson Laboratories (stock: 002020; strain: C57Bl/6J-Apc<sup>Cmin</sup>/J). Blm<sup>Cmin</sup>/ mice have been previously reported (11). The pink-eyed unstable mouse model (18) was a gift from Dr. A.J.R. Bishop, University of Texas Health Science Center at San Antonio. Heterozygous Msh2<sup>−/−</sup> mice (19) were obtained from the laboratory of Dr. Winfried Edelmann, Albert Einstein College of Medicine, New York. Apc<sup>Minc</sup>/, Blm<sup>Cmin</sup>/, Msh2<sup>−/−</sup>, and BLM-Tg lines were intercrossed to generate mice of the required genotypes, all on congenic C57Bl/6J backgrounds. Animals were bred in a barrier facility and were maintained according to the NIH animal care and use guidelines. Both male and female mice were included in experimental study groups for subsequent analyses. All experiments involving animals received prior approval from the OSU Institutional Animal Care and Use Committee (IACUC), OLAW Assurance #A3261-01. Animal work was conducted in accordance with the established criteria of our animal use protocol, #2012A00000021, approved by IACUC. Mice were euthanized at 16 weeks by CO₂ inhalation, followed by cervical dislocation. Intestines were removed, rinsed in PBS, and cut into sections corresponding to the duodenum, jejunum, ileum, cecum, and colon (large intestine). Tissues were opened longitudinally, washed twice in PBS, and examined under a dissecting microscope. Gross numbers of adenomas/intestinal polyps were counted. Tissues were fixed in 10% formalin overnight, blocked in paraffin, sectioned, and stained with hematoxylin and eosin. Slides were evaluated to confirm tumors and determine gastrointestinal neoplasia (GIN). The criteria for GIN are as described previously (20).

Animal dissection

Mice were euthanized at 16 weeks by CO₂ inhalation, followed by cervical dislocation. Intestines were removed, rinsed in PBS, and cut into sections corresponding to the duodenum, jejunum, ileum, cecum, and colon (large intestine). Tissues were opened longitudinally, washed twice in PBS, and examined under a dissecting microscope. Gross numbers of adenomas/intestinal polyps were counted. Tissues were fixed in 10% formalin overnight, blocked in paraffin, sectioned, and stained with hematoxylin and eosin. Slides were evaluated to confirm tumors and determine gastrointestinal neoplasia (GIN). The criteria for GIN are as described previously (20).

Dissection of the RPE/scoring reversion events

These have been described previously (18).

Statistical analysis

All statistical analyses were performed with Prism 6.1.

Results

BLM expression rescues the embryonic lethality of the Blm<sup>Cmin</sup> knockout mouse

A transgenic mouse was generated that expressed human BLM on a congenic C57Bl/6J background, hereafter designated BLM-Tg. qPCR was used to establish the allelic status of the BLM transgene in sibling mice bred from the established colony (Supplementary Fig. S1). Protein expression levels correlated with the allelic status of the transgene; homozygous (BLM<sup>+/-</sup>) mice expressed approximately twice as much BLM as hemizygous (BLM<sup>+/-</sup>) mice. The BLM-Tg also rescued the embryonic lethality of the conventional Blm<sup>Cmin</sup> knockout mouse (11). Mating of BLM<sup>+/-</sup>/Blm<sup>Cmin</sup> animals yielded BLM<sup>+/-</sup>/Blm<sup>Cmin</sup> mice at normal Mendelian ratios.
Long-term expression of BLM for over 24 months had no apparent deleterious effects on animal health. BLM-Tg mice (n = 3) were subjected to full necropsy and phenotypic analyses. No overt phenotypic abnormalities were detected; only age- or strain-related lesions were observed (data not shown). Tumors were not apparent in any tissue at necropsy, and there was no evidence of architectural destruction or invasion which would suggest neoplastic transformation.

Transgenic expression of BLM reduces adenoma numbers in ApcMin/+ mice

The BLM-Tg line was crossed to ApcMin/+ mice, also on a C57Bl/6J background. Animals were maintained in a barrier environment, and four different groups of litter mates were generated: (i) ApcMin/+; BlmCin/-, (ii) ApcMin/+; BLM+/T, (iii) ApcMin/+; BLM+/T, and (iv) BLM+/T and/or wild-type mice. Mice were euthanized after 16 weeks and gross intestinal adenomas were counted with a dissecting microscope to differentiate adenomas in the duodenum, jejunum, ileum, cecum, and colon. Pathology studies confirmed and categorized adenomas as low- or high-grade and scored GIN according to the criteria of Boivin and colleagues as an early marker for neoplasia (20).

BLM-Tg significantly reduced gross numbers of intestinal adenomas in ApcMin/+ litter mates (Fig. 1A; Table 1). ApcMin/+ mice developed a mean of 46.44 ± 11.42 adenomas, compared with 24.44 ± 8.22 for ApcMin/+; BLM+/T and 17.24 ± 8.26 for ApcMin/+; BLM+/T mice (P < 0.0001 for both groups; Mann-Whitney U test). Tumor attenuation of the Min phenotype was dose-dependent; ApcMin/+; BLM+/T mice developed significantly less adenomas than ApcMin/+; BLM+/T mice (P = 0.016; Mann-Whitney U test). Suppression of adenoma formation by BLM-Tg was most evident in the jejunal and ileal segments of the gastrointestinal tract (Fig. 1B), which is not surprising, as these regions comprise the predominant site of adenoma formation in the ApcMin/+ mode (17). There was no difference in mean adenoma numbers between male and female mice. Adenomas were not observed in control groups of BLM-Tg or wild-type mice.

Despite the reduction in total adenomas by BLM-Tg, there was no significant reduction in the levels of GIN between groups (Fig. 1A), although the trend was suggestive. Compare means of 4.69 ± 4.22 for ApcMin/+ to 2.71 ± 2.05 for ApcMin/+; BLM+/T, and 3.00 ± 3.33 for ApcMin/+; BLM+/T mice. However, these sample sizes confer only a 30% power to detect differences between means of 1.56 with a significance level (α) of 0.05 (two-tailed).

We would require 80 or more animals in each group to have 80% power to detect a difference between means of 1.34 with a significance level (α) of 0.05 (two-tailed).

BLM-Tg increases survival in the ApcMin/+ mouse model of intestinal tumorigenesis

Survival of ApcMin/+ mice was increased by the BLM transgene (Fig. 2). Median survival for ApcMin/+ mice was 137 days compared with 196 days for ApcMin/+; BLM+/T mice and 221 days for ApcMin/+; BLM+/T mice (P = 0.0001; log-rank test for both groups). Survival times between ApcMin/+; BLM+/T and ApcMin/+; BLM+/T mice were also significantly different (P = 0.0053; log-rank test), indicating a dose-dependent effect of the transgene. Although BLM-Tg significantly extended survival of ApcMin/+ mice, most likely due to reduced intestinal tumor burden, they died earlier than BLM-Tg litter mate controls, which are all still alive after 350 days (Fig. 2). It is not surprising that BLM overexpression was unable to mitigate the persistent intestinal tumorigenesis that is characteristic of the Min phenotype.

Transgenic BLM does not affect tumor etiology

While genetic background modifies tumor penetrance in the ApcMin/+ model (21), adenomas rarely progress to carcinoma (22). Comparative histopathologic evaluation of intestinal lesions from ApcMin/+; BLM+/T and ApcMin/+; BLM+/T mice was consistent with the well-characterized etiology of tumor development in the ApcMin/+ model (Fig. 3A–E; refs. 17, 20). Most adenomas were classified as low-grade; no adenocarcinomas were observed. Although more high-grade adenomas developed in ApcMin/+ (10/282) compared with ApcMin/+; BLM+/T (1/148) or ApcMin/+; BLM+/T (1/101) mice, the numbers of adenomas assessed were insufficient to determine significance.

To more fully understand the nuanced effects of Blm/BLM dosage on tumor initiation versus progression in the ApcMin/+ intestine, we employed the BlmCin/- knockout mouse model (11). We examined tumors from aged ApcMin/+; BlmCin/- mice (n = 20) to investigate how Blm haploinsufficiency affected tumor progression within the same intestinal model. Adenomas with high-grade dysplasia (Fig. 3G) were observed in 15 ApcMin/+; BlmCin/- mice, and 4 mice also developed adenocarcinomas that invaded the serosa (Fig. 3H). There was a statistically significant increase in both carcinomas and high-grade dysplasia in the ApcMin/+; BlmCin/- mice (P < 0.0001). These data suggest that Blm/BLM dosage can modulate tumor burden and progression in the
mouse. Although BLM-Tg most likely suppresses adenoma formation by inhibiting progression from GIN to intestinal adenoma, it may also have subtle effects on the initiation of dysplasia that this study is not sufficiently powered to reveal.

Transgenic BLM does not attenuate intestinal tumor numbers in mismatch repair–deficient ApcMin-/- mice

A well-documented aspect of the ApcMin-/- phenotype on the C57Bl-6J background is that inactivation of the wild-type Apc locus by LOH is essential for subsequent intestinal adenoma formation (17). Given the known roles of Blm/BLM in HR, we investigated if our transgenic BLM could likewise reduce numbers of intestinal adenomas in an ApcMin-/- model that was not dependent on LOH as a second-hit mechanism of inactivation. It has been observed that when BLM-/- is combined with mismatch repair (MMR)–null mouse models, either Mlh1-/- or Msh2-/-, the mechanism of Apc inactivation changes from that of LOH to intrinsic mutation. Analyses of adenomas from Mlh1-/-; ApcMin-/- and Msh2-/-; ApcMin-/- mice demonstrated intragenic (point) mutation of the wild-type Apc allele in 81% and 85% of cases, respectively (23, 24). This shift is most likely due to the characteristic mutator phenotypes inherent to these specific models of MMR deficiency. Analyses of respective control groups of ApcMin-/- mice were combined to confirm LOH in all of the adenomas examined (23, 24).

We combined our ApcMin-/-; BLM-Tg model with the Msh2-null allele, Msh22N/- (19), and generated all possible combinations of ApcMin-/-; BLM+/-; Msh22N/- animals. Mice transgenic for BLM are collectively represented as BLM+/-; they were not stratified as BLM+/- and BLM-/- for this analysis. The increased intestinal tumor burdens that developed in cohorts of ApcMin-/-; BLM+/-; Msh2-/- and ApcMin-/-; Msh2-/- mice resulted in a severe decline in animal survival, compelling necropsy of these groups at 11 to 12 weeks. Control groups of ApcMin-/-; BLM-/- and ApcMin-/-; BLM+/-; Msh2-/- mice were analyzed at the standard 16- to 17-week time point. Intestinal adenoma counts for the control groups were: ApcMin-/-; BLM-/-, 49.5 ± 12.0; ApcMin-/-; BLM+/-, 23.7 ± 8.9; and ApcMin-/-; BLM+/-; Msh2-/-, 24.1 ± 10.3 (Fig. 4). Adenoma numbers for ApcMin-/- and ApcMin-/-; BLM+/- groups are similar to those of Fig. 1, indicating that introduction of the Msh22N/- allele onto the ApcMin-/-; BLM+/- background did not alter tumor susceptibility in the intestine. It is also evident that heterozygosity for Msh2 does not perturb adenoma development in the intestines of ApcMin-/- mice which is consistent with published data (24, 25). The ApcMin-/-; BLM+/-; Msh2-/- and ApcMin-/-; Msh2-/- groups presented comparable adenoma counts of 272.0 ± 28.5 and 288.2 ± 32.3, respectively (Fig. 4). This striking increase in intestinal adenomas is a characteristic feature of MMR-deficient ApcMin-/- mouse phenotypes (23–26). Our data suggest that when Apc is inactivated by intragenic mutagenesis in this model, rather than by LOH, transgenic BLM has no significant effect on the outcome of intestinal adenoma development.

Overexpression of BLM modulates DNA repair by downregulating HR

Given the known role of BLM in maintaining genomic integrity (1, 2), we hypothesized that BLM-Tg ameliorated tumorigenesis in ApcMin-/- mice by suppressing HR. To investigate this possible mechanism, BLM-Tg mice were crossed to the pink-eyed unstable (pun/un) mouse model which measures in vivo HR levels. In this model, a somatic intrachromosomal deletion within the mouse p gene restores melanin production in the otherwise transparent cells of the retinal pigment epithelium (RPE), generating a clone of brown cells, or eye-spot (18). This deletion event occurs spontaneously and is dependent on HR. Thus, the number of RPE eye-spots represents an in vivo read-out for HR. Cohorts of pun/un; BLM+/- mice were euthanized after 20 days, and RPE eye-spots were counted. Representative examples are shown in Fig. 5A and B. In contrast with ApcMin-/- mice, BLM-Tg dosage does not appear to be a critical modifier in the pun/un model; differences in eye-spot numbers for pun/un; BLM+/- and pun/un; BLM-/- were not significant. Mitotic cell division is essentially complete in the RPE by P20 (27), so it is possible that the restricted developmental window of this tissue is insufficient to highlight subtle differences in HR between pun/un; BLM+/- and pun/un; BLM-/- genotypes. Therefore, these mice were combined and analyzed as one group (Fig. 5C). The number of eye-spots in control pun/un; BLM+/- mice of 6.9 ± 3.2 is comparable with previous reports (18, 28), whereas BLM overexpression reduces HR 2-fold, resulting in 3.4 ± 1.9 eye-spots per RPE in pun/un; BLM+/- mice (P < 0.0001; Mann–Whitney U test). Although it was not possible to directly measure HR in the intestinal epithelial compartment of

Table 1. Mean number of intestinal adenomas per region in ApcMin-/-, ApcMin-/-; BLM-/- and ApcMin-/-; BLM+/- mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>du.</th>
<th>je.</th>
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<th>co.</th>
<th>co.</th>
<th>All tumors</th>
</tr>
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<tbody>
<tr>
<td>ApcMin-/-</td>
<td>27</td>
<td>7.84</td>
<td>13.56</td>
<td>23.1</td>
<td>0.44</td>
<td>1.37</td>
<td>46.44</td>
</tr>
<tr>
<td>ApcMin-/-; BLM-/-</td>
<td>18</td>
<td>4.00</td>
<td>7.44</td>
<td>11.83</td>
<td>0.28</td>
<td>0.94</td>
<td>24.44</td>
</tr>
<tr>
<td>ApcMin-/-; BLM+/-</td>
<td>17</td>
<td>3.30</td>
<td>4.12</td>
<td>8.47</td>
<td>0.29</td>
<td>1.06</td>
<td>17.24</td>
</tr>
<tr>
<td>BLM-Tg &amp; wild-type</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tr>
</tbody>
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Abbreviations: ce., cecum; co., colon; du., duodenum; il., ileum; je., jejunum.


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Our intestinal model, our data suggest that the observed reduction in adenoma numbers is also due to modulation of HR by BLM-Tg.

**Discussion**

Rescue of the Blnm<sup>Cim/Cim</sup> embryonic lethal phenotype by BLM-Tg indicates that expression of this human ortholog is sufficiently regulated, within the physiologic context of our model, to direct normal development in Blnm-null mice. Our findings that BLM-Tg reduces adenoma numbers in the Apc<sup>Min</sup>/+ mouse model of intestinal tumorigenesis (Fig. 1) are consistent with the known role of BLM in HR and its requirement for maintaining genomic integrity (2, 11). Moreover, BLM-Tg expression suppressed adenoma formation in the Apc<sup>Min</sup>-/+ model by a dose-dependent mechanism (Fig. 1), suggesting that augmentation of the HR pathway may be a viable objective for attenuating tumor suppression in such in vivo milieus, notably the intestinal compartment. Reduction of intestinal tumor burden results in accompanying dose-dependent increases in median survival times for Apc<sup>Min</sup>-/−BLM<sup>+/+</sup> and Apc<sup>Min</sup>-/−BLM<sup>++/++</sup> mice (Fig. 2).

Adenomas that developed in Apc<sup>Min</sup>-/−BLM<sup>+/+</sup> mice were pathologically identical to those from Apc<sup>Min</sup>-/− animals (Fig. 3), indicating that the BLM-Tg did not affect tumor origin or skew tumor spectrum in this intestinal model. Furthermore, long-term expression does not adversely alter the wild-type phenotype of BLM<sup>+/+</sup> and BLM<sup>++/++</sup> mice. Mice (n = 12) have now been aged for over 24 months without deleterious effects on survival or overt signs of tumorigenesis. This is somewhat surprising because data from the p<sup>mm</sup> model indicate that, mechanistically, BLM-Tg overexpression downregulates HR 2-fold (Fig. 5). However, the crucial function of HR in mediating error-free repair of DNA damage, it is possible that aging BLM-Tg mice may ultimately prove more susceptible to spontaneous tumorigenesis. Perhaps if aged mice were challenged with radiomimetic agents, the inflicted DNA damage might exceed their (lowered) threshold for HR repair, consequently resulting in an increased susceptibility to tumorigenesis. Transgenic mouse lines have also been generated for Wtm, another member of the RecQ helicase family (29). Although the wild-type Wtm transgene has yet to be tested in models of tumorigenesis, it has no effect on HR in RPE cells of the p<sup>mm</sup> mouse (30).

The elevated BLM levels observed in our Apc<sup>Mim</sup>-/−BLM-Tg model most likely reduce adenoma formation through suppression of HR, thus maintaining heterozygosity of the wild-type Apc allele. We used the p<sup>mm</sup> and MMR-deficient Msh2−/− models to further investigate the mechanism of tumor reduction in the Apc<sup>Mim</sup>-/−BLM<sup>+</sup> mice, rather than attempting to correlate a reduction in surrogate markers of HR, such as Rad51 foci, with reduction in adenoma burden. These genetic models presented a more relevant system for assessing the effects of the BLM-Tg on HR in vivo. A 2-fold reduction of eye-spots in RPE cells of p<sup>mim</sup>-/−BLM<sup>+</sup> mice (Fig. 5) suggests that BLM-Tg directly modulates HR in this tissue. Our interpretation of the observed reduction in adenoma numbers in the Apc<sup>Mim</sup>-/−BLM<sup>+</sup> model is that it is caused by the effect of BLM-Tg on HR in the intestinal epithelia, thus suppressing LOH of the wild-type Apc allele. This conclusion is supported by the data from the Apc<sup>Mim</sup>-/−BLM<sup>+</sup>;Msh2−/− mice. When Apc is activated by point mutation, due to innate MMR deficiency, thus precluding the requirement for inactivation of the wild-type Apc allele by LOH, there are no observable differences in intestinal adenoma numbers between Apc<sup>Mim</sup>-/−BLM<sup>+</sup>;Msh2−/−

**Figure 3.** Histopathology of intestinal tumor development associated with Blm/BLM in Apc<sup>Mim</sup>-/+ mice. A-F, although BLM-Tg reduces adenoma numbers on the Apc<sup>Mim</sup>-/+ background, it does not alter the underlying histopathology of emerging adenomas: A, Apc<sup>Mim</sup>-/+ jejunum, boxed area highlights GIN; B, Apc<sup>Mim</sup>-/+;BLM<sup>++/++</sup> jejunum, boxed area highlights GIN; C, Apc<sup>Mim</sup>-/+;BLM<sup>++/++</sup> cecum, adenoma; D, Apc<sup>Mim</sup>-/+;BLM<sup>++/++</sup> colon, adenoma; E, Apc<sup>Mim</sup>-/+;BLM<sup>++/++</sup> jejunum, adenoma; F, Apc<sup>Mim</sup>-/+;BLM<sup>++/++</sup> ileum, adenoma. G-H, Blm haploinsufficiency drives tumor development and accelerates the development of intestinal adenomas with high-grade dysplasia and invasive carcinoma: G, Apc<sup>Mim</sup>-/+;Blnm<sup>Cim/Cim</sup>, adenoma with high-grade dysplasia; H, Apc<sup>Mim</sup>-/+;Blnm<sup>Cim/Cim</sup>, invasive adenocarcinoma. All sections were stained with hematoxylin and eosin. Scale bars each represent 100 μm.

**Figure 4.** Transgenic BLM does not attenuate intestinal tumor numbers in MMR-deficient Apc<sup>Mim</sup>-/+ mice. Box and whiskers plot showing total adenoma numbers arising in Apc<sup>Mim</sup>-/+ and Apc<sup>Mim</sup>-/+;BLM<sup>+</sup> mice on Msh2-deficient backgrounds. Significant values are indicated above each bracket: ****, P < 0.0001; NS, not significant. Statistical analyses were performed using the Mann-Whitney U test.
Modulation of BLM Suppresses Intestinal Adenoma Formation

Figure 5.
Expression of BLM-Tg downregulates HR in the p<sup>−/−</sup> mouse RPE. Eye-spots consist of pigmented clones of revertant RPE cells in which melanin production has been restored by an intrachromosomal recombination event. They are easily visible on the transparent RPE background. A and B, representative examples of eye-spots in p<sup>−/−</sup>/BLM<sup>Tg</sup> mice. These are comprised of: A, single or simple multiples of revertant RPE cells although (B) sometimes rarer, more complex configurations are observed. Eye-spots separated by a single nonrevertant (clear) cell are scored as a single unit.

C, BLM-Tg suppresses HR in p<sup>−/−</sup> RPE cells. p<sup>−/−</sup>/BLM<sup>Tg</sup> and p<sup>−/−</sup>/BLM<sup>Tg/</sup> mice have been combined and represented as a single group. p<sup>−/−</sup>/BLM<sup>Tg</sup>, because analyses demonstrated both genotypes are similarly effective in this model. Mean ± SD are shown for each group. p<sup>−/−</sup>/RPE mice have 6.9 ± 3.2 eye-spots, n = 277, 40 RPE; p<sup>−/−</sup>/BLM<sup>Tg</sup> mice have 3.4 ± 1.9 eye-spots, n = 192, P = 0.0001. Scale bars each represent 50 μm.

...and Apc<sup>−/−</sup>/Msh2<sup>−/−</sup> mice (Fig. 4). If BLM-Tg was affecting adenoma formation through other mechanisms unrelated to HR, one would predict that adenoma numbers should still differ between these two models. This is not the case.

Consistent with this model, levels of GIN are also reduced between Apc<sup>−/−</sup> and Apc<sup>−/−</sup>/BLM-Tg genotypes, although they do not meet statistical significance (Fig. 1A). If correct, BLM-Tg would act before the potential onset of GIN, since GIN pathologically precedes adenomas in the Apc<sup>−/−</sup> model and since LOH of the wild-type Apc allele is a fundamental requirement for GIN development on a C57Bl/6J background [31]. In addition to perturbing LOH, and hence subsequent levels of GIN and adenomas, it is possible that BLM-Tg may selectively target neoplastic cells after they have emerged as larger lesions on the Apc<sup>−/−</sup> background. It remains unclear whether elevating BLM levels could eliminate, perhaps through apoptosis, single or small populations of nascent cells that have acquired two mutant alleles of Apc.

Data from our Apc<sup>−/−</sup>/BLM<sup>Tg</sup> models suggest that levels of specific DNA repair proteins may be titrated to achieve positive therapeutic outcomes in the context of specific hereditary cancer syndromes, exemplified by FAP. There are many inhibitors readily available that target the HR repair pathway and downregulate HR (reviewed in refs. 32, 33). However, we are unaware of any small-molecule inhibitors, or other reagents, that effect upregulation of endogenous BLM and thus, might prove more suitable for therapeutic applications. With this in mind, we are investigating expression profiles of our BLM-Tg model to determine if there are other molecular targets that are more amenable to therapeutic modulation.

Our study establishes that BLM expression can be effectively manipulated in a mouse model of intestinal tumorigenesis to successfully attenuate the tumor phenotype. We show that overexpression of human BLM reduces intestinal adenoma formation in the Apc<sup>−/−</sup> mouse model and propose that the mechanism is through downregulation of the HR repair pathway. This presents the potential to explore new avenues for intestinal tumor prevention by controlling levels of BLM expression or other genes of the DNA repair pathways. Our data demonstrate the therapeutic potential of titrating levels of specific DNA repair proteins that may be protective against tumor formation and suggest that this approach of modulating fundamental DNA repair pathways may be a viable pharmacologic strategy for cancer prevention.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.A. McIlhatton, K. Murnan, G.P. Boivin, J. Groden
Writing, review, and/or revision of the manuscript: M.A. McIlhatton, G.P. Boivin, J. Groden
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.M. Croce
Study supervision: M.A. McIlhatton, J. Groden
Other (generated BLM transgene for the BLM transgenic mouse used in the study): D. Carson

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Genetic Manipulation of Homologous Recombination In Vivo Attenuates Intestinal Tumorigenesis

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