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Gata2 −77 enhancer regulates adult hematopoietic stem cell survival

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Letter to the Editor

GATA2 is a master transcription factor that governs hematopoietic stem cell (HSC) generation in the mammalian embryo and confers multi-lineage differentiation of hematopoietic stem and progenitor cells [1–4]. Previously, we demonstrated that *Gata2* expression in fetal liver myeloid progenitors (MPs) is controlled by its upstream −77 enhancer [5, 6]. While $-77^{-/-}$ fetal liver HSCs exhibited normal or greater than normal long-term repopulating activity, myeloid progenitor (MP) differentiation potential was impaired, thus causing anemia and embryonic lethality. However, whether the −77 enhancer has essential functions to regulate adult hematopoiesis has not been established.

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Author Contributions

The contributions of individual authors are listed below: XY for experimental design & execution as well as manuscript preparation; YIC and GK for experimental execution; EAR for histopathology analysis and manuscript preparation; YZ, KDJ, and CM for technical or material support; EHB and JZ for experimental design and manuscript preparation.

Conflict of Interest Disclosures

We declare no competing financial interests.

Methods are described in Supplementary Information.

We analyzed adult hematopoiesis in 6-week old wild-type (WT) control, $-77^{+/-}$, and $Gata2^{+/-}$ mice. $Gata2^{+/-}$ mice served as a positive control for reducing *Gata2* expression in adult hematopoietic stem and progenitor cells (HSPCs) [7, 8]. By contrast to normal fetal liver hematopoiesis in $-77^{+/-}$ embryos [5], the HSC compartment was enlarged in $-77^{+/-}$ bone marrow (BM) and spleen (SP), while the multipotent progenitor (MPP) compartment was elevated only in SP (Fig. 1A). $-77^{+/-}$ HSCs and MPPs exhibited decreased apoptosis (Fig. 1B and 1C) and increased quiescence (Fig. 1D and 1E). Our data suggest that increased $-77^{+/-}$ MPPs in SP may result from increased HSC numbers, decreased apoptosis, and/or increased quiescence. By contrast, HSC and MPP compartments were reduced in $Gata2^{+/-}$ mice (Fig. 1A), and these cells exhibited increased apoptosis (Fig. 1B) and quiescence (Fig. 1C). Quantification of Lin^{-} $Sca1^{+}$ $cKit^{+}$ (LSK) cells and MPs revealed that similar to HSCs and MPPs, $-77^{+/-}$ LSK cells expanded in SP (Fig. S1A) and exhibited decreased apoptosis (Fig. S1C) and increased quiescence (Fig. S1D). $Gata2^{+/-}$ LSKs were reduced in BM and exhibited increased apoptosis and quiescence, consistent with the previous report [8]. The MP compartment was largely normal in $-77^{+/-}$ and $Gata2^{+/-}$ mice (Fig. S1B). These analyses revealed unexpected phenotypes in $-77^{+/-}$ adult HSCs and MPPs that were not predictable from our analysis of $-77^{+/-}$ embryos [5] nor from $Gata2^{+/-}$ mice [8].

We conducted a serial competitive reconstitution analysis of $-77^{+/-}$ HSC functions *in vivo*. WT, $-77^{+/-}$, and $Gata2^{+/-}$ BM cells ($CD45.2^{+}$) were mixed with competitor cells ($CD45.1^{+}$) at 1:1 ratio and transplanted into lethally irradiated recipients ($CD45.1^{+}$). Consistent with what we found with $-77^{+/-}$ mice, $-77^{+/-}$ BM cells displayed an increase ($p<0.001$) in multi-lineage reconstitution of primary recipients in comparison to WT BM cells (Fig. 2A). At 16-week post-transplant, recipients were sacrificed, and donor-derived hematopoiesis was analyzed. All $-77^{+/-}$-derived cells were overrepresented in the primary recipient BM (Fig. 2B), including myeloid, T- and B-lineage cells (Fig. 2C), as well as HSCs, MPPs, common myeloid progenitors (CMPs), granulocyte-macrophage progenitors (GMPs), megakaryocyte-erythroid progenitors (MEPs), and common lymphoid progenitors (CLPs) (Fig. 2D). In secondary recipients, the reconstitution capacity of $-77^{+/-}$ BM cells was comparable to that of WT BM cells (Fig. S2A). Although expansion of donor-derived HSC and MPP compartments persisted in $-77^{+/-}$ recipients, $-77^{+/-}$-derived progenitor compartments were indistinguishable from those of WT recipients (Fig. S2B). These results suggest a defect in stem to progenitor cell transition, which was not detected in $-77^{+/-}$ embryos [5]. Consistent with prior reports [7, 8], $Gata2^{+/-}$ BM cells exhibited decreased reconstitution ($p < 0.01$) in primary and secondary recipients (Fig. 2 and S2). In aggregate, our data demonstrate a unique −77 enhancer function to regulate adult HSC activity that was not predicted from analysis of $77^{+/-}$ embryos or $Gata2^{+/-}$ mice.

To determine whether the −77 enhancer regulates hematopoietic stem and progenitor cells (HSPCs) by conferring *Gata2* expression, its only known activity thus far, *Gata2* mRNA levels were quantified in WT, $-77^{+/-}$, and $Gata2^{+/-}$ LSK and MP cell populations using qRT-PCR. Consistent with the prior report [8], *Gata2* expression was reduced in $Gata2^{+/-}$ LSKs and MPs. Surprisingly, *Gata2* expression in $-77^{+/-}$ LSKs and MP populations was also lower than that of WT cells (Fig. S3). We analyzed transcript levels of genes localized adjacent to *Gata2*, *Eefsec*, *Rab7*, and *Rpn1*. These genes are known not to be regulated by −77 in fetal liver cells [5]. The mRNA levels of these genes in $-77^{+/-}$ and $Gata2^{+/-}$ LSK and

MPs were indistinguishable from those in WT cells (Fig. S3). Furthermore, we sorted WT and $-77^{+/-}$ HSCs and MPPs and quantified mRNA levels of HSC stemness genes and apoptosis genes using qRT-PCR (Fig. S4). We found that *c-Kit*, *Myb*, *Mpl*, and *Gata2* were upregulated in $-77^{+/-}$ HSCs (Fig. S4A), while *c-Kit*, *Myb*, and *Mpl* were upregulated and *Gata2* was downregulated in $-77^{+/-}$ MPPs (Fig. S4B). For the apoptosis genes, genes promoting cell survival were upregulated and genes promoting apoptosis were downregulated in $-77^{+/-}$ HSCs and MPPs. Our data suggest that the qualitatively different phenotypes of $-77^{+/-}$ and $Gata2^{+/-}$ HSCs is associated with the opposite regulation of *Gata2* expression in HSCs. This may reflect downregulation of *Gata2* expression in some cells of the $-77^{+/-}$ mice vs. in all cells of the $Gata2^{+/-}$ mutants. Alternatively, this phenotypic difference may reflect a previously unrecognized −77 enhancer function in HSCs.

In addition to their roles in normal hematopoiesis, both −77 and *Gata*2 itself are linked to oncogenic RAS-driven cancers. In 2% of human AML with poor prognosis, a chromosomal inversion [inv(3)] repositions *GATA2* −77 enhancer next to *MECOM*, increasing *MECOM* expression, which encodes EVI1, and lowers *GATA2* expression, inducing AML [6, 9, 10]. Activating receptor tyrosine kinase (RTK)/Ras mutations characterize ~98% of inv(3) patients [11]. Synergism between $Gata2^{+/-}$ and Evi1 overexpression [12], and synergism between oncogenic Nras and Evi1 overexpression [13] were reported in leukemogenesis. Moreover, in non-small cell lung cancer, GATA2 promotes the survival of oncogenic KRAS-dependent cancer cells, and thus its downregulation constitutes a synthetic lethality in these cells [14]. It remains unknown, however, if $-77^{+/-}$ or $Gata2^{+/-}$ cooperates with oncogenic *Kras* to promote leukemogenesis. We tested this possibility by generating 4 cohorts of mice, *Mx1-Cre* (control), $Kras^{LSL\ G12D/+}$*; Mx1-Cre* (Kras), $Kras^{LSL\ G12D/+}$*;* $-77^{+/-}$*; Mx1-Cre* (Kras;−77), and $Kras^{LSL\ G12D/+}$*;* $Gata2^{+/-}$*; Mx1-Cre* (Kras;Gata2). At 6-weeks of age, Kras; −77 and Kras;Gata2 mice displayed largely similar myeloproliferative neoplasm (MPN) phenotypes as Kras mice, including splenomegaly (Fig. S5A), increased white blood cell count and decreased platelet number in peripheral blood (PB) (Fig. S5B), and increased monocytes and/or neutrophils in various hematopoietic tissues (BM, SP, and PB) (Fig. S5C). At the moribund stage, all the Kras, Kras;−77, and Kras;Gata2 mice developed MPN (Fig. S5D and S5E). They also developed acute T-cell lymphoblastic leukemia (T-ALL) (defined as thymus weight >150 mg as we reported previously [15]) with various penetrances (Fig. S5F). All of these mice had a comparable survival (Fig. S5G). Upon transplantation, Kras, Kras;−77, and Kras;Gata2 BM cells reconstituted recipients indistinguishably (Fig. S6A). The recipients developed a high-incidence of T-ALL and MPN with a lower frequency (Fig. S6B–S6E). Again, no significant difference was detected in their survival (Fig. S6F). Our data demonstrate that neither $-77^{+/-}$ nor $Gata2^{+/-}$ heterozygous mutants significantly impact oncogenic *Kras*-induced leukemogenesis in these mouse models.

In summary, our study evaluated *Gata2* −77 enhancer function in adult hematopoiesis and oncogenic *Kras*-driven leukemia. We identified a role of −77 enhancer to regulate adult HSC survival. These $-77^{+/-}$ adult hematopoietic phenotypes did not impact survival of oncogenic *Kras* mice.

Sincerely,

Supplementary Material

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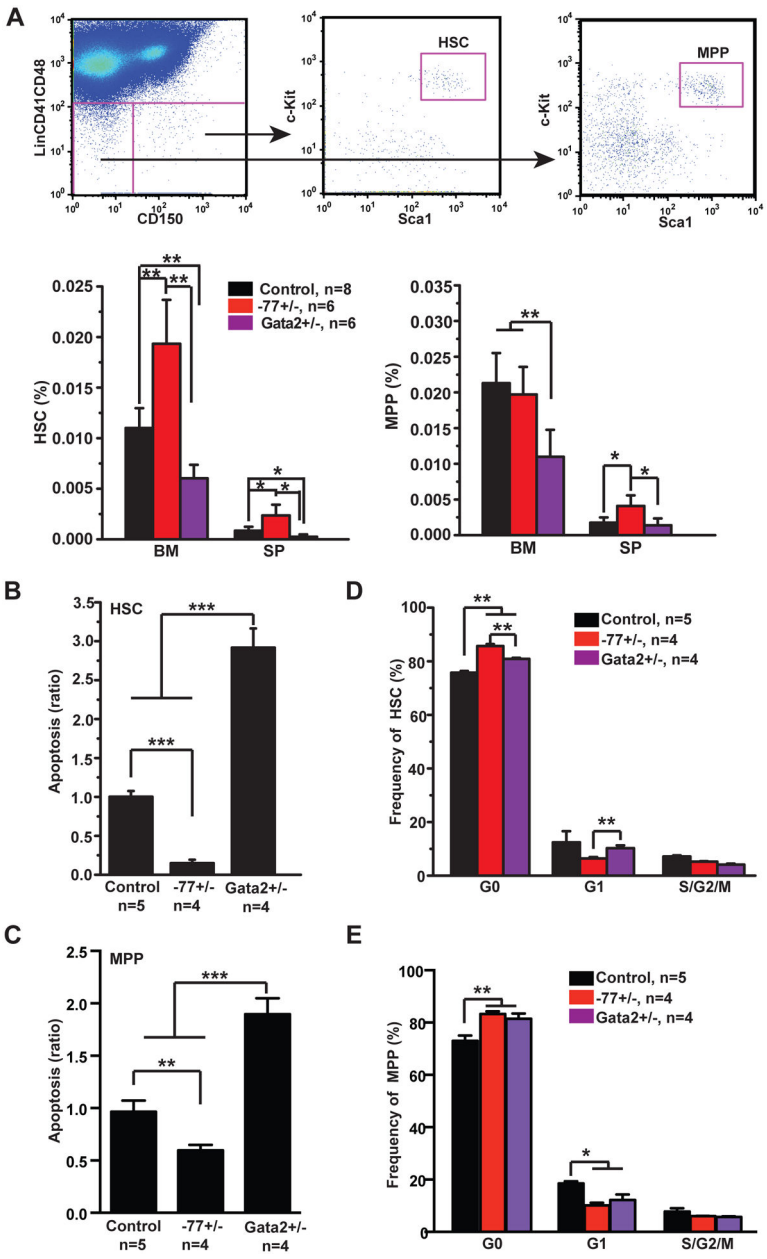

Figure 1. Increased HSC survival in *Gata2* $-77^{+/-}$ mice.

Control (wild-type C57BL/6), *Gata2* $-77^{+/-}$ (−77) and *Gata2*$^{+/-}$ (Gata2+/−) mice were sacrificed at 6-weeks of age. (A) Gating strategy for hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs). HSCs are defined as Lin^{-} $Sca1^{+}$ c-Kit^{+} $CD41^{-}$ $CD48^{-}$ $CD150^{+}$ cells and MPPs are defined as Lin^{-} $Sca1^{+}$ c-Kit^{+} $CD41^{-}$ $CD48^{-}$ $CD150^{-}$ cells. HSCs and MPPs were quantified in bone marrow (BM) and spleen (SP). (B, C) Quantification of apoptotic HSCs (B) and MPPs (C) from BM using Annexin V and DAPI. (D, E) Cell cycle analysis of BM HSCs (D) and MPPs (E) using Ki67 and DAPI. The results are presented as means + SD. * P<0.05, ** P<0.01; *** P<0.001.

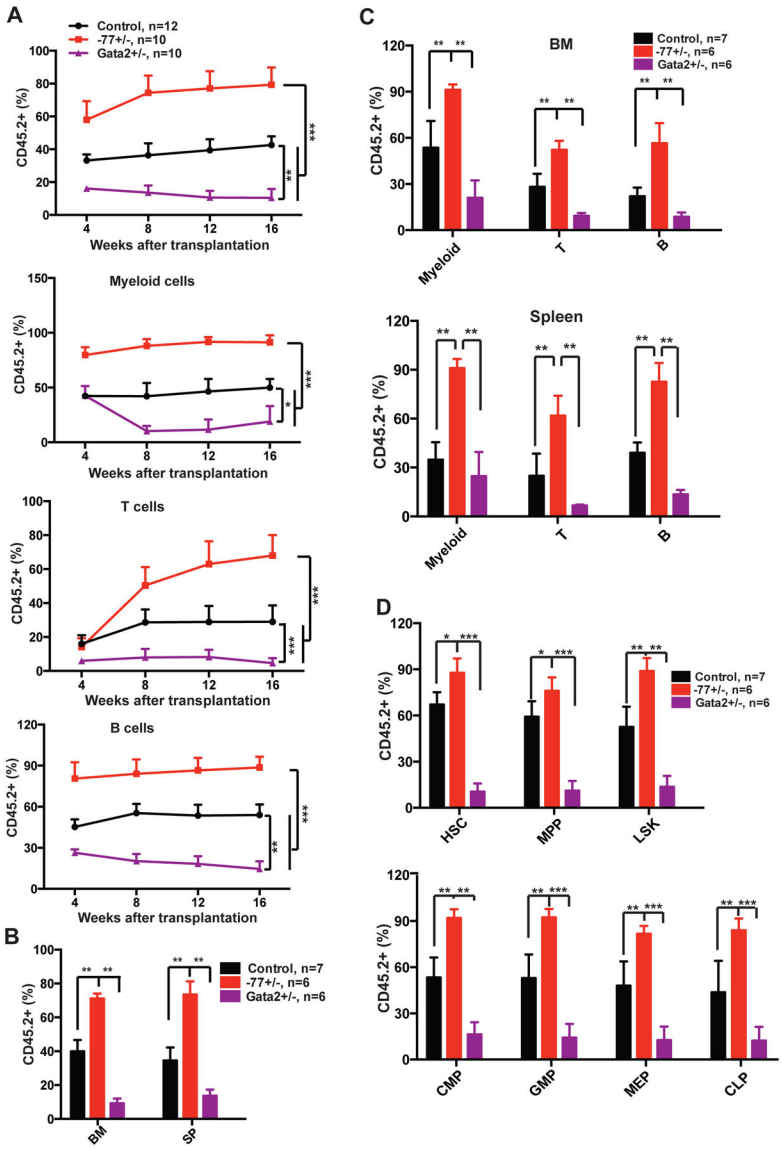

Figure 2. *Gata2* $-77^{+/-}$ bone marrow cells exhibit increased long-term reconstitution activity in primary recipients.

1×10^6 bone marrow (BM) cells ($CD45.2^+$) from 6-week old control (wild-type C57BL/6), *Gata2* $-77^{+/-}$ (−77), and *Gata2*$^{+/-}$ (Gata2+/−) mice were mixed with 1×10^6 competitor BM cells ($CD45.1^+$) and injected into lethally irradiated $CD45.1^+$ recipients. (A) Quantification of donor-derived myeloid, T- and B-cells in peripheral blood of primary recipients 4, 8, 12 and 16 weeks after transplantation. (B-D) Primary recipients were sacrificed 16 weeks posttransplant for terminal evaluation. (B) Quantification of donor-derived cells in BM and spleen (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 hematopoietic stem cells (HSCs), multipotent progenitors (MPPs), Lin^- $Sca1^+$ $c\text{-}Kit^+$ (LSKs), common myeloid progenitors (CMPs), granulocyte-macrophage progenitors (GMPs), megakaryocyte-

erythroid progenitors (MEPs), and common lymphoid progenitors (CLPs) in BM of primary recipients. The results are presented as means + SD. * P<0.05, ** P<0.01; *** P<0.001.