NEOPLASIA
www.neoplasia.com

Volume 16 Number 2 February 2014 pp. 129–136 129

Strain Background Determines Lymphoma Incidence in *Atm* Knockout Mice[1,2]

Paula C. Genik*, Helle Bielefeldt-Ohmann†, Xianan Liu‡, Michael D. Story§, Lianghao Ding§, Jamie M. Bush¶, Christina M. Fallgren* and Michael M. Weil*

*Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO; †Australian Infectious Diseases Research Centre, Queensland Institute of Medical Research, Australian School of Veterinary Science, University of Queensland, Gatton, Queensland, Australia; ‡Department of Environmental Health and Safety, Iowa State University, Ames, IA; §Department of Radiation Oncology, Division of Molecular Radiation Biology and Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX; ¶Department of Anatomic Pathology, IDEXX Laboratories, Inc, Westbrook, ME

Abstract

About 10% to 30% of patients with ataxia-telangiectasia (A-T) develop leukemias or lymphomas. There is considerable interpatient variation in the age of onset and leukemia/lymphoma type. The incomplete penetrance and variable age of onset may be attributable to several factors. These include competing mortality from other A-T–associated pathologies, particularly neurodegeneration and interstitial lung disease, and allele-specific effects of *ataxia-telangiectasia mutated* (*ATM*) gene mutations. There is also limited evidence from clinical observations and studies using *Atm* knockout mice that modifier genes may account for some variation in leukemia/lymphoma susceptibility. We have introgressed the Atm^{tm1Awb} knockout allele (Atm^{-}) onto several inbred murine strains and observed differences in thymic lymphoma incidence and latency between $Atm^{-/-}$ mice on the different strain backgrounds and between their F1 hybrids. The lymphomas that arose in these mice had a pattern of sequence gains and losses that were similar to those previously described by others. These results provide further evidence for the existence of modifier genes controlling lymphomagenesis in individuals carrying defective copies of *Atm*, at least in mice, and the characterized Atm^{-} congenic strain set provides a resource with which to identify these genes. In addition, we found that fewer than expected $Atm^{-/-}$ pups were weaned on two strain backgrounds and that there was no correlation between body weight of young $Atm^{-/-}$ mice and lymphoma incidence or latency.

Neoplasia (2014) 16, 129–136

Abbreviations: A-T, ataxia-telangiectasia; ATM, ataxia-telangiectasia mutated; CGH, comparative genomic hybridization; GISTIC, Genomic Identification of Significant Targets in Cancer; Gzm, granzyme; PBL, peripheral blood lymphocyte; Prkdc, protein kinase, DNA-activated, catalytic polypeptide; *Tcr*, T cell receptor gene

Address all correspondence to: Dr Michael M. Weil, Radiation Cancer Biology and Oncology Section, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, 1618 Campus Delivery, MRB 483, Fort Collins, CO 80523. E-mail: Michael.Weil@ColoState.EDU

[1]This work was supported by National Institutes of Health grant R03 CA135528 (to M.M.W.) and the Ataxia-Telangiectasia Children's Project (to M.M.W.). The authors disclose no potential conflicts of interest.

[2]This article refers to supplementary materials, which are designated by Tables W1 to W3 and Figures W1 and W2 and are available online at www.neoplasia.com.

Received 5 December 2013; Revised 7 February 2014; Accepted 10 February 2014

DOI 10.1593/neo.131980

Introduction

Cancer follows respiratory injury as the major cause of mortality in ataxia-telangiectasia (A-T), and 10% to 30% of patients with A-T develop leukemias or lymphomas, generally of T cell origin. Non-Hodgkin lymphoma is the most common malignancy, followed by T cell acute lymphocytic leukemia [1]. T-prolymphocytic leukemia has been reported in older patients. However, most patients with A-T do not develop leukemia or lymphoma, and the age of onset varies for those who do. The reason for this incomplete penetrance is unknown, but there is some evidence that modifier genes may play a role. Concordance between members of the same family for leukemia or lymphoma type and for the age of onset has been reported [2,3]. This concordance could also be explained by differing phenotypic effects of specific *ataxia-telangiectasia mutated* (*ATM*) mutations, and indeed, missense mutations and small, in-frame deletions are found in some concordant families. However, in other families with concordant members, both *ATM* alleles are inactivated by truncating mutations, and ATM protein is not detectable. In addition, a particular *ATM* mutation may cause leukemia or lymphoma in some families yet lead to different leukemia/lymphoma types with different ages of onset in other families.

Since the cloning of the gene defective in A-T, *ATM*, in 1995 [4], at least five laboratories have created mice with *Atm* knockout alleles [5–9]. Mice with a conditional *Atm* knockout allele [10] and at least two *Atm* knock-in alleles have also been engineered [11,12]. Mice homozygous for the *Atm* knockout alleles (referred to here as $Atm^{-/-}$ mice) develop malignant thymic lymphomas resulting in diminished life spans. On the basis of a literature survey, Reliene and Schiestl [13] found that different laboratories report differing life spans for $Atm^{-/-}$ mice. To explain the discrepancies, they focused on differences in husbandry conditions (specific pathogen-free as opposed to conventional facilities) and differences in commercial rodent chows. The possibility that the strain background for the *Atm* knockout allele may be a factor in varying life spans was also considered but somewhat discounted because $Atm^{-/-}$ mice on the 129S6/SvEvTac background survive for about the same time as $Atm^{-/-}$ mice on mixed backgrounds.

To determine if genetic background influences thymic lymphoma susceptibility or latency in $Atm^{-/-}$ mice, we introgressed the Atm^{tm1Awb} knockout allele onto three additional inbred strain backgrounds. We then monitored lymphoma development in the four different $Atm^{-/-}$ inbred strains and in three $Atm^{-/-}$ F1 hybrid strains. Here, we report strain-specific differences in lymphoma susceptibility and latency in $Atm^{-/-}$ mice that suggest a role for modifier genes in these phenotypes. In addition, we surveyed the lymphomas for recurrent sequence gains and losses and for granzyme (Gzm) gene rearrangements. We found sequence gains and losses that were largely in agreement with those previously reported [14,15] and no evidence for previously reported rearrangements of the *GzmB* and *GzmC* genes [16].

Materials and Methods

Generation of Atm^{tm1Awb} *Congenic Strains*

129S6/SvEvTac Atm^{tm1Awb} mice originally created by Barlow et al. [5] served as the donor strain for the *Atm* knockout allele. The A/J Atm^{tm1Awb} and C57BL/6J Atm^{tm1Awb} congenic strains were generated by five marker-directed backcrosses using a chromosome elimination "speed congenic" strategy we have previously described [17]. The marker-directed backcrosses were followed by two conventional backcrosses and five intercross generations. BALB/cByJ Atm^{tm1Awb} congenic mice were generated by 13 generations of conventional backcrosses, followed by 5 intercross generations. These congenic strains are available from the Jackson Laboratory (Bar Harbor, ME). $Atm^{-/-}$ mice are infertile, so the congenic strains are maintained with $Atm^{+/-}$ breeders. For the remainder of this report, we will refer to the background strains as 129S6, C57BL/6, BALB/c, and A/J. F1 hybrids were generated by matings of 129S6 and C57BL/6 mice, 129S6 and A/J mice, and C57BL/6 and A/J mice. We will refer to these hybrids as 129SB6F1, 129SAF1, and B6AF1 regardless of the maternal and paternal strains used in the crosses.

$Atm^{-/-}$ and $Atm^{+/+}$ mice used in this study were littermates generated from crosses of $Atm^{+/-}$ mice. The mice on all strain backgrounds were bred contemporaneously and were weaned into common cages. Consequently, mice of any given strain were housed in the same cages as similarly aged mice of other strains.

Genotyping

Genotyping was performed on DNA isolated from tail snips and amplified using *Atm*-F (5′-GACTTCTGTCAGATGTTGCTGCC-3′), *Atm*-R (5′-CGAATTTGCAGGAGTTGCTGAG-3′), and *Atm*-Neo (5′-GGGTGGGATTAGATAAATGCCTG-3′). This three-primer set yields a 161-bp amplimer from the wild-type *Atm* allele and a 441-bp amplimer from the knockout allele.

Histopathology

Tissues were fixed in 10% buffered formaldehyde for 48 to 72 hours, followed by transfer into and storage in 70% ethanol until processed and embedded in paraffin. Sections (6 μm) were stained with hematoxylin and eosin and examined on a Nikon Eclipse 51E microscope (Nikon Instruments Inc, Melville, NY) equipped with a Nikon DS-Fi1 camera with a DS-U2 unit and NIS-Elements F software.

Gzm Gene Rearrangement

Reverse transcription–polymerase chain reaction (PCR) was used to detect fusion transcripts resulting from rearrangements of the *GzmB* and *GzmC* genes. Approximately 2 μg of total RNA prepared using an RNeasy Kit (Qiagen Sciences, Germantown, MD) was reverse transcribed (SuperScript II; Invitrogen, Grand Island, NY), and the first strand cDNA was amplified using different primer pair combinations of *GzmB* forward primers (*GzmB*-F1 to *GzmB*-F7) and *GzmC* reverse primers (*GzmC*-R1 to *GzmC*-R7, listed in Table W1). To confirm the expression of *GzmB* and *GzmC* genes and the suitability of the primers, primers of *GzmB*-F1 through *GzmB*-F7 were used in PCR control reactions with *GzmB*-R1, and *GzmC*-R1 through *GzmC*-R7 were assessed with *GzmC*-F1.

Array Comparative Genomic Hybridization

Genomic DNA was extracted from five tumors isolated from 129SAF1 mice and six tumors isolated from 129SB6F1 mice using a DNeasy Blood and Tissue Kit (Qiagen Sciences) according to the manufacturer's instructions. DNA isolated from the tail of each respective animal was used as reference DNA. DNA concentration and sample buffer quality was determined using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). Samples containing contaminants as indicated by low 260:230 ratios were repurified using a DNA clean-up and concentrator kit (Zymo Research, Irvine, CA). Labeling reactions were prepared using the Roche NimbleGen Labeling Protocol (Roche NimbleGen, Madison, WI)