GATA3 is redundant for maintenance and self-renewal of hematopoietic stem cells

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Abstract

GATA3 has been identified as a master regulator of T helper cells, as well as being important for early thymic progenitors and T cell commitment. However, Gata3 expression initiates already at the hematopoietic stem cell (HSC) level, implicating a potential role also in regulation of HSCs. Herein we used a conditional Gata3 knockout strategy in which Gata3 expression was completely deleted from the earliest stage of embryonic hematopoietic development after emergence of HSCs from hemogenic endothelium. Through a detailed analysis of HSCs at the phenotypic and functional level, we demonstrate that steady state levels of HSCs are normal in Gata3^{fl/fl}Vav-Cre^{+/-} mice. Moreover, through long-term primary and secondary transplantation experiments, we also unequivocally demonstrate that Gata3 has a redundant role in post-transplantation HSC self-renewal.

Introduction

GATA 1-3 are expressed at distinct stages in the hematopoietic hierarchy. GATA1 is required for erythropoiesis, megakaryocytes and eosinophils, GATA2 for primitive hematopoietic stem/progenitor cells, and GATA3 for multiple stages of T-cell development. However, Gata3 is expressed already in HSCs, multipotent progenitors and early thymic progenitors (ETPs). While the role of GATA3 has been carefully investigated in T-lymphopoiesis, its role in HSCs has been much less explored. Recent studies showing that Gata3 null fetal liver cells can normally generate short-lived myeloid cells 10 weeks after transplantation are compatible with GATA3 having little or no role in HSC regulation. However, the HSC phenotype was not directly analysed in
neither the fetal liver nor the bone marrow of transplanted mice, nor was the defining stem cell property to self-renew upon transplantation into secondary recipients.

Methods

Mice

Wild type (WT) transplantation recipients and competitor cells were on a pure C57BL/6 (CD45.1) background. Gata3^{flo/fl}, Vav-iCre and R26R-eYFP mice have been described.\textsuperscript{13,14, 15} Animal experiments were approved by the local ethics committee at University of Oxford and the UK Home Office.

FACS analysis

Bone marrow (BM) cells were stained according to previously described protocols.\textsuperscript{16} For specification of antibodies, see Supplemental methods.

Gene expression analysis

Global gene expression analysis was performed using Mouse Genome 430 2.0 Arrays. Gata3 deletion efficiency was evaluated with dynamic array Biomark Fluidigm analysis (Supplemental information). Gata3 was amplified and analysed for gene expression using a dynamic array (Biomark Fluidigm) as previously described.\textsuperscript{17} 100 cells were sorted / well; 2-4 wells / genotype. Data were analyzed using BioMark\textsuperscript{TM} Real-Time PCR Analysis Software v2.0 (Fluidigm) and the ΔCt method was applied.\textsuperscript{18} Hprt TaqMan® Gene Expression Assay ID Mm01337569_m1 was used.
Statistical analysis

Statistical significances were determined with 2-tailed Mann-Whitney test.

Results and discussion

We not only confirmed that *Gata3* mRNA is expressed in highly purified LSKFLT3−CD48−CD150+ HSCs, but that it is subsequently down-regulated in lymphoid-primed multipotent progenitors (LMPPs) in which thymic-seeding progenitors are thought to reside, before being upregulated again in LIN−CD25 KIThiFLT3+ ETPs (Figure 1A). *GATA3fl/fl* mice were crossed with *Vav-iCre* mice, ensuring *Gata3* deletion shortly after the emergence of definitive HSCs from the hemogenic endothelium. *Vav-iCre* mice crossed with *R26R-eYFPfl/fl* mice demonstrated that *Vav-iCre* ensures recombination in virtually all (>99%) BM LSK cells (Supplemental Figure). BM cellularities of control (*Gata3fl/flVav-Cre+/+*) and *Gata3* deficient (*Gata3fl/flVav-Cre+/−*) littermates were indistinguishable (Figure 1B), as was the frequencies of LSKCD150+CD48− HSCs (Figure 1C and 1D), in which complete *Gata3* deletion was confirmed (Figure 1E). These findings suggest that *Gata3* null HSCs expand normally after their emergence in the embryo, and remain unaffected in postnatal BM.

BM cells from *Gata3fl/flVav-Cre+/+* or *Gata3fl/flVav-Cre+/−* mice were transplanted in competition with WT BM cells into lethally irradiated recipients. As evidence of the complete recombination induced by *Vav-Cre*, *Gata3* null BM cells failed to contribute to T cell reconstitution, whereas B cell (CD19+) and myeloid (Gr1+Mac1+) reconstitution was unaffected 4-6 months (Figure 1F and 1G) and 11 months (Figure 1H) after transplantation. The myeloid and B cell lineages remained reconstituted to a similar
degree in Gata3-deleted as non-deleted BM cells, as late as 8 months after transplantation into secondary recipients (Figure 1I), demonstrating a dispensable role for GATA3 in HSC self-renewal.

Vav-Cre induced recombination in the present studies ensured highly efficient hematopoietic recombination\textsuperscript{14} while allowing viable Gata3 null embryos to develop despite of Gata3 deletion occurring already shortly after emergence of HSCs in the early embryo. This approach combined with state of the art HSC phenotypical analysis, and long-term HSC reconstitution assessment, including secondary transplantations, unequivocally establishes that GATA3 is dispensable for HSC regulation at multiple levels, including steady state maintenance and self-renewal, as well as the expansion that takes place during development and post-transplantation.\textsuperscript{22}

GATA2 and GATA3 can partially rescue the Gata1-deficient erythroid phenotype.\textsuperscript{23, 24} As GATA2 has been demonstrated to play a key role in HSC regulation,\textsuperscript{5} it remains plausible that a role of GATA3 in HSC regulation might be redundant to that of GATA2, and that this could be revealed in Gata2-deficient HSCs.

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Authorship Contributions and Disclosure of Conflicts of Interest
SEWJ and NBV designed and conceptualized the overall research, analyzed the data and wrote the manuscript, NBV performed the transplantations and phenotypic FACS analysis; SD performed gene expression analysis; SD, PSW and TBJ performed FACS analysis of stem/progenitors and peripheral blood; SL performed affymetrix analysis. The authors declare that they do not have any financial conflicts of interests.
References


Figure legends

Figure 1. Phenotypic and functional characterization of HSCs in Gata3\textsuperscript{fl/fl} Vav-Cre\textsuperscript{tg/+} mice.

A. GATA3 mRNA expression in one week old wild type LSKFLT3\textsuperscript{CD150\textsuperscript{hi}CD48\textsuperscript{lo}} HSCs, LSKFLT3\textsuperscript{hi}IL7R\textsuperscript{lo} LMPPs, and Lin\textsuperscript{-}CD44\textsuperscript{CD25\textsuperscript{-}KIT\textsuperscript{+}FLT3\textsuperscript{+}} ETPs. Data are expressed as mean (SEM) normalized RMA expression (see Methods). n = 3 experiments.

B. BM cellularity in 1 week old Gata3\textsuperscript{fl/fl}Vav-Cre\textsuperscript{+/+} and Gata3\textsuperscript{fl/fl}Vav-Cre\textsuperscript{tg/+} mice. Data are expressed as mean (SD), n=3-4 mice/genotype. ns, non-significant.

C. FACS profiles from representative mouse showing gating strategy for LSKCD150\textsuperscript{hi}CD48\textsuperscript{lo} cells in 1 week old mice. Numbers indicate percentage of total BM cells within the indicated gate. n=3-4 mice/genotype.

D. Bar graphs show mean (SD) frequency of LSKCD150\textsuperscript{hi}CD48\textsuperscript{lo} cells. n=3-4 mice/genotype. ns, non-significant.

E. Bar graphs show mean (SEM) Gata3 mRNA expression levels (relative to Hprt) in LSKCD150\textsuperscript{hi}CD48\textsuperscript{lo} HSCs isolated from 1-2 week old Gata3\textsuperscript{fl/fl} Vav-Cre\textsuperscript{+/+} or Gata3\textsuperscript{fl/fl} Vav-Cre\textsuperscript{tg/+} mice.

F-I. 0.5-2 million BM cells from 1-2 weeks old Gata3\textsuperscript{fl/fl} Vav-Cre\textsuperscript{+/+} or Gata3\textsuperscript{fl/fl} Vav-Cre\textsuperscript{tg/+} mice (CD45.2) were transplanted into lethally irradiated (900cGy) WT (CD45.1) recipients together with a similar dose of competitor WT (CD45.1) adult BM cells. (F) Representative FACS analysis of peripheral blood 4 months after transplantation. Numbers indicate percentage of indicated gate within reconstituted T (CD4\textsuperscript{+/CD8\textsuperscript{+}}), B (CD19\textsuperscript{+}) and myeloid (Gr1\textsuperscript{+}Mac1\textsuperscript{+}) cells. n=3-6 mice/group.
(G-H) Mean percentage (SEM) of test cell (CD45.2+) reconstitution within the T, B and myeloid blood cell lineages, respectively, 4-6 (G) or 11 (H) months after transplantation. G, n = 6-9 mice/group; H, n=2-3 mice/group. (I) 8 months after transplantation one half femur equivalent from each primary recipient were transplanted into one secondary WT (CD45.1) recipient. Data show mean (SEM) percentage of test cell reconstitution within T, B and myeloid reconstituted cells, respectively. N = 3-6 mice / group. 1°, primary reconstitution. 2°, secondary reconstitution.

*P < 0.05, ***P < 0.001, ns indicates non-significant.
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