nal for macrophage activation to a trigger of rapid cell death. Selective killing of activated macrophages prevents the secretion of chemokines and cytokines that alert the remainder of the immune system to the presence of the pathogen. This may explain why anthrax infections proceed undetected until the terminal stage, when vast bacteremia occurs. Future research should focus on the balance between macrophage activation and apoptosis, as it seems to play a key role in the pathogenesis of anthrax and other deadly infections.

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REPORTS

Enhanced Tumor Formation in Mice Heterozygous for *Blm* Mutation

Kathleen Heppner Goss,[1,2]* Mary A. Risinger,[1] Jennifer J. Kordich,[1,2] Maureen M. Sanz,[4] Joel E. Straughen,[1] Lisa E. Slovek,[1] Anthony J. Capobianco,[1] James German,[4] Gregory P. Boivin,[3] Joanna Groden[1,2]†

Persons with the autosomal recessive disorder Bloom syndrome are predisposed to cancers of many types due to loss-of-function mutations in the *BLM* gene, which encodes a recQ-like helicase. Here we show that mice heterozygous for a targeted null mutation of *Blm*, the murine homolog of *BLM*, develop lymphoma earlier than wild-type littermates in response to challenge with murine leukemia virus and develop twice the number of intestinal tumors when crossed with mice carrying a mutation in the *Apc* tumor suppressor. These observations indicate that *Blm* is a modifier of tumor formation in the mouse and that *Blm* haploinsufficiency is associated with tumor predisposition, a finding with important implications for cancer risk in humans.

Bloom syndrome (BS) is characterized by small stature, immunodeficiency, male infertility, and predisposition to cancer of many tissue types (*1*). Cells from persons with BS show increased somatic recombination, chromosome breakage, and site-specific mutations (*1–3*). The BS locus, *BLM*, encodes BLM, an adenosine triphosphate–dependent, 3′-5′ helicase with homology to the recQ DEXH-box–containing DNA and RNA helicases (*4*); loss of BLM helicase activity is responsible for the genomic instability of BS cells (*5*, *6*). BLM resolves Holliday junctions, suppresses recombination in vitro, and is required for the fidelity of DNA double-strand break repair (*7–9*).

We have used gene targeting by homologous recombination to disrupt the mouse *Blm* gene to simulate BLM^{Ash}, a BS-causing mutant allele of *BLM* carried by approximately 1% of Ashkenazi Jews (*4*, *10*, *11*). BLM^{Ash} contains a frameshift mutation in exon 10 of *BLM* that results in premature translation termination (*4*). In contrast to work with two mouse models of BS previously reported (*12*, *13*), we used a gene-targeting construct in which exons 10, 11, and 12 of *Blm* were replaced with a hypoxanthine phosphoribosyltransferase *(Hprt)* cassette (fig. S1A). Germ line transmission of this mutant allele, Blm^{Cin}, followed blastocyst injection of targeted embryonic stem cells to generate heterozygous mice (fig. S1B). Crosses to generate $Blm^{Cin/Cin}$ mice were unsuccessful, indicating that homozygous disruption of *Blm* results in embryonic lethality (*14*). Western blots of protein lysates from $Blm^{+/+}$ testes, an abundant source of *Blm* RNA and BLM protein (*12*, *13*), displayed a specific band of approximately 190 kD when probed with a COOH-terminal antiserum to BLM (fig. S1C). Lysates from $Blm^{Cin/+}$ testes had an approximately 50% reduction in BLM in comparison to $Blm^{+/+}$ testes. Lysates of heterozygous tissues were similarly evaluated with an NH_2-terminal antibody to BLM and revealed no smaller immunoreactive proteins (fig. S1D). This reduction of full-length BLM and the absence of truncated BLM in $Blm^{Cin/+}$ mice confirm that we had generated a null allele. This allele allowed us to examine the biological consequences of *Blm* haploinsufficiency, that is, a reduction in wild-type (WT) *Blm* gene dosage and its gene product.

BS somatic cells exhibit increases in chromosome aberrations, sister chromatid exchanges (SCEs), homologous chromatid interchanges, and micronuclei that are a consequence of chromosome breakage (*1*, *15*, *16*). Although the cytogenetic analysis of somatic cells from human *BLM* heterozygotes remains to be completed (*17*), spermatozoa from two of three obligate heterozygotes have been shown to display excess numbers of chromosome breaks and rearrangements (*18*). To learn whether *Blm* haploinsufficiency affects genomic stability, we cultured primary lung fibroblasts from

[1]Department of Molecular Genetics, Biochemistry, and Microbiology; [2]Howard Hughes Medical Institute; [3]Department of Pathology and Laboratory Medicine; University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267, USA. [4]Department of Pediatrics, Weill Medical College of Cornell University, New York, NY 10021, USA.

*Present address: Department of Surgery, Division of Epithelial Pathobiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA.
†To whom correspondence should be addressed. E-mail: Joanna.Groden@uc.edu

$Blm^{Cin/+}$ and $Blm^{+/+}$ mice with bromodeoxyuridine (BrdU) for two cell cycles. SCE analyses (*19*) revealed no statistically significant difference between the SCE frequency in $Blm^{Cin/+}$ and $Blm^{+/+}$ cells (*17*). However, the number of micronuclei in these BrdU-treated cultures revealed a twofold increase in $Blm^{Cin/+}$ cells as compared to $Blm^{+/+}$ cells (table S1). A similar effect was observed in untreated fibroblast cultures from two WT and two $Blm^{Cin/+}$ mice (table S1). These results suggest that mouse cells heterozygous for Blm^{Cin} have a subtle increase in genomic instability presumably related to the reduced BLM level.

To investigate the effect of *Blm* haploinsufficiency on tumor formation, we injected 9 $Blm^{+/+}$ and 15 $Blm^{Cin/+}$ newborn mice with murine leukemia virus (MLV). All $Blm^{Cin/+}$ mice developed metastatic T cell lymphoma and had an average life-span of 117.3 ± 33.0 days (Fig. 1). Although all of the WT littermates also developed T cell lymphoma, the WT mice had a longer average life-span of 184.4 ± 93.1 days. Histological analyses of four tumors of each genotype revealed no substantial morphological differences between tumors, that all lymphomas were CD8-positive, and that most were CD4-positive (*14*). Thus, *Blm* haploinsufficiency enhances T cell tumorigenesis in mice in response to viral challenge.

Because the gastrointestinal tract is a common site of cancer in human BS (*1*, *20*), we tested the effect of *Blm* haploinsufficiency on $Apc^{Min/+}$-mediated intestinal tumorigenesis. $Apc^{Min/+}$ mice carry a premature stop codon in one allele of the *Apc* tumor suppressor gene (Apc^{Min}), develop multiple intestinal adenomas, and are a murine model of familial adenomatous polyposis coli (*21*, *22*). $Blm^{Cin/+}$ female mice on the 129/SvEv background (backcross generation N_3) were crossed with $Apc^{Min/+}$ male mice (C57BL/6). The progeny were killed at 4 months of age and examined for intestinal tumors (*23*). $Apc^{Min/+};Blm^{+/+}$ mice (n = 14) developed an average of 14.2 ± 10.2 gastrointestinal tumors per animal whereas $Apc^{Min/+};Blm^{Cin/+}$ mice (n = 8) developed twice that number (31.4 ± 19.1) (Fig. 2A). Tumor size was similar in the $Apc^{Min/+};Blm^{Cin/+}$ and $Apc^{Min/+};Blm^{+/+}$ mice (1.3 mm ± 0.2 and 1.4 mm ± 0.1, respectively). Most tumors arose in the small intestine, although 4 of 8 $Apc^{Min/+};Blm^{Cin/+}$ mice and 1 of 14 $Apc^{Min/+};Blm^{+/+}$ mice developed one colon tumor each. No intestinal tumors were observed in any $Apc^{+/+}$ mice.

The tumors from $Apc^{Min/+};Blm^{Cin/+}$ and $Apc^{Min/+};Blm^{+/+}$ animals were classified as adenomas with either low- or high-grade dysplasia, based on gland architecture, nuclear/cytoplasmic ratio, amount of interglandular stroma, nucleus location, prominence of nucleoli, and the presence of mucus secretion. Intestinal specimens from mice of both genotypes showed evidence of intraepithelial neoplasia and low-grade adenomas in the small intestine. All colonic adenomas (four $Apc^{Min/+};Blm^{Cin/+}$ and one $Apc^{Min/+};Blm^{+/+}$) displayed high-grade dysplasia (Fig. 2B and fig. S2). Only $Apc^{Min/+};Blm^{Cin/+}$ mice developed tumors with high-grade dysplasia in the small intestine (5 of 223 tumors evaluated, Fig. 2B and fig. S2). Representative histological sections of the stomach, cecum, brain, mammary gland, testis, and thymus, as well as blood smears, were examined in $Apc^{Min/+};Blm^{+/+}$ and $Apc^{Min/+};Blm^{Cin/+}$ mice; no neoplasia outside the intestinal tract was observed.

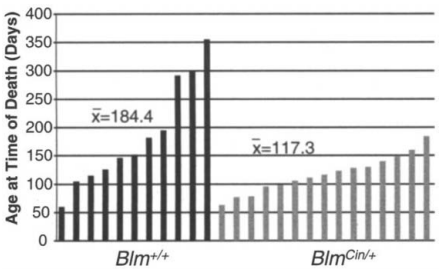

Fig 1. $Blm^{Cin/+}$ mice die earlier from MLV-induced T cell lymphoma than $Blm^{+/+}$ mice do. Three litters of $Blm^{+/+}$ and $Blm^{Cin/+}$ newborn littermates (Black Swiss in the F_7 generation) were injected intraperitoneally with 100 μl of MLV at a concentration of 1×10^5 plaque-forming units per ml and were monitored for lymphoma development. The age in days at the time of death is plotted for each $Blm^{+/+}$ (black bars, n = 9) and $Blm^{Cin/+}$ (gray bars, n = 15) mouse. The mean ($\bar{x}$) age at the time of death is shown for each group of mice; $P < 0.05$, Student's t test.

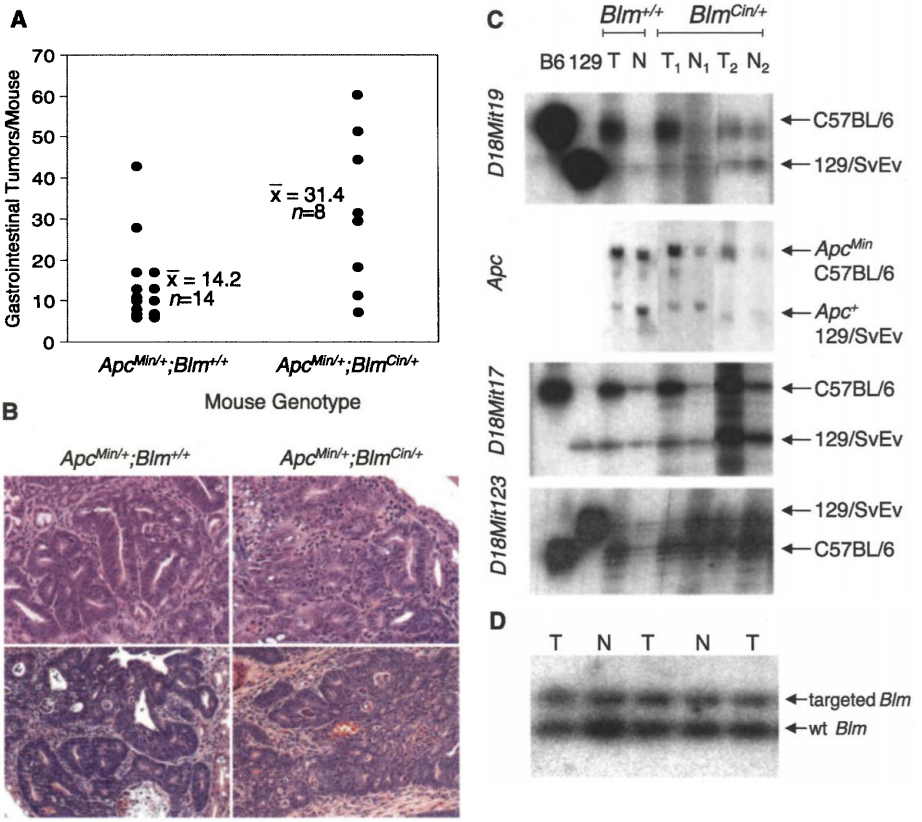

Fig 2. Haploinsufficiency of *Blm* modifies the tumor phenotype of $Apc^{Min/+}$ mice. (**A**) Quantification of tumors from $Apc^{Min/+};Blm^{+/+}$ (n = 14) and $Apc^{Min/+};Blm^{Cin/+}$ (n = 8) mice. Animals were killed at 4 months of age, and the mean number of grossly visible gastrointestinal tumors (≥ 1 mm) per mouse is shown. Statistical analysis was performed with the nonparametric Wilcoxon rank sum test ($P < 0.05$). (**B**) Histological analysis of small intestinal tumors (top panels) and colonic tumors (bottom panels) from $Apc^{Min/+};Blm^{+/+}$ (left) and $Apc^{Min/+};Blm^{Cin/+}$ (right) mice. Sections are stained with hemotoxylin and eosin (magnification 1000×). (**C**) Analysis of *Apc* and chromosome 18 microsatellite loci *D18Mit19*, *D18Mit17*, and *D18Mit123* in DNA from microdissected adenoma tissue (T) and adjacent normal tissue (N). The pair on the left is from an $Apc^{Min/+};Blm^{+/+}$ mouse; the pairs in the middle and on the right are from two $Apc^{Min/+};Blm^{Cin/+}$ mice. DNA from C57BL/6 (B6) and 129/SvEv (129) mice are included as controls; bands corresponding to the C57BL/6 and 129/SvEv alleles are highlighted with arrows. (**D**) PCR/Southern analysis of *Blm* in DNA microdissected from adenoma tissue (T) and adjacent normal tissue (N) from an $Apc^{Min/+};Blm^{Cin/+}$ mouse. Bands representing the WT alleles (400 base pairs) and targeted alleles (780 base pairs)are shown.

Intestinal adenomas from $Apc^{Min/+}$;$Blm^{Cin/+}$ mice were evaluated for loss of heterozygosity (LOH) at the *Apc* locus, a feature typical of $Apc^{Min/+}$ adenomas (*24*, *25*). Five adenomas from $Apc^{Min/+}$;$Blm^{+/+}$ mice and 20 adenomas from $Apc^{Min/+}$;$Blm^{Cin/+}$ mice were analyzed (Fig. 2C). The mean ratio of the Apc^{+} to the Apc^{Min} allele in tumors from $Apc^{Min/+}$;$Blm^{Cin/+}$ mice was not different from that in tumors from $Apc^{Min/+}$;$Blm^{+/+}$ mice. Each was consistent with published values (*24*, *25*), demonstrating loss of the WT *Apc* allele in all tumors from *Blm* heterozygous mice.

We next investigated the mutational mechanisms responsible for loss of the normal *Apc* allele in the intestinal tumors by LOH analysis. *Apc* maps to chromosome 18 and is located on the genetic map at 15.0 centimorgans (*26*). We used quantitative polymerase chain reaction (PCR) with simple sequence length polymorphism markers (*19*) for three loci to examine allelic loss on chromosome 18 in our set of 25 tumors (Fig. 2C). Tumors in $Apc^{Min/+}$;$Blm^{+/+}$ mice and 18 of 20 tumors in $Apc^{Min/+}$;$Blm^{Cin/+}$ mice were characterized by LOH of *Apc* and all markers proximal and distal to *Apc* on chromosome 18 (Fig. 2C). Two tumors from $Apc^{Min/+}$;$Blm^{Cin/+}$ mice, of which one is shown, remained heterozygous at the proximal marker *D18Mit19* (Fig. 2C). These results indicate that *Apc* loss in $Apc^{Min/+}$ tumors with two WT *Blm* alleles is characterized by LOH of chromosome 18 but that in some $Blm^{Cin/+}$ tumors, loss of the normal *Apc* allele occurs by somatic recombination.

To test whether the tumors from $Apc^{Min/+}$;$Blm^{Cin/+}$ mice retained the WT *Blm* allele, we performed a quantitative PCR-based assay on 14 tumors. The ratio of the WT to the targeted allele in each tumor sample from $Apc^{Min/+}$;$Blm^{Cin/+}$ mice was not significantly different from that in adjacent normal tissue (Fig. 2D). Western blots of 10 tumor lysates from $Apc^{Min/+}$;$Blm^{Cin/+}$ mice evaluated with antiserum to COOH-terminus of BLM confirmed that BLM expression had been retained (fig. S3A). Similarly, immunofluorescence of MLV-induced lymphomas from $Blm^{Cin/+}$ mice demonstrated nuclear BLM staining (fig. S3B). These results suggest that mutation of the remaining WT *Blm* allele was not required for tumor formation in either T cells or intestinal tissues.

Mutation of one allele of *Blm* has measurable consequences for the phenotype of murine somatic cells and for the tumor susceptibility of the mouse. Our data demonstrate that *Blm* haploinsufficiency is sufficient to affect tumor formation in susceptible mice, and probably alter genomic stability. These effects have not been described in other targeted *Blm* mice (*12*, *13*). These data also suggest that *Blm* haploinsufficiency could promote tumor formation in tissues other than those studied here. Additionally, although none of the tumors in $Apc^{Min/+}$;$Blm^{Cin/+}$ mice were invasive at the 4-month end point of our experiments, it is possible that these tumors would progress to malignancy if given more time. Our results are also important for human populations: About 1 in 100 Ashkenazi Jews carry one mutant allele of *BLM* (*25*, *26*). An accompanying paper by Gruber *et al.* (*27*) demonstrates that carriers of BLM^{Ash} have a more than twofold increase in the occurrence of colorectal cancer. Together, these studies suggest that *Blm*/*BLM* mutation is an important modifier of intestinal cancer predisposition and that individuals carrying one mutant allele of *BLM* may have one of the noteworthy clinical hallmarks of BS—namely, increased cancer predisposition.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5589/2051/DC1
Materials and Methods
Figs. S1 to S3
Table S1

24 May 2002; accepted 19 August 2002

Cleavage of *Scarecrow-like* mRNA Targets Directed by a Class of *Arabidopsis* miRNA

Cesar Llave, Zhixin Xie, Kristin D. Kasschau, James C. Carrington*

Micro-RNAs (miRNAs) are regulatory molecules that mediate effects by interacting with messenger RNA (mRNA) targets. Here we show that *Arabidopsis thaliana* miRNA 39 (also known as miR171), a 21-ribonucleotide species that accumulates predominantly in inflorescence tissues, is produced from an intergenic region in chromosome III and functionally interacts with mRNA targets encoding several members of the *Scarecrow-like* (*SCL*) family of putative transcription factors. miRNA 39 is complementary to an internal region of three *SCL* mRNAs. The interaction results in specific cleavage of target mRNA within the region of complementarity, indicating that this class of miRNA functions like small interfering RNA associated with RNA silencing to guide sequence-specific cleavage in a developmentally controlled manner.

Micro-RNAs in eukaryotes are ~21- to 22-ribonucleotide RNAs that arise from short stem-loop precursors through the activity of the double-stranded ribonuclease Dicer (*1*–*6*). The miRNAs from *lin-4* and *let-7* genes are involved in translational control through interaction with 3′-proximal sequences in target mRNAs in *Caenorhabditis elegans* (*7*–*11*). However, the range of functions for other miRNAs in plants, animals, and microorganisms has yet to be determined.

Arabidopsis contains numerous small RNAs, many of which resemble miRNAs identified in animals (*12*, *13*). Several of