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Aberrant TCRδ rearrangement underlies the T-cell lymphocytopenia and t(12;14) translocation associated with ATM deficiency

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Key Points
- ATM-deficient T-cell lymphocytopenia is in part caused by defects in TCRδ rearrangements.
- Aberrant TCRδ arrangement is required for the recurrent t(12;14) translocations, but not chromosome 14 amplification, in ATM-deficient thymic lymphomas.

Introduction

As a master regulator of the DNA damage response, ataxia-telangiectasia mutated (ATM) kinase is rapidly activated by DNA double-strand breaks and phosphorylates substrates involved in both DNA repair and checkpoint control. Loss of ATM causes ataxia-telangiectasia (A-T) syndrome characterized by primary immunodeficiency and greatly increased risk for T-cell lymphomas/leukemia. Both A-T and T-cell prolymphoblastic leukemia patients with somatic mutations of ATM frequently carry inv(14;14) between the T-cell receptor α/β (TCRα/β) and immunoglobulin H loci, but the molecular origin of this translocation remains elusive. ATM-deficient mice recapitulate lymphocytopenia of A-T patients and routinely succumb to thymic lymphomas with t(12;14) translocation, syntenic to inv(14;14) in humans. Here we report that deletion of the TCRδ enhancer (Eδ), which initiates TCRδ rearrangement, significantly improves αβ T cell output and effectively prevents t(12;14) translocations in ATM-deficient mice. These findings identify the genomic instability associated with V(D)J recombination at the TCRδ locus as the molecular origin of both lymphocytopenia and the signature t(12;14) translocations associated with ATM deficiency. (Blood. 2015;125(17):2665-2668)

Study design

All animal work was conducted with approval from the institutional Animal Care and Use Committee of Columbia University. ATM−/− and Eδ−/− mice were described previously. T-cell development was characterized in 4- to 6-week-old mice as previously described. Briefly, single-cell suspensions of thymus and spleen were counted and stained with fluorochrome-conjugated antibodies against CD4, CD8, CD3, TCRβ, or TCRγ. For DN staining, thymocytes were counterstained with phycoerythrin-conjugated anti-CD4, CD8, B220, TCRγδ, Ter119 and CD19 antibodies, then with fluorochrome-conjugated anti-CD44 and anti-CD25 antibodies. Flow cytometry data were collected on FACSCalibur (BD) and analyzed with FlowJo. For cytogenetic analyses, single cell suspensions were incubated with colcemid (10 μg/mL) for 4 to 10 hours and fixed and stained using ASI applied spectral imaging paints. Images were acquired and analyzed with Isis and Metafer 4 (MetaSystems, Newton, MA). Comparative genomic hybridization (CGH)

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Results and discussion

ATM<sup>−/−</sup> Eδ<sup>−/−</sup> mice were born at the expected ratio and indistinguishable from ATM<sup>−/−</sup> littermates. In thymuses of ATM<sup>−/−</sup> Eδ<sup>−/−</sup> mice, γδ T-cell frequency was reduced, but not abolished, consistent with the role of Eα in residual γδ T-cell development<sup>10</sup> (Figure 1A-B). Although thymus cellularity was reduced by ~50% in ATM<sup>−/−</sup> mice, both thymus cellularity and the number of mature SP T cells were significantly rescued in ATM<sup>−/−</sup> Eδ<sup>−/−</sup> mice (Figure 1C-D). This is unexpected, given that >90% of thymocytes are αβ T cells. Further analyses showed that this rescue was mostly due to moderate increases of DNIV (CD25<sup>+</sup>CD44<sup>+</sup>) percentage and marked increases of DP thymocyte number without changes in the SP to DP ratio (Figure 1C-D and supplemental Figure 1A-B). TCRδ undergoes V(D)J recombination in DNII-DNIII (CD25<sup>+</sup>).<sup>10</sup> ATM is particularly important for inversionsal V(D)J recombination,<sup>12</sup> and Vδ5 is located downstream of Cδ and requires inversionsal rearrangement.<sup>15</sup> The configuration of the TCRα/δ locus also determines that if DNA breaks in the TCRδ loci are unrepaired, then Eα would separate from Vαs and disable TCRα V(D)J recombination (Figure 2C). These findings reveal an important role of genomic instability at the TCRδ locus in αβ T-cell development and T-cell lymphocytopenia associated with ATM deficiency, and they suggest that suppression of TCRδ rearrangement might improve T-cell counts in A-T patients.

Despite improved T-cell counts, the kinetics of succumbing to thymic lymphomas was similar in ATM<sup>−/−</sup> Eδ<sup>−/−</sup> mice and ATM<sup>−/−</sup> controls (Figure 2A). ATM<sup>−/−</sup> Eδ<sup>−/−</sup> thymic lymphomas consisted of immature (surface TCRβ<sup>−/−</sup>) DP T cells similar to ATM<sup>−/−</sup> controls (supplemental Figure 1C). Southern blot identified clonal TCRβ rearrangements and focal amplification centromeric to the TCRα/δ loci (chromosome 14) in all ATM<sup>−/−</sup> mice and ATM<sup>−/−</sup> controls (supplemental Figures 1D and 2A).<sup>3</sup> ATM<sup>−/−</sup> thymic lymphomas were derived from immature T cells that had not yet undergone TCRα rearrangement, evidenced by retention of the Cδ<sup>−/−</sup>.<sup>5</sup> All ATM<sup>−/−</sup> Eδ<sup>−/−</sup> and 2 of 5 ATM<sup>−/−</sup> Eδ<sup>−/−</sup> lymphomas (545 and 2280) retained at least 1 Cδ, suggesting that the chromosome 14 amplifications most likely occurred during TCRδ rearrangement in those tumors, consistent with the ability for Eα to drive residual TCRδ rearrangements in the absence of Eδ.<sup>10</sup> Notably, the other 3 ATM<sup>−/−</sup> Eδ<sup>−/−</sup> lymphomas showed homozygous deletion of Cδ, indicative of biallelic TCRα rearrangements (Figure 2C). Southern blot further confirmed that all 3 tumors (497, 713, and 745) rearranged the proximal Jα segments (Figure 2C). Thus, TCRδ rearrangement is not required for
Figure 2. Aberrant TCRβ rearrangement is required for t(12;14), but not chromosomal 14 amplifications, in murine ATM-deficient thymic lymphomas. (A) Survival curve for ATM+/− (n = 10), ATM−/− Es−/+ (n = 1), and ATM−/− Es−/− (n = 8) mice with median survival at 99, 112, and 137 days, respectively. *P* values were calculated using the log-rank test. (B) Representative chromosome 12 (green) and chromosome 14 (red) paint analyses of no. 745 ATM−/− Es−/− lymphomas (without t(12;14)) and no. 900 ATM−/− Es−/− lymphomas with t(12;14) translocation. More than 20 metaphases were analyzed for each lymphoma, and t(12;14) in >50% metaphases was used as the criterion for clonal translocations. The results are summarized in panel E. (C) Southern blot mapping the rearrangements of C5 and Jκ. The diagram shows the TCRβ locus, EcoRI (black arrows), and probe locations (black dashed line for Southern probes and red dashes for adjacent CGH probes). A total of 10 μg EcoRI-digested genomic DNA from either tumor (T) or the corresponding kidney (K) was analyzed on each lane. Loading control probe detected a fragment of the single-copy DNA. (D) CGH analyses of ATM−/− Es−/− thymic lymphomas 745 and 545. Each tumor DNA was analyzed with matched kidney control DNA on a 244K mouse CGH array from Agilent. The log ratio of tumor vs kidney control at each probe location is displayed in centromere-to-telomere orientation for each chromosome (from 1 to 19 then 20). (E) Summary of chromosome X/Y, from left to right). The red arrows and labels indicate recurrent amplification of Notch1, chromosome 14 upstream of TCRβ locus, and trisomy 15. Green arrows and labels indicate recurrent deletion of the telomeric portion of chromosome 12 and the Pten locus, as well as deleitional rearrangement in TCR β loci. (E) Summary of chromosome 12/14 paint analyses of ATM−/− Es−/− and ATM−/− Es−/− lymphomas. (F) Quantitative PCR results that measured the expression of IL7R, Jak1, Notch1, Pten, Bcl11b, and Psmc6 genes from ATM−/− Es−/− (n = 3), ATM−/− Es−/− (n = 4), ATM−/− Es−/− (n = 5) lymphomas. Psmc6 locates at the amplification area within chromosome 14 and is used as a marker for the amplification here. Bcl11b is within the region that is hemizygotically deleted in chromosome 12. Briefly, total RNA was extracted using Trizol (Invitrogen, CA) from a single-cell suspension derived from ATM−/− and ATM−/− thymic lymphomas 745 and 545. Each tumor DNA was analyzed with matched kidney control DNA on a 244K mouse CGH array from Agilent. The log ratio of tumor vs kidney control at each probe location is displayed in centromere-to-telomere orientation for each chromosome (from 1 to 19 then 20).
chromosome 14 amplification and T-cell lymphomagenesis in ATM-deficient mice. In the absence of the TCRδ rearrangement, aberrant TCRα rearrangement is able to promote chromosome 14 amplifications and oncogenic transformation of ATM-deficient T cells.

We next investigated the origin of the t(12;14) translocations characteristic of ATM deficiency. Chromosome paint identified clonal t(12;14) translocations in all ATM−/−Ehδ−/− lymphomas and the 2 C6-positive ATM−/−Ehδ−/− lymphomas (545 and 2280), but not in the 3 ATM−/−Ehδ−/− lymphomas (497, 713, and 745) with biallelic TCRα rearrangements (Figure 2B,E). CGH analyses of 2 C6-positive (2280 and 545) and 2 C6-negative (497 and 745) ATM−/−Ehδ−/− lymphomas confirmed focal amplification upstream of the TCRαδ locus (Figure 2D and supplemental Figure 2B) and also revealed a unique hemizygous deletion of chromosome 14 downstream of the TCRαδ locus only in C8-negative (497 and 745) tumors (Figure 2D and supplemental Figure 2B). CGH probe mapping showed that the deletion started at the proximal Jα (Figure 2C-D).

Together with the absence of the t(12;14) translocation, this result suggests that when chromosome 14 amplification occurs in DP T cells during TCRα rearrangement, the break in chromosome 12 is no longer available to form the t(12;14) translocation, causing the telomeric portion of chromosome 14 to be lost and pointing to concurrent immunoglobulin H and TCRδ rearrangement in DN T cells as the molecular origin for t(12;14). This further implies that t(12;14) and the syntenic inv(14;14) are not essential for T-cell transformation. Despite the lack of t(12;14), both C6-negative (497 and 745) tumors displayed hemizygous deletion of chromosome 12 telomeric regions, supporting the existence of potential tumor-suppressor genes (eg, Bcl11b). In this context, SKY analyses identified t(12;15) translocations involving chromosome 15 in tumor 745 (Figure 2B and supplemental Figure 2C). Finally, CGH also indicated that murine ATM−/−thymic lymphomas, regardless of Ehδ status, display genetic changes that have been associated with human T-cell acute lymphoblastic leukemia (T-ALL), including trisomy of chromosome 15 containing c-Myc, amplification and overexpression of Notch1, deletion of Pten, and overexpression of IL7R (Figure 2F), highlighting ATM-deficient murine thymic lymphomas as a bona fide animal model for human T-ALL. In summary, our study identifies aberrant TCRδ rearrangement in the absence of ATM together with the unique configurations of the TCRαδ locus as one of the major causes to both T-cell–specific lymphocytopenia and the t(12;14) and related inv(14;14) associated with ATM deficiency and potentially in other T-ALL.

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Authorship

Contributions: W.J., F.W.A., and S.Z. designed experiments; S.Z. wrote the paper; M.G. performed the SKY analyses for tumor 745; W.J., B.J.L., C.L., R.L.D., and S.Z. performed the rest of the experiments.

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