Gata2 +9.5 enhancer regulates adult hematopoietic stem cell self-renewal and T-cell development

Xiaona You,[1,2] Yun Zhou,[1,2] Yuan-I Chang,[1] Guangyao Kong,[1] Erik A. Ranheim,[3] Kirby D. Johnson,[2,4] Alexandra A. Soukup,[2,4] Emery H. Bresnick,[2,4] and Jing Zhang[1,2]

[1]McArdle Laboratory for Cancer Research, [2]Wisconsin Blood Cancer Research Institute, and [3]Department of Pathology and Laboratory Medicine, University of Wisconsin–Madison, Madison, WI; and [4]Carbone Cancer Center, Department of Cell and Regenerative Biology, University of Wisconsin School of Medicine and Public Health, Madison, WI

Key Points

- Gata2 +9.5 enhancer regulates adult hematopoietic stem cell self-renewal.
- Gata2 +9.5 enhancer regulates T-cell development at the lymphoid-primed MPP3 stage.

Mammalian GATA2 gene encodes a dual zinc finger transcription factor, which is essential for hematopoietic stem cell (HSC) generation in the aorta, gonad, mesonephros (AGM) region, HSC self-renewal, and specification of progenitor cell fates. Previously, we demonstrated that Gata2 expression in AGM is controlled by its intronic +9.5 enhancer. Gata2 +9.5 deficiency removes the E-box motif and the GATA site and depletes fetal liver HSCs. However, whether this enhancer has an essential role in regulating adult hematopoiesis has not been established. Here, we evaluate Gata2 +9.5 enhancer function in adult hematopoiesis. $+9.5^{+/-}$ bone marrow cells displayed reduced T cell reconstitution in a competitive transplant assay. Donor-derived analysis demonstrated a previously unrecognized function of the +9.5 enhancer in T cell development at the lymphoid-primed multipotent progenitor stage. Moreover, $+9.5^{+/-}$ adult HSCs displayed increased apoptosis and reduced long-term self-renewal capability in comparison with wild-type (WT) HSCs. These phenotypes were more moderate than those of $Gata2^{+/-}$ HSCs. Consistent with the phenotypic characterization, Gata2 expression in $+9.5^{+/-}$ LSKs was moderately higher than that in $Gata2^{+/-}$ LSKs, but lower than that in WT LSKs. Our data suggest that +9.5 deficiency compromises, without completely abrogating, Gata2 expression in adult HSCs.

Introduction

Mammalian GATA2 gene encodes a dual zinc finger transcription factor, which is essential for hematopoietic stem cell (HSC) generation from hemogenic endothelium in the aorta, gonad, mesonephros (AGM) region and HSC self-renewal.[1-3] Previously, we demonstrated that Gata2 expression in AGM is controlled by part of its intronic +9.5 enhancer.[2,4,5] Gata2 +9.5 deficiency removes the E-box motif and the GATA site and depletes fetal liver HSCs. $+9.5^{-/-}$ mouse embryos died at E13-14, revealing the important +9.5 enhancer function in embryonic HSC generation.[2,6,7] Furthermore, a +9.5 Ets motif mediates hematopoietic regeneration in response to myeloablation.[8] However, whether this enhancer has broad functions to regulate adult hematopoiesis has not been established. Here, we report that Gata2 +9.5 enhancer regulates adult HSC self-renewal and its haploinsufficiency impacts T cell development starting at the multipotent progenitor 3 (MPP3) stage.

The full-text version of this article contains a data supplement.
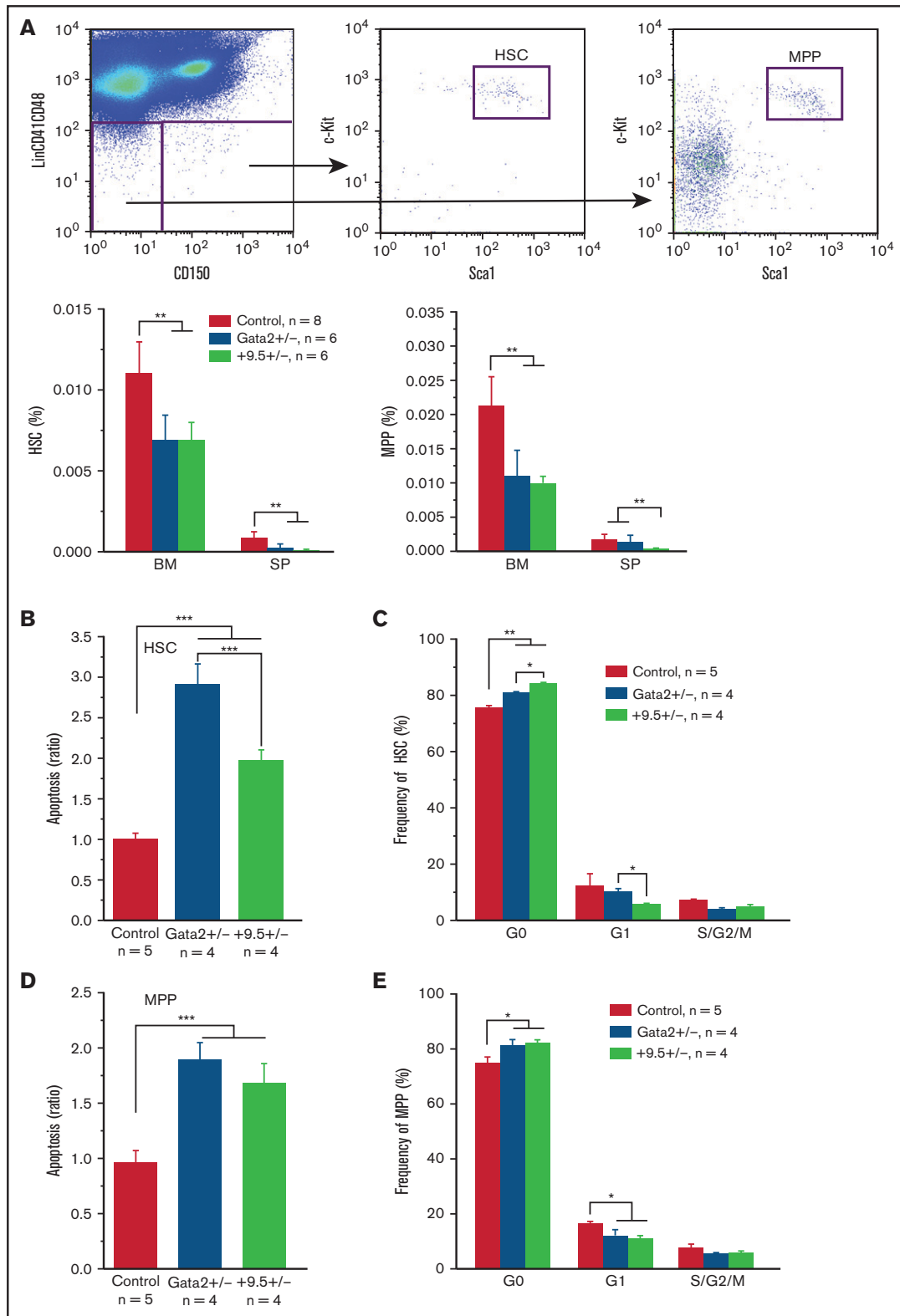
Figure 1. Increased apoptosis in *Gata2 +9.5*$^{+/-}$ and *Gata2*$^{+/-}$ HSPCs. Six-week-old WT C57BL/6 (Control), *Gata2 +9.5*$^{+/-}$ (+9.5$^{+/-}$), and *Gata2*$^{+/-}$ (Gata2$^{+/-}$) mice were analyzed. (A) Gating strategy for HSCs and MPPs. HSCs and MPPs are defined based on LSK SLAM markers as described in Methods. Their frequencies were quantified in BM and SP. (B,D) Quantification of apoptotic BM HSCs (B) and MPPs (D) using Annexin V and 4′,6-diamidino-2-phenylindole (DAPI). (C,E) Cell cycle profiling of BM HSCs (C) and MPPs (E) using Ki67 and DAPI. The results are presented as mean ± standard deviation (SD). *$P <$ .05; **$P <$ .01; ***$P <$ .001.
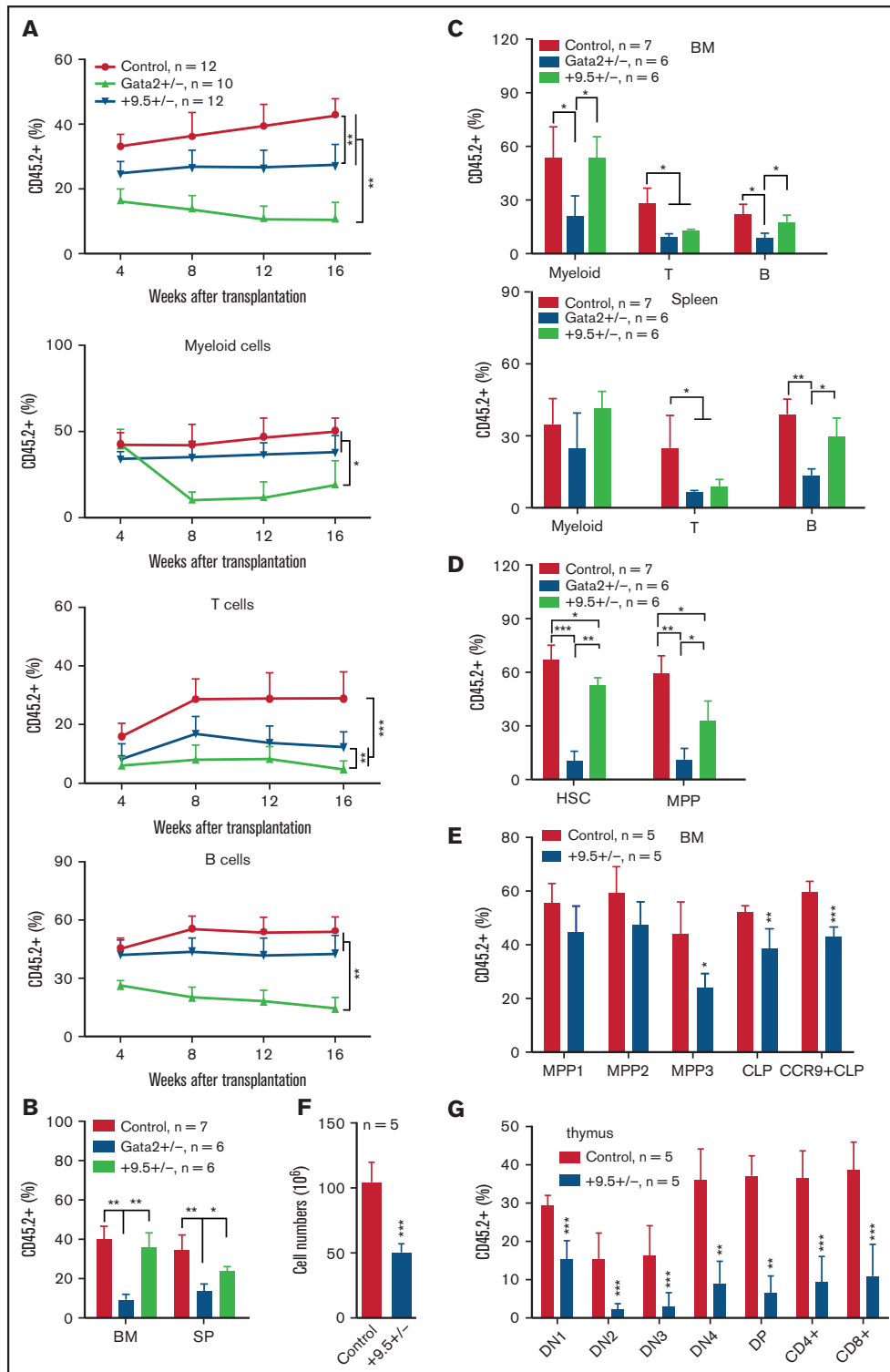
Figure 2. *Gata2 +9.5^{+/−}* BM cells display decreased reconstitution in primary recipients. A total of 1×10^6 CD45.2$^+$ BM cells from 6-week-old WT C57BL/6J (Control), *Gata2 +9.5^{+/−}* (+9.5^{+/−}), and *Gata2^{+/−}* (Gata2^{+/−}) mice were mixed with 1×10^6 CD45.1$^+$ competitor BM cells and injected into lethally irradiated recipients (CD45.1$^+$). (A) Quantification of donor-derived myeloid, T, and B cells in recipient peripheral blood every 4 weeks after transplantation. (B-D) Terminal evaluation was performed in primary recipients 16 weeks after transplant. (B) Quantification of donor-derived cells in BM and SP of primary recipients. (C) Quantification of donor-derived myeloid, T, and B cells in BM and SP of primary recipients. (D) Quantification of donor-derived HSCs and MPPs in BM of primary recipients. The results are presented as means ± SD. *$P < .05$; **$P < .01$; ***$P < .001$. (E-G) Recipients were killed at 8 weeks after transplant. (E) Quantification of donor-derived MPP1, MPP2, and MPP3; CLPs; and CCR9$^+$ CLPs in BM. (F) Quantification of thymic cell numbers. (G) Quantification of donor-derived T cells in thymus. The results are presented as means + SD. *$P < .05$; **$P < .01$; ***$P < .001$.

Methods

Mice

Gata2 germline heterozygous deletion mice (*Gata2*^{+/−})[3] and *Gata2* +9.5 *cis*-element germline heterozygous deletion mice (+9.5[+/−])[6] were maintained in a pure C57BL/6J genetic background (>N10). CD45.1[+] isogenic recipient mice were purchased from Jackson Laboratories. All animal experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* and approved by an Animal Care and Use Committee at University of Wisconsin-Madison. The program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Additional methods are described in supplemental Methods.

Results and discussion

We analyzed adult hematopoietic stem and progenitor cells (HSPCs) in 6-week-old wild-type (WT) control, +9.5[+/−], and *Gata2*^{+/−} mice. *Gata2*^{+/−} mice served as a positive control for reducing *Gata2* expression in adult hematopoietic compartment.[9,10] In contrast to normal numbers of fetal liver HSCs and MPPs in E12.5 +9.5[+/−] embryos,[6] both HSC and MPP compartments were significantly decreased in +9.5[+/−] bone marrow (BM) and spleen (SP) compared with those in WT, but largely comparable to those in *Gata2*^{+/−} mice (Figure 1A). The +9.5[+/−] HSC apoptosis was at a level intermediate to those of control and *Gata2*^{+/−} HSCs (Figure 1B), whereas their quiescence was higher (Figure 1C). By contrast, both +9.5[+/−] and *Gata2*^{+/−} MPPs displayed increased apoptosis (Figure 1D) and quiescence (Figure 1E) in comparison with WT MPPs. Quantification of Lin[−]Sca1^{+} cKit[+] (LSK) cells revealed that similar to HSCs and MPPs, +9.5[+/−] and *Gata2*^{+/−} LSK cells decreased in BM (supplemental Figure 1A), whereas the MP compartment was largely normal in +9.5[+/−] and *Gata2*^{+/−} mice (supplemental Figure 1B). In addition, consistent with the prior report,[10] *Gata2*^{+/−} LSKs exhibited increased apoptosis (supplemental Figure 1C) and quiescence (supplemental Figure 1D). The +9.5[+/−] LSKs showed a similar apoptotic rate and elevated quiescence compared with *Gata2*^{+/−} LSKs. These analyses revealed phenotypes in +9.5[+/−] adult HSCs and LSKs resembling those in *Gata2*^{+/−} cells but with significant quantitative differences.[10]

We performed a serial competitive reconstitution analysis of +9.5[+/−] HSC functions in vivo. WT, +9.5[+/−], and *Gata2*^{+/−} BM cells (CD45.2[+]) were mixed with competitor cells (CD45.1[+]) at a 1:1 ratio and transplanted into lethally irradiated recipients (CD45.1[+]). Consistent with what we found with +9.5[+/−] fetal liver cells, +9.5[+/−] cells in peripheral blood (PB) were significantly decreased ($P < .01$) in comparison with WT cells (Figure 2A). Interestingly, this reduced reconstitution mainly resulted from decreased donor-derived T cells, whereas donor-derived myeloid and B cells were comparable to those in recipients transplanted with WT cells. We killed recipients 16 weeks after transplant and analyzed donor-derived hematopoiesis. The +9.5[+/−]-derived cells were comparable in BM but lower in SP of primary recipients (Figure 2B). As with PB, +9.5[+/−]-derived T cells were reduced in BM and SP (Figure 2C). Moreover, we observed a moderate but significant reduction of +9.5[+/−]-derived HSCs and MPPs in comparison with WT recipients (Figure 2D). Consistent with reduced donor-derived HSCs and MPPs in primary recipients, +9.5[+/−]-derived cells displayed a decrease in multilineage reconstitution in secondary recipients (supplemental Figure 2A). Similar to the primary

transplantation, +9.5[+/−]-derived HSCs and MPPs were moderately decreased in BM of secondary recipients (supplemental Figure 2B). Consistent with previous studies,[9,10] *Gata2*^{+/−} BM cells exhibited dramatically reduced multilineage reconstitution ($P < .01$) in both primary and secondary recipients (Figure 2; supplemental Figure 2).

Our results demonstrated a previously unrecognized +9.5 enhancer function in T-cell development. To identify the relevant T-cell developmental stage(s), we analyzed donor-derived linage-biased MPPs and the downstream common lymphoid progenitors (CLPs) in control and +9.5[+/−] recipients 8 weeks after transplant as previously described.[11] MPP1 can generate all lineages of blood cells, MPP2 loses megakaryocyte/erythroid potential, whereas MPP3 is responsible for generating downstream CLPs and lymphocytes. We found that donor-derived MPP1 and MPP2 were comparable between control and +9.5[+/−] recipients (Figure 2E). By contrast, donor-derived MPP3 and CLP compartments were reduced in +9.5[+/−] recipients compared with control (Figure 2E). In particular, donor-derived CCR9^{+} CLPs, which migrate to the thymus and initiate thymic T-cell development, were also reduced in +9.5[+/−] recipients (Figure 2E). Not surprisingly, thymic T-cell cellularity in +9.5[+/−] recipients was decreased to ∼50% of that in control (Figure 2F). We analyzed thymic T-cell development from the Thy1.2^{+}CD4^{−}CD8^{−} double-negative (DN) cells to CD4^{+}CD8^{+} double-positive cells and to CD4^{+} and CD8^{+} T cells. DN thymocytes can be further divided into 4 successive subpopulations depending on the status of CD44 and CD25 expression: CD44^{+}CD25^{−} (DN1), CD44^{+}CD25^{+} (DN2), CD44^{−}CD25^{+} (DN3), and CD44^{−}CD25^{−} (DN4). Donor-derived thymic T cells were reduced at all stages in +9.5[+/−] recipients compared with control (Figure 2G). These results indicate that +9.5 regulates T-cell development at the MPP3 stage.

In addition, our data demonstrated that +9.5[+/−] HSCs and MPPs displayed more moderate phenotypes in self-renewal compared with *Gata2*^{+/−} cells, suggesting that in addition to the +9.5 partial enhancer we characterized, there is additional element(s) regulating *Gata2* expression in these cells. In support of our conclusion, heterozygous mutations in the Ets motif of +9.5 enhancer reduce *GATA2* expression and cause GATA2 deficiency syndromes in human.[5,6] These mutations impair hematopoietic regeneration in adult mice after myeloablation.[8] To determine the impact of +9.5 enhancer deletion on *Gata2* expression, we quantified *Gata2* mRNA levels in WT, +9.5[+/−], and *Gata2*^{+/−} LSK and MP cells using quantitative reverse transcriptase-polymerase chain reaction. Corroborating the prior study,[10] *Gata2* expression was decreased in *Gata2*^{+/−} LSKs and MPs. As expected, *Gata2* expression in +9.5[+/−] MP populations was comparable to that in *Gata2*^{+/−} MPs, whereas *Gata2* expression in +9.5[+/−] LSKs was moderately higher than that in *Gata2*^{+/−} LSKs but still lower than that of WT LSKs (supplemental Figure 3). Thus, the reduced self-renewal in +9.5[+/−] HSPCs is associated with *Gata2* downregulation.

In summary, we evaluated *Gata2* +9.5 enhancer function in adult hematopoiesis and demonstrated a +9.5 enhancer function to regulate adult HSC self-renewal. The impact of +9.5[+/−] on HSCs is less than *Gata2*^{+/−}, suggesting that +9.5 deficiency compromises, but does not completely abrogate, *Gata2* expression in adult HSCs. In sharp contrast to the +9.5 enhancer, *Gata2* −77 enhancer regulates adult HSC survival and the impact of −77[+/−] on HSCs is opposite to that of *Gata2*^{+/−}.[12] Moreover, *Gata2* +9.5 enhancer regulates T-cell development at the lymphoid-primed MPP3 stage, and T-cell development has not been analyzed in −77 enhancer mutant mice.

Authorship

Contribution: X.Y. provided experimental design and execution and wrote the manuscript; Y.Z., Y.-I.C., and G.K. executed the experiments; E.A.R. provided histopathology analysis and wrote the manuscript; Y.Z., K.D.J., and A.A.S. provided technical or material support; and E.H.B. and J.Z. provided experimental design and wrote the manuscript.

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ORCID profiles: X.Y., 0000-0001-9996-1466; G.K., 0000-0001-6662-0016; A.A.S., 0000-0002-4149-2393; J.Z., 0000-0003-1194-0666.

Correspondence: Jing Zhang, Room 7453, WIMR II, McArdle Laboratory for Cancer Research, 1111 Highland Ave, University of Wisconsin–Madison, Madison, WI 53705; e-mail: zhang@oncology.wisc.edu.

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