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LETTER TO THE EDITOR

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Haploinsufficiency of Bcl11b suppresses the progression of ATM-deficient T cell lymphomas

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Abstract

Bcl11b is a transcription factor important for T cell development and also a tumor-suppressor gene that is hemizygously inactivated in ~10 % human T cell acute lymphoblastic leukemia (T-ALL) and several murine T-ALL models, including $ATM^{-/-}$ thymic lymphomas. Here we report that heterozygous loss of Bcl11b ($Bcl11b^{+/-}$) unexpectedly reduced lethal thymic lymphoma in $ATM^{-/-}$ mice by suppressing lymphoma progression, but not initiation. The suppression was associated with a T cell-mediated immune response in $ATM^{-/-}Bcl11b^{+/-}$ mice, revealing a haploid insufficient function of Bcl11b in immune modulation against lymphoma and offering an explanation for the complex relationship between Bcl11b status with T-ALL prognosis.

Correspondence/findings

Bcl11b is a transcription factor that is "monoallelically" inactivated in ~10 % of human T cell acute lymphoblastic leukemia (T-ALL) [1–3] and several murine T-ALL models, including $ATM^{-/-}$ thymic lymphomas [4–6]. Both human and murine T-ALLs retain at least one intact copy of Bcl11b, indicating that Bcl11b is a bona fide haploinsufficient tumor-suppressor gene. Complete loss of the Bcl11b gene abrogates T cell development and gain of NK cell phenotype, revealing a critical role of Bcl11b in T cell lineage commitment [7–10]. Conditional inactivation of both copies of Bcl11b in double-positive (DP) T cells leads to overproduction of innate $CD8^+$ single-positive (SP) T cells [11] and compromises T-regulatory cell function [12]. Yet, hemizygous loss of Bcl11b has no measurable impact on T cell function [7–10] and the mechanism by which it promotes T-ALL remains elusive.

To address this, we characterized $Bcl11b^{+/-}ATM^{-/-}$ mice [6, 13]. ATM kinase is a master regulator of the DNA damage responses [14]. $ATM^{-/-}$ mice routinely succumb to immature T cell lymphomas sharing molecular features with human T-ALL. Of the $ATM^{-/-}$ thymic lymphomas, 80 % hemizygously deleted Bcl11b as a result of non-reciprocal t(12;14) translocation [6, 15]. Conditional inactivation of Bcl11b in T cells via LckCre eliminates recurrent t(12;14) translocations from $ATM^{-/-}$ thymic lymphomas, suggesting Bcl11b as the target of the large chromosome 12 deletion [16]. $ATM^{-/-}Bcl11b^{+/-}$ mice were born at expected frequency (Additional file 1: Figure S1A). Thymocyte development and peripheral T and B cell repertoire in young (4 week) $ATM^{-/-}Bcl11b^{+/-}$ mice were indistinguishable from that of $ATM^{-/-}$ mice, displaying reduced surface TCRβ/CD3ε expression in DP cells and a partial blockade at the DP to SP transition characteristic for ATM-deficiency [6, 17] (Additional file 1: Figure S1B).

While we initially expected Bcl11b-deficiency to accelerate ATM-deficient thymic lymphomas based on its frequent inactivation in T-ALLs, hemizygous-deletion of Bcl11b prevented lethal thymic lymphoma in ~50 % $ATM^{-/-}$ mice and only 2/11 $ATM^{-/-}Bcl11b^{+/-}$ mice developed overt thymic lymphomas (Fig. 1a). The median survival of $ATM^{-/-}Bcl11b^{+/-}$ mice was significantly longer than that of $ATM^{-/-}$ controls (167 vs. 106 days, $p < 0.01$) (Fig. 1a). Analyses of 3- and 10-month-old $ATM^{-/-}Bcl11b^{+/-}$ mice revealed clonal expansion of immature ($surfaceTCR\beta^{low}$) thymocytes in 3-month, but not 10-month-old $ATM^{-/-}Bcl11b^{+/-}$ mice (Fig. 1b, c). T cell lymphomas from 3-month-old $ATM^{-/-}Bcl11b^{+/-}$ mice retained both WT and null alleles of Bcl11b, consistent with the lack-of-LOH in T-ALL (Fig. 1d). Despite the clonal

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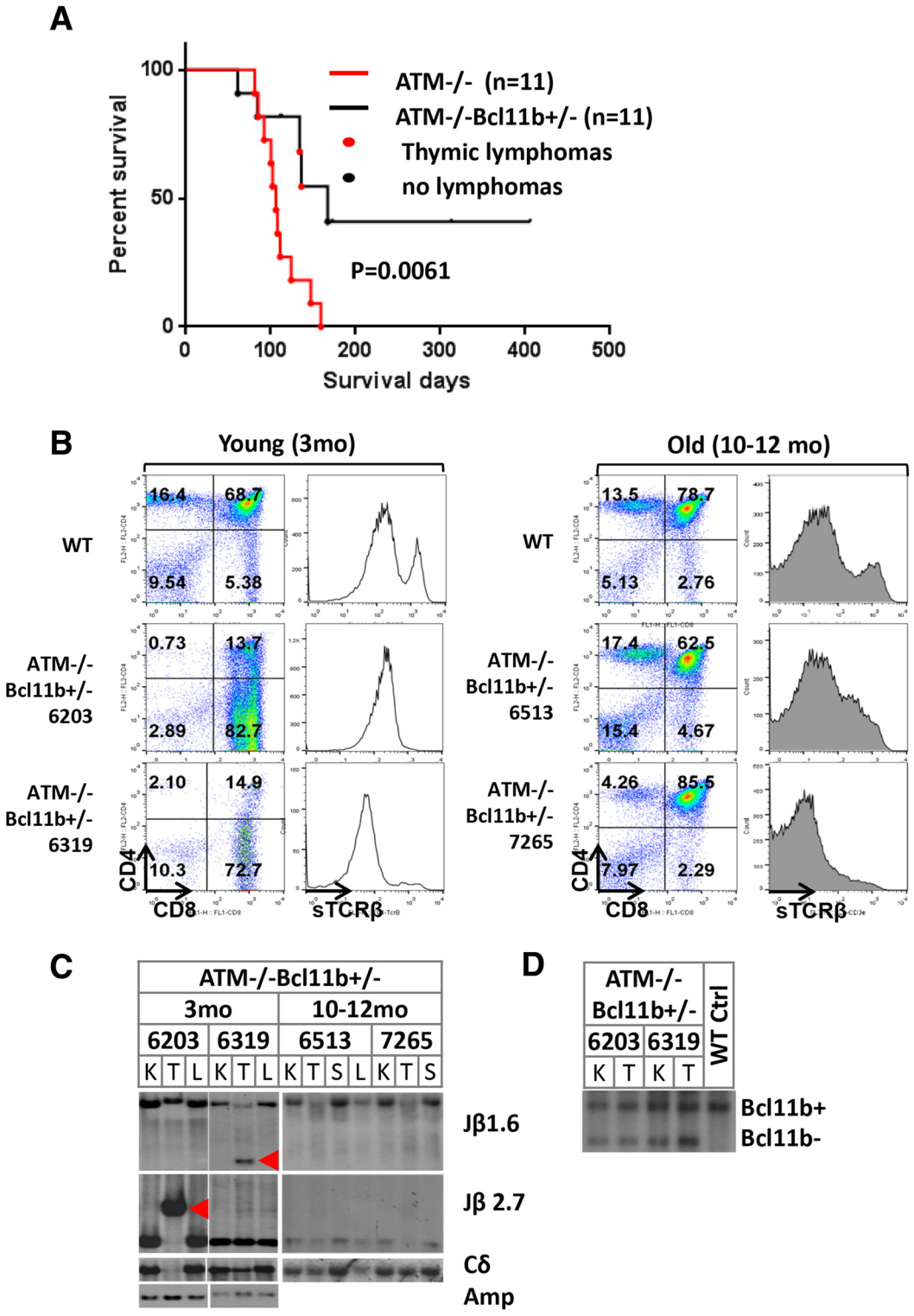

Fig. 1 Heterozygous loss of Bcl11b suppresses the progression, but not the initiation of ATM-deficient thymic lymphomas. **a** Thymic lymphoma-free survival of $ATM^{-/-}$ and $ATM^{-/-}Bcl11b^{+/-}$ mice. Median survival of $ATM^{-/-}$ and $ATM^{-/-}Bcl11b^{+/-}$ cohorts was 106 and 169 days, respectively. *p* value for log-ranking test is 0.0061. **b** Representative flow cytometry analyses of the thymus from control and $ATM^{-/-}Bcl11b^{+/-}$ mice at 3 or 10 months of age. **c** Southern blot analyses of EcoRI digested genomic DNA from kidney (K), thymus (T), enlarged submandibular lymph nodes (L), or spleen (S) from $ATM^{-/-}Bcl11b^{+/-}$ mice probed with TCRβ probes (Jβ1.6 or 2.7), TCRδ constant region (Cδ) or chromosome 14 amplification region (Amp) [6]. **d** Southern blot analyses of Bcl11b locus on KpnI digested genomic DNA with Bcl11b probe [13]

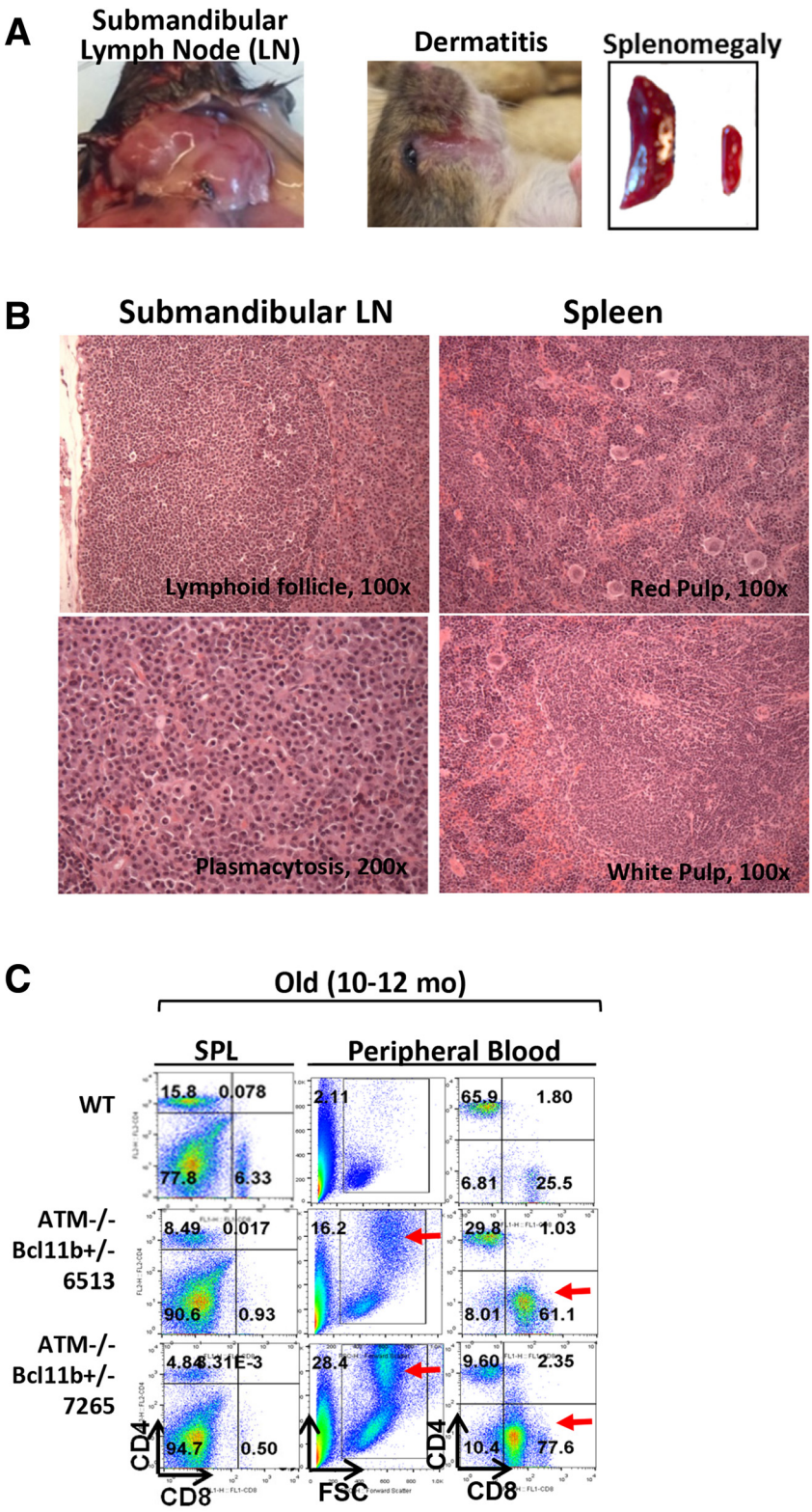

Fig. 2 Heterozygous loss of Bcl11b induces an inflammatory/immune response in ATM$^{-/-}$ mice. **a** Representative pictures of enlarged submandibular lymph nodes, dermatitis, and splenomegaly in ATM$^{-/-}$Bcl11b$^{+/-}$ mice. **b** Histopathologic analysis of the submandibular lymph nodes and spleen in ATM$^{-/-}$Bcl11b$^{+/-}$ mice. **c** Flow cytometry analysis shows significant enrichment of CD8^{+} SP T cells in the peripheral blood, but not the spleen of 10-month-old ATM$^{-/-}$Bcl11b$^{+/-}$ mice

expansion in 3-month-old $ATM^{-/-}Bcl11b^{+/-}$ mice, most thymic lymphomas failed to progress to lethal disease (Additional file 1: Figure S1C), suggesting that heterozygous Bcl11b-deficiency suppresses the progression, but not the initiation of $ATM^{-/-}$ thymic lymphomas.

Notably, almost all $ATM^{-/-}Bcl11b^{+/-}$ mice developed variable degrees of splenomegaly, excessive submandibular lymph node (LN) enlargement that manifested to fatal airway obstruction (non-lymphoma-related death), and dermal inflammation commencing at 2–3 months of age (Fig. 2a). In contrast to normal lymphocyte profiles in 3-month-old $ATM^{+/+(+/-)}Bcl11b^{+/-}$ mice (Additional file 1: Figure S2A and S2B), histologic analyses consistently revealed a lymphocyte-mediated inflammatory/immune disorder in $ATM^{-/-}Bcl11b^{+/-}$ mice characterized by marked plasmacytosis with reactive germinal centers ($B220^{+}IgM^{+}$ $Bcl6^{+}CD138^{-}$ B cells) in submandibular LN, reactive follicular hyperplasia of the white pulp and increased extramedullary hematopoiesis in the red pulp in the spleen (Fig. 2b and Additional file 1: Figure S2C), and acute and chronic dermal inflammation in the skin. While a cell-autonomous function of Bcl11b deletion on epidermal integrity cannot be ruled out [13], the splenic and LN changes noted raised the possibility of an autoimmune disorder, which could have contributed to the lack of tumor progression in $ATM^{-/-}Bcl11b^{+/-}$ mice. Correspondingly, 10-month tumor-free $ATM^{-/-}Bcl11b^{+/-}$ mice accumulated activated $CD8^{+}$ T cell in PB (Fig. 2c).

Our data suggest that in an ATM-deficient background, heterozygous Bcl11b deficiency tilts immune homeostasis and limits the expansion, but not the initiation of ATM-deficient thymic lymphomas. Notably, homozygous Bcl11b deletion suppressed melanoma in murine models [18]. Given the role of Bcl11b in T cell lineage commitment, $CD8^{+}$ T cell development, and T-reg function, our data suggest that heterozygous Bcl11b deficiency can modulate anti-tumor immune response despite the lack of measurable T cell development defects in $Bcl11b^{+/-}$ mice [7–10]. This role of Bcl11b in immune modulation and tumor suppression might explain the discrepancies between Bcl11b status (mutation, deletion, and downregulation) and T-ALL prognosis in different studies [1–3, 19]. It also suggests that Bcl11b is likely lost later during T-ALL development, as early deletion likely causes autoimmune dysfunction, analogous to TNFAIP3 (A20) in DLBCL [20].

Additional file

Additional file 1: Figure S1. Characterization of T cell development in $ATM^{-/-}Bcl11b^{+/-}$ mice. (A) Actual and expected offspring from breeding between $Bcl11b^{+/-}ATM^{+/-}$ and $ATM^{+/-}$ mice. The *p* value calculated based on chi-squared test is 0.89. (B) Representative flow cytometric analysis of pre-malignant thymocytes from WT, $ATM^{-/-}$ and $ATM^{-/-}Bcl11b^{+/-}$ mice (4–6 weeks). **Figure S2.** Analyses of autoimmunity in $ATM^{-/-}Bcl11b^{+/-}$ mice. (A) Flow cytometric analysis of the spleen, bone marrow, and lymph nodes from 3-month and 10-month-old $ATM^{-/-}Bcl11b^{+/-}$ mice. Notably, the frequency of the $CD11b^{+}$ myeloid cells increases in the spleen of $ATM^{-/-}Bcl11b^{+/-}$ mice, consistent with increased extramedullary hematopoiesis in the red pulp seen in Fig. 2b. (B) Flow cytometric analyses of the thymus and spleen (SPL) from 3-month-old littermate $ATM^{+/+}Bcl11b^{+/+}$ (WT), $Bcl11b^{+/-}$, $ATM^{+/-}Bcl11b^{+/-}$ mice. Double negative (DN) staining was performed on gated CD8-CD4-CD19-TCRγ/δ^{-} thymocytes. Homozygous deletion of Bcl11b dramatically increased the number of phenotypical NK cells [8, 9]. The percentage of $NK1.1^{+}$ or $CD8^{+}$ cells does not significantly increase in the thymus and spleen of 3-month-old $Bcl11b^{+/-}$ mice. (C) Immunohistochemical staining with germinal center marker Bcl6 and plasma cell marker CD138 of the enlarged submandibular lymph nodes. (PDF 2082 kb)

Abbreviations

ALL: acute lymphoblastic leukemia; ATM: ataxia telangiectasia mutated; BM: bone marrow; DN: double negative; DP: double positive; LN: lymph node; PB: peripheral blood; SP: single positive; SPL: spleen.

Authors' contributions

WJ, KP, and SZ designed the experiments. KP, WJ, and SZ wrote the manuscript. GB performed the histopathologic analysis. KP, WJ, BJL, DGL, CL, and SZ performed the experiments.

Competing interests

The authors declare that they have no competing interests.

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