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Tcrδ translocations that delete the *Bcl11b* haploinsufficient tumor suppressor gene promote atm-deficient T cell acute lymphoblastic leukemia

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Abbreviations: ATM, Ataxia Telangiectasia mutated; DSB, DNA double strand break; T-ALL, T cell acute lymphoblastic leukemia; TCR, T cell receptor; A-T, Ataxia Telangiectasia, Ea, TCRa transcriptional enhancer; ALL, acute lymphoblastic leukemia; SKY, spectral karyotyping.

ATM is the master regulator of the cellular response to DNA double strand breaks (DSBs). Deficiency of ATM predisposes humans and mice to $\alpha\beta$ T lymphoid cancers with clonal translocations between the *T cell receptor (TCR) α/δ* locus and a 450 kb region of synteny on human chromosome 14 and mouse chromosome 12. While these translocations target and activate the *TCL1* oncogene at 14q32 to cause T cell pro-lymphocytic leukemia (T-PLL), the *TCRα/δ*;14q32 translocations in ATM-deficient T cell acute lymphoblastic leukemia (T-ALL) have not been characterized and their role in cancer pathogenesis remains unknown. The corresponding lesion in Atm-deficient mouse T-ALLs is a chromosome t(12;14) translocation with *Tcrδ* genes fused to sequences on chromosome 12; although these translocations do not activate *Tcl1*, they delete the *Bcl11b* haploinsufficient tumor suppressor gene. To assess whether *Tcrδ* translocations that inactivate one copy of *Bcl11b* promote transformation of Atm-deficient cells, we analyzed $Atm^{-/-}$ mice with mono-allelic *Bcl11b* deletion initiating in thymocytes concomitant with *Tcrδ* recombination. Inactivation of one *Bcl11b* copy had no effect on the predisposition of $Atm^{-/-}$ mice to clonal T-ALLs. Yet, none of these T-ALLs had a clonal chromosome t(12;14) translocation that deleted *Bcl11b* indicating that *Tcrδ* translocations that inactivate a copy of *Bcl11b* promote transformation of *Atm*-deficient thymocytes. Our data demonstrate that antigen receptor locus translocations can cause cancer by deleting a tumor suppressor gene. We discuss the implications of these findings for the etiology and therapy of T-ALLs associated with ATM deficiency and TCRα/δ translocations targeting the 14q32 cytogenetic region.

Introduction

The cellular response to DSBs coordinates DNA repair with activation of cell cycle checkpoints to maintain genomic stability and suppress malignant transformation[1] The Ataxia Telangiectasia mutated (ATM) protein kinase is the master regulator of this response.[2] Upon activation by DSBs, ATM induces phosphorylation of numerous proteins to stimulate DSB repair, activate cell cycle checkpoints, and induce apoptosis if DSBs persist. Inherited *ATM* deficiency in humans causes Ataxia Telangiectasia (A-T), a fatal disease involving progressive neurological degeneration, immunodeficiency, elevated chromosomal instability, and increased predisposition to lymphoid malignancies.[3] The average life expectancy of people with A-T is ~25 years, with chronic lung infection and lymphoid cancers the major causes of death.[3] Although childhood acute lymphoblastic leukemia (ALL) is the most common malignancy that occurs in humans with A-T, their cancer genomes have not been characterized beyond the identification of clonal translocations that target a 450 kb region of 14q32 and involve TCRα/δ loci on chromosome 14 or TCRβ loci on chromosome 7.[3] In contrast, while adult T-PLLs arise less often in A-T humans, these cancers have been more extensively characterized and found to harbor clonal translocations in which *TCRα* genes are fused within 160 kb of the *TCL1* oncogene at 14q32.1 or the MTCP1 oncogene on the X chromosome.[3-7] *TCRα;TCL1* and *TCRα;MTCP1* translocations are also recurrent clonal lesions in T-PLLs with acquired inactivation of *ATM*[8,9] *TCRα;TCL1* and *TCRα;MTCP1* translocations likely drive transformation by placing *TCL1* or *MTCP1* under control of the *TCRα* transcriptional enhancer (Eα). Demonstrations that enforced expression of *TCL1* or *MTCP1* causes T cell lymphoma in

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mice support this notion and provide mouse models of T-PLL.[10,11] The absence of similar characterization and models for the recurrent 14q23 translocations in T-ALLs of A-T children provides barriers for elucidating the etiology of ATM-deficient T-ALLs for the development of therapeutics that could cure these fatal lymphoid malignancies.

Germline Atm-deficient ($Atm^{-/-}$) mice are useful model to assess the role of 14q23 translocations in the pathogenesis of T-ALLs that develop in A-T patients. Like A-T children, $Atm^{-/-}$ mice exhibit immunodeficiency, elevated chromosomal instability, and increased predisposition to T-ALLs arising in the thymus.[12-15] These phenotypes result from loss of ATM functions in promoting DSB repair, activating cell cycle checkpoints, and inducing apoptosis during TCR recombination and proliferation of developing $\alpha\beta$ T cells.[16-20] $Atm^{-/-}$ mice invariably succumb by six months of age to T-ALLs with clonal translocations that typically involve the *Tcrα/δ* locus on chromosome 14, the *Tcrβ* locus on chromosome 6, and/or the immunoglobulin heavy chain (*Igh*) locus on chromosome 12 that recombines D and J segments in thymocytes.[19-23] The most frequent clonal translocation in $Atm^{-/-}$ T-ALLs is a t(12;14) chromosome translocation that occurs in ~60% of these cancers.[19-23] The initial cytogenetic characterization of $Atm^{-/-}$ T-ALLs suggested that these clonal t(12;14) translocations occur between *Tcrα/δ* loci and a region of mouse chromosome 12 that harbors *Tcl1* and shares synteny with human chromosome 14q32.[22] This led to the hypothesis that these lesions fuse *Tcrα* genes to chromosome 12 sequences near *Tcl1* and drive transormation through the ability of Eα to activate expression of *Tcl1* or another oncogene in this region of chromosome 12.[22,24] However, our molecular analysis of $Atm^{-/-}$ T-ALLs revealed that these recurrent t(12;14) translocations fuse *Tcrδ* genes to chromosome 12 sequences, deleting 5-30 Mb of the telomeric end of chromosome 12.[21] Notably, these deletions often included *Tcl1*, and deletions that did not include *Tcl1* did not increase Tcl1 expression.[21] We also showed that these clonal *Tcrδ* translocations arise independent of Eα.[21] Thus, our data counter the notion that the clonal recurrent t(12;14) translocations of $Atm^{-/-}$ T-ALLs promote transformation by targeting and activating *Tcl1* or another oncogene on chromosome 12.

The recurrent clonal *TCRα/δ* translocations of T-ALLs that arise in A-T humans and $Atm^{-/-}$ mice are selected from many other translocations formed in ATM-deficient thymocytes, indicating that these TCRα/δ translocations effect genetic changes that promote malignant transformation of immature $\alpha\beta$ T cells.[3] In this context, t(12;14) translocations are not the most frequent translocations observed in non-malignant $\alpha\beta$ T cells of $Atm^{-/-}$ mice,[24] despite being the most prevalent clonal translocation in $Atm^{-/-}$ T-ALLs. The recurrent t(12;14) translocations of $Atm^{-/-}$ T-ALLs nearly always delete one copy of *Bcl11b*, resulting in reduced Bcl11b expression.[21] In mice, both copies of *Bcl11b* are required to inhibit development of T-ALL following γ-radiation,[25] demonstrating that *Bcl11b* is a haploinsufficient tumor suppressor gene. Consistent with this notion, TCRδ translocations or mutations that inactivate a single copy of *BCL11B* are recurrent clonal lesions in human T-ALLs.[26-28] Therefore, we have speculated that *TCRδ* translocations that delete *BCL11B* promote development of ATM-deficient T-ALL.[21,29] To test this hypothesis, we sought to determine the effect of pervasive mono-allelic *Bcl11b* inactivation on the spontaneous predisposition of $Atm^{-/-}$ mice to T-ALLs with clonal t(12;14) translocations that delete *Bcl11b*.

Results

Both $\alpha\beta$ and $\gamma\delta$ T lymphocytes develop in the thymus from $CD4^-CD8^-$ (double negative, DN) thymocytes.[30] Assembly and expression of TCRγ and TCRδ genes in DN thymoctyes signals differentiation into $\gamma\delta$ T cells.[30] In contrast, assembly and expression of TCRβ genes in DN cells signals differentiation into $CD4^+CD8^+$ (double positive, DP) thymocytes, in which assembly and expression of functional TCRα genes signals differentiation into $CD4^+$ or $CD8^+$ (single positive, SP) thymocytes that become mature $CD4^+$ or $CD8^+$ $\alpha\beta$ T cells.[30] The *Lckcre* transgenic mouse provides an experimental approach to pervasively delete and inactivate "floxed" genes in DN thymocytes concomitant with *Tcrδ* and *Tcrβ* recombination.[31,32] We have shown that *Lckcre* expression in $Atm^{-/-}$ mice does not alter their predisposition to clonal T-ALLs or the frequency of clonal t(12;14) translocations in these Atm-deficient T-ALLs.[23] Consequently, to assess whether *Tcrδ* translocations that inactivate one allelic copy of *Bcl11b* promote transformation of Atm-deficient thymocytes, we established and analyzed $Lckcre^{+/-}Atm^{-/-}Bcl11b^{flox/WT}$ (*LAb*), $Lckcre^{+/-}Atm^{-/-}$ (*LA*), and *Lckcre-Bcl11b*$^{flox/WT}$ (*Lb*) mice. We detected *Lckcre*-mediated deletion of $Bcl11b^{flox}$ alleles in total thymocytes and splenocytes of *LAb* and *Lb* mice, with near complete deletion in thymocytes (data not shown), confirming pervasive mono-allelic inactivation of *Bcl11b* in developing T cells. Thus, we created and aged parallel cohorts of 26 *LAb*, 8 *LA*, and *16 Lb* mice to evaluate their predisposition to T-ALL. We analyzed only eight cohort *LA* mice since we had previously characterized a larger cohort of *LA* mice.[23] We show here that our current cohort *LA* mice survived cancer-free between 82-138 days with a median age of cancer free-survival of 97 days (**Fig. 1A**), similar to the published median ages of cancer-free survival of *LA* and $Atm^{-/-}$ mice.[19-23] Our cohort *LAb* mice survived cancer-free between 74-294 days with a median age of cancer-free survival of 117 days (**Fig. 1A**), which was not significantly different than the median age of cancer-free survival of cohort *LA* mice. All cohort *LA* and *LAb* mice were euthanized due to thymic cancers that caused respiratory stress, except for one *LAb* mouse that developed large masses of cancer cells in the spleen and lymph nodes (**Table 1**; data not shown). All cohort *Lb* mice survived cancer-free during the one-year study, except for one that succumbed to a thymic malignancy detected during necropsy (**Fig. 1A**). Our data indicate that pervasive mono-allelic inactivation of *Bcl11b* starting in DN thymocytes does not accelerate the mortality of $Atm^{-/-}$ mice from thymic malignancies.

Similar to A-T children,[3] $Atm^{-/-}$ and *LA* mice succumb to mainly clonal T-ALLs that arise from a single thymocyte and do not express surface TCRβ or TCRα but do express CD4 and CD8 or just CD8.[14,19-23] To determine the effect of *Lckcre-*

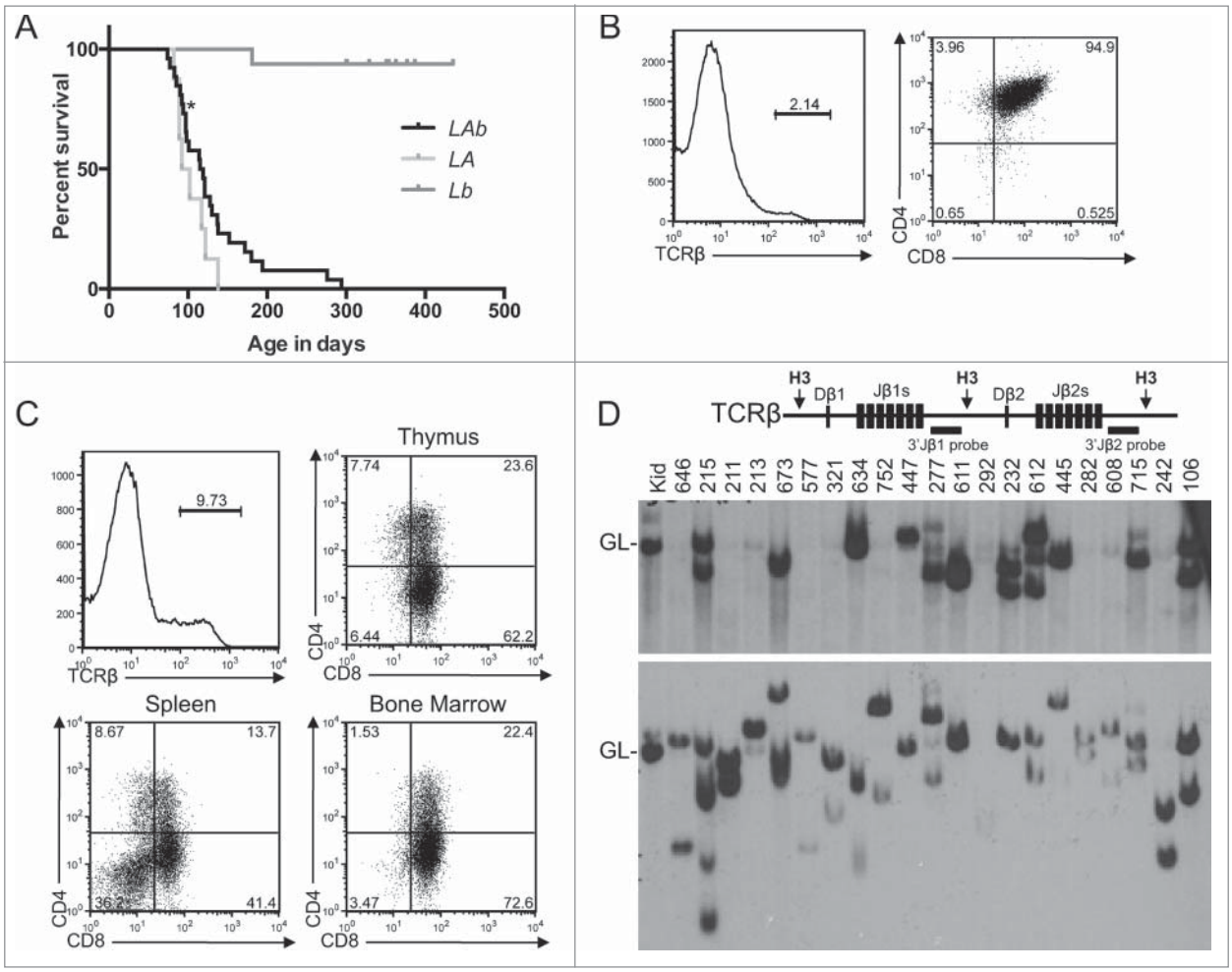

Figure 1. T-ALL Predisposition of *Atm*$^{-/-}$ Mice with Pervasive Mono-Allelic Deletion of *Bcl11b* initiating in DN Thymocytes. (**A**) Kaplan-Meier curve depicting the cancer-free survival of parallel cohorts of 26 *LAb*, 8 *LA*, and *16 Lb* mice. All cohort mice succumbed to thymic T-ALLs except for *LAb* mouse #211 that succumbed to a T-ALL in peripheral lymphoid tissues (indicated by asterisk). These mice were of a mixed C57BL6 and 129SvEv background. (**B-C**) Flow cytometry analysis of *LAb* T-ALLs no. 321 (**B**) and no. 277 (**C**) showing surface expression of TCRβ or CD4 and CD8. This analysis was conducted as described (33). Gates were drawn using normal thymocytes or splenocytes. The percentages of cells in each gate are indicated. (**D**) Schematic of the *Tcr*β locus (top) and Southern blot analyses of *Tcr*β rearrangements (bottom). Top, shown are relative locations of the indicated *Tcr*β segments, *Hind*III restriction sites (H3), and 3′Jβ1 and 3′Jβ2 probes. Bottom, Southern blots of *Hind*III-digested DNA from the indicated *LAb* thymic cancers (and *LAb* splenic cancer #211) or from the kidney of a *WT* mouse using the 3′Jβ1 or 3′Jβ2 probe as previously described.33 Germline (GL) bands for each probe are indicated. The membrane was hybridized with the 3′Jβ1 probe and then stripped and hybridized with the 3′Jβ2 probe, revealing which *LAb* cancers lack 3′Jβ1-hybridizing band(s) due to Vβ-to-Dβ2-Jβ2 rearrangements on both alleles. The images were cropped from a larger blot. Mouse no. 634 was removed from the cohort for incorrect genotyping.

mediated mono-allelic deletion of *Bcl11b* on the predisposition of *Atm*$^{-/-}$ mice to clonal T-ALLs from thymocytes of later stages of $\alpha\beta$ T cell development, we analyzed TCRβ, CD4, and CD8 expression and TCRβ rearrangements in *LAb* T-ALLs. First, we used flow cytometry to analyze expression of TCRβ, CD4, and CD8 proteins on cells isolated from the thymuses, spleens, lymph nodes, and bone marrow of euthanized cohort *LAb* mice. All 19 *LAb* thymic cancers assayed were TCRβ^- and either CD4$^+$CD8$^+$ or CD8$^+$ (**Fig. 1B**,**C**; **Table 1**). Many mice that succumbed to thymic cancers also harbored cells in their spleens, lymph nodes, and bone marrow with identical TCRβ, CD4, and CD8 expression as their malignant thymocytes (**Fig. 1C**; data not shown), suggesting dissemination of a single T-ALL. The one *LAb* peripheral lymphoid malignancy was TCRβ^- and CD4$^+$CD8$^+$ (**Table 1**). Next, we conducted Southern blot analysis to identify *Tcr*β rearrangements in representative *LAb* malignancies. *Tcr*β rearrangements cause deletion or changes in the size of 3′Jβ1-hybridizing bands and changes in the sizes of 3′Jβ2-hybridizing bands.[33] For normal DN thymocytes to survive, proliferate, and differentiate into DP thymocytes, *Tcr*β recombination must occur on at least one allele.[34] We found that all but three *LAb* cancers analyzed contained one or two rearranged *Tcr*β alleles and therefore arose from a single thymocyte (**Fig. 1D**). Of the remaining three: one (no. 215) had four *Tcr*β rearrangements indicative of two distinct cancers or a single cancer that continued *Tcr*β recombination; one (no. 447) had only

Table 1. Analysis of *LAb* tumor cohort

Mouse	Age at Death	Gross phenotype	Surface expression
8	94	thymic lymphoma	n.d.
95	138	thymic lymphoma	n.d.
106	92	thymic lymphoma	TCRβ^- DP/CD8$^+$
211	98	disseminated disease	TCRβ^- DP
213	121	thymic lymphoma	TCRβ^- CD8$^+$
215	77	thymic lymphoma	TCRβ^- DP/CD8$^+$
232	137	thymic lymphoma	TCRβ^- DP
242	114	thymic lymphoma	TCRβ^- CD8$^+$
277	115	thymic lymphoma	TCRβ^- CD8$^+$
282	119	thymic lymphoma	TCRβ^{int} DP
292	101	thymic lymphoma	TCRβ^{int} DP/CD8$^+$
321	97	thymic lymphoma	TCRβ^- DP
445	172	thymic lymphoma	TCRβ^- DP/CD8$^+$
447	130	thymic lymphoma	TCRβ^- CD8$^+$
538	85	thymic lymphoma	n.d.
557	74	thymic lymphoma	TCRβ^- DP
608	194	thymic lymphoma	TCRβ^- CD8$^+$
611	294	thymic lymphoma	TCRβ^- DP/CD8$^+$
612	180	thymic lymphoma	TCRβ^{int} DP/CD8$^+$
646	97	thymic lymphoma	TCRβ^- DP/CD8$^+$
673	90	thymic lymphoma	TCRβ^- CD4/8 var
715	83	thymic lymphoma	TCRβ^- DP
751	276	thymic lymphoma	n.d.
752	152	thymic lymphoma	TCRβ^- DP/CD8$^+$
771	121	thymic lymphoma	n.d.
938	128	thymic lymphoma	n.d.

germline *Tcrβ* allele(s) consistent with malignant transformation prior to *Tcrβ* recombination or a cancer with aberrant *Tcrβ* rearrangement on one allele; and one (no. 292) lacked detectable *Tcrβ* alleles suggesting aberrant *Tcrβ* rearrangements on both alleles (**Fig. 1D**). These flow cytometry and Southern blot analyses of *LAb* T-ALLs indicate that pervasive mono-allelic inactivation of *Bcl11b* in DN cells does not effect the predisposition of *Atm*$^{-/-}$ mice to clonal T-ALLs arising from thymocytes of later stages of $\alpha\beta$ T cell development.

To determine the effect of *Lckcre*-mediated mono-allelic deletion of *Bcl11b* on the frequency of clonal t(12;14) translocations that delete *Bcl11b* in *Atm*$^{-/-}$ T-ALLs, we conducted Spectral Karyotyping (SKY) and PCR on representative *LAb* T-ALLs. SKY is a molecular cytogenetic method of analyzing metaphase spreads that identifies chromosome translocations.[35] We and others have previously shown that the clonal t(12;14) translocations that delete the telomeric end of chromosome 12 occur in ~60% of T-ALLs that arise in *Atm*$^{-/-}$ and *LA* mice.[21-23] In contrast, we show here that only one of the 10 *LAb* T-ALLs analyzed by SKY (no. 211) harbored a clonal t(12;14) translocation (**Fig. 2A**, **Table 2**). Yet, this T-ALL also contained a clonal t(14;12) translocation and lacked a normal chromosome 12 (**Fig. 2A**, **Table 2**), neither of which is observed in *Atm*$^{-/-}$ or *LA* T-ALLs with the clonal t(12;14) translocation that deletes the telomeric end of chromosome 12 through *Bcl11b*.[21-23] To assess whether *LAb* T-ALL no. 211 had a mono-allelic deletion of the *Bcl11b* locus, we conducted PCR with primers that amplify *Bcl11b* and distinguish among wild-type (*WT*), "floxed" (*flox*),

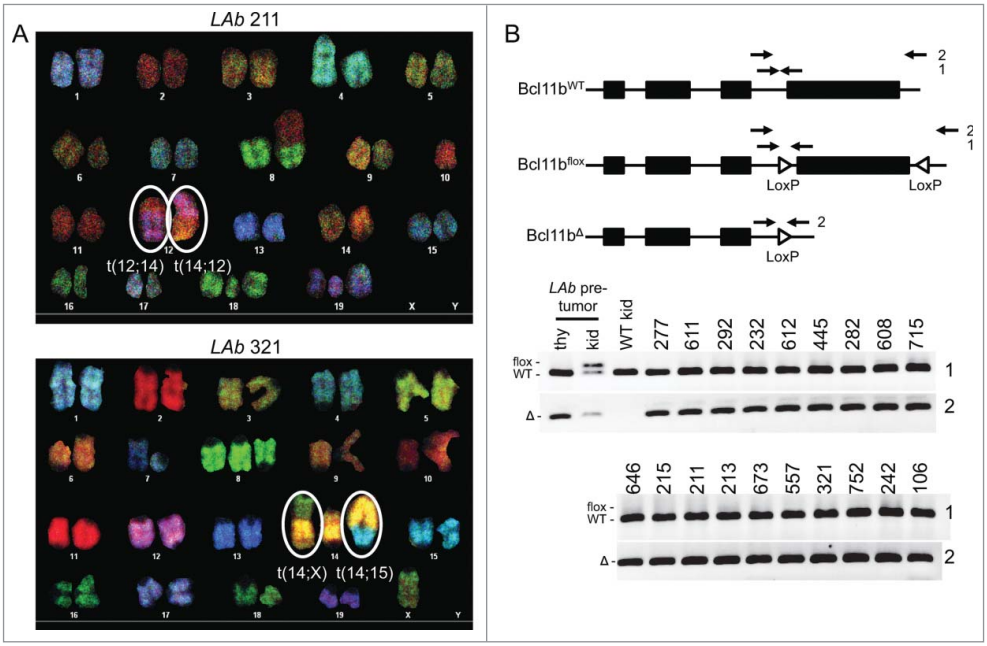

Figure 2. *LAb* T-ALLs Lack Clonal Chromosome t(12;14) Translocations that Delete *Bc111b*. (**A**) SKY images of metaphase spreads prepared from *LAb* T-ALLs #211 or #321. The clonal chromosome t(12;14) and t(14;12) translocations of *LAb* T-ALL #211 and the clonal chromosome t(14;X) and t(14;15) translocations of *LAb* T-ALL #321 are circled. (**B**) Schematics (top) and PCR analyses (bottom) of *Bcl11b*^{WT}, *Bcl11b*^{flox}, and *Bcl11b*$^{\Delta}$ alleles. Top, shown for each allele are locations of the *Bcl11b* exons, *loxP* sites, and PCR primers. The no. 1 primer set amplifies distinct bands from *Bcl11b*^{WT} and *Bcl11b*^{flox} alleles, while the no. 2 primer set amplifies a band from only *Bcl11b*$^{\Delta}$ alleles. Bottom, Images of PCR no. 1 or no. 2 products from genomic DNA of the indicated *LAb* T-ALLs, *LAb* thymocytes, *LAb* kidney, or *WT* kidney. The images were cropped from a larger blot.

Table 2. LAb SKY summary

Tumor	Clonal Translocations	Non-clonal Translocations
8	t(12;1)	none
211	t(12;14) t(14;12) t(4;4)	t(15;9) t(4;15) t(1;2) t(14;16) t(17;19) t(2;8) t(8;11) t(4;16)
213	none	t(12;19) t(2;7) t(4;2) t(14;1) t(14;2) t(14;12) t(19;Y)
215	none	t(7;19) t(12;1) t(6;13) t(12;14) t(14;6) t(2;2) t(11;14) t(10;12) t(19;Y) t(12;19;1) t(6;1) t(12;7)
277	t(12;16) t(14;16)	t(2;6) t(6;2) t(12;12)
292	t(12;1) t(11;3)	none
321	t(14;15) t(14;X)	none
608	none	t(17;19) t(12;16) t(12;Y) t(15;19;16) t(12;1) t(19;5) t(14;1)
611	t(14;14)	none
751	t(6;19) t(19;6) t(12;Y)	none

and *Lckcre*-deleted (Δ) *Bcl11b* alleles (**Fig. 2B**). We found equal intensities of $Bcl11b^{WT}$ and $Bcl11b^{\Delta}$ products (**Fig. 2B**), indicating that neither the clonal t(12;14) translocation nor the clonal t (14;12) translocation deleted either copy of *Bcl11b*. Four other *LAb* T-ALLs (nos. 8, 277, 292, and 751) also had clonal translocations involving chromosome 12 (**Table 2**), but each retained a normal chromosome 12 and their $Bcl11b^{WT}$ and $Bcl11b^{\Delta}$ loci (**Fig. 2B**; data not shown). The other five *LAb* T-ALLs analyzed by SKY and PCR each harbored two normal copies of chromosome 12 (**Table 2**) and retained their $Bcl11b^{WT}$ and $Bcl11b^{\Delta}$ loci (**Fig. 2B**). In addition, none of the other 12 *LAb* T-ALLs that we analyzed only by PCR had deletions of either their $Bcl11b^{WT}$ or $Bcl11b^{\Delta}$ loci (**Fig. 2B**). These data indicate that pervasive mono-allelic inactivation of *Bcl11b* initiating in $Atm^{-/-}$ DN cells concomitant with *Tcrδ* rearrangements precludes development of T-ALLs with t(12;14) translocations that delete *Bcl11b* or with independent deletion of *Bcl11b*.

Discussion

The selection of clonal t(12;14) translocations that delete the telomeric end of chromosome 12 in $Atm^{-/-}$ T-ALLs indicates that thee recurrent lesions effect genetic changes that promote malignant transformation of Atm-deficient thymocytes. Our finding that pervasive mono-allelic deletion of *Bcl11b* initiating in $Atm^{-/-}$ DN thymocytes concomitant with *Tcrδ* rearrangements precludes the development of T-ALLs with t(12;14) translocations that delete *Bcl11b* demonstrates that inactivation of a single copy of *Bcl11b* is a major genetic change that drives malignant transformation of $Atm^{-/-}$ thymocytes. However, the inability of such pervasive mono-allelic *Bcl11b* deletion to cause more rapid onset of T-ALL and/or promote the development of polyclonal T-ALLs indicates that formation of t(12;14) translocations that delete *Bcl11b* is not a limiting factor in pathogenesis of $Atm^{-/-}$ T-ALLs. Consistent with this notion, t(12;14) translocations and analogous t(14;14) translocations/inversions arise in ~1% of ATM-deficient αβ T cells in mice and humans, respectively, however $Atm^{-/-}$ mice and A-T children succumb to T-ALLs that arise from a single immature αβ T cell at ages in their lives when billions of mature αβ T cells have already developed. The $Atm^{-/-}$ T-ALLs with clonal t(12;14) translocations that delete *Bcl11b* also harbor additional clonal oncogenic lesions, such as *Pten* deletion or *Notch1* activation, that differ among these malignancies.[21] Since chromosome t(12;14) translocations in $Atm^{-/-}$ αβ T cells arise concomitant with Tcrβ-mediated proliferation and differentiation of DN thymocytes,[36] it is likely that the acquisition and selection of additional oncogenic lesions during DN-to-DP thymocyte expansion is necessary and rate- limiting for transformation of $Atm^{-/-}$ thymocytes lacking one copy of *Bcl11b*. The t(12;14) translocations of $Atm^{-/-}$ αβ T cells arise from aberrant repair between DSBs induced by the RAG proteins at *Tcrδ* loci on chromosome 14 and DSBs induced by other factors on the telomeric end of chromosome 12. RAG DSBs induced at *Igh* loci near the chromosome 12 telomere contribute to generation of t(12;14) translocations.[24] However, considering that $Atm^{-/-}$ mice lacking Rag2 and expressing a TCRβ transgene that drives thymocyte proliferation still succumb to T-ALLs with clonal translocation/deletion of the region of chromosome 12 where *Bcl11b* resides,[19] DSBs arising from DNA replication errors also likely contribute to formation of these oncogenic translocations.

Our data reveal that antigen receptor locus translocations can drive malignant transformation of lymphocytes through deletion and inactivation of a tumor suppressor gene. It has long been known that clonal antigen receptor locus translocations found in lymphoid malignancies promote transformation by unleashing the activities of oncogenes. Therefore, therapies for human leukemias and lymphomas are being developed that target and inactivate these oncogenes or their downstream signaling pathways or targets. In addition to their t(12;14) translocations that delete *Bcl11b*, $Atm^{-/-}$ T-ALLs harbor di-centric chromosome 14 derivatives with amplification of sequences centromeric of *Tcrα/δ* loci.[21] Although we also observed this amplicon in the one human T-ALL analyzed,[21] the amplified sequences lack any known oncogenes. In addition, *LAb* T-ALLs contain neither dicentric chromosome 14 derivatives nor amplification of sequences centromeric of *Tcrα/δ* loci (data not shown). Accordingly, our data suggest that the clonal *TCRα/δ;14q32* translocations in T-ALLs arising in A-T children most likely promote transformation of *ATM*-deficient thymocytes through deletion and inactivation of one allelic copy of *BCL11B*. Although strong evidence indicates that BCL11B is a haploinsufficient suppressor of T-ALL, the mechanisms by which bi-allelic *BCL11B* expression inhibits transformation and by which mono-allelic expression of BCL11B leads to T-ALL are unknown. Expression of *Bcl11b* in

thymocytes silences stem/progenitor cell gene expression programs to prevent TCR-independent self-renewal and cellular proliferation before initiation of TCR recombination and thereby promotes $\alpha\beta$ T cell development.[37-39] In human and mouse T lineage cells developed beyond the DN thymocyte stage, decreased BCL11B expression appears to compromise the cellular response to DNA replication stress leading to increased apoptosis of cells from DNA damage during S phase.[40] Since ATM stimulates DNA repair and induces apoptosis if DNA damage is too severe, mono-allelic BCL11B expression in ATM-deficient thymocytes could lead to increased frequency of additional oncogenic lesions arising from the aberrant repair of DSBs induced during DN-to-DP thymocyte proliferation. *LAb* mice provide a useful pre-clinical model to elucidate how inherited ATM deficiency and acquired BCL11B haploinsufficiency cooperate to cause T-ALL, and to design and test efficacy of T-ALL therapies that target intrinsic properties of these cancer cells.

Materials and Methods

Mice

Lck-cre[31] *Atm*$^{-/-}$,[41] and *Bcl11b*$^{flox/flox}$[42] mice of a mixed 129SvEv and C57BL/6 were bred to create experimental animals. Mice of both sexes and normal weight were studies. Expect for cohort mice, all mice were analyzed between 4-6 weeks of age. Cohort mice were monitored and euthanized on signs of distress. These studies were conducted under the approval and monitoring of the Children's Hospital of Philadelphia IACUC.

Flow cytometry

Cells were stained in PBS with 3% FBS using APC-anti-TCRβ, FITC-anti-CD8, PE-anti-CD4, PE-Cy7-anti-B220, FITC-anti-CD43, and APC-anti-IgM antibodies (BD Pharmingen). Data were collected using a FACSCalibur with CellQuest software (BD Biosciences) and analyzed by FlowJo software (Tree Star). Statistics were performed in Microsoft Excel or Graphpad Prism 5 using a two-tailed unpaired Student's t-test.

Kaplan-meier analysis

Curves were generated in Prism 5 and compared using the log-rank (Mantel-Cox) test.

Southern blotting

DNA (20 μg) was digested with 100 units of indicated enzymes (New England Biolabs), separated on 0.8% TAE gels, transferred onto Zeta-probe (BioRad), and hybridized with[32] P-labeled 3′Jβ1, 3′Jβ2, 5′Jα, 5′Tp53 or 3′Tp53 probes as described.[33]

Spectral karyotyping

Metaphases were prepared as described.[23] Spectral karyotyping was performed per instructions (Applied Spectral Imaging, ASI). Slides were examined with a BX61 microscope (600× magnification) from Olympus controlled by a LAMBDA 10-B Smart Shutter (Sutter Instrument). Images were captured using a LAMBDA LS light source (Sutter) and a COOL-1300QS camera (ASI), and analyzed by Case Data Manager Version 5.5 (ASI).

PCR. Reactions were performed as describe.[42] The no. 1 PCR primer set used the following two oligos: 5′-ACTGCACACGTGACTCCAAG-3′ and 5′-AAGCCATGTGTGTTCTGTGC-3′ primers. The no. 2 PCR primer set used the following two oligos: 5′-CGTGTTCCCTTGCCGTCGGGGGAGG-3′ and 5′-GCT TCCCTCTACGTCACTTGCGAGT-3′.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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