HEMATOPOIESIS AND STEM CELLS

Adult hematopoietic stem cells lacking Hif-1α self-renew normally

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Key Points

- Hif-1α is dispensable for cell-autonomous HSC survival.
- HSCs do not require intrinsic Hif-1α to respond to hematopoietic injury.

The hematopoietic stem cell (HSC) pool is maintained under hypoxic conditions within the bone marrow microenvironment. Cellular responses to hypoxia are largely mediated by the hypoxia-inducible factors, Hif-1 and Hif-2. The oxygen-regulated α subunits of Hif-1 and Hif-2 (namely, Hif-1α and Hif-2α) form dimers with their stably expressed β subunits and control the transcription of downstream hypoxia-responsive genes to facilitate adaptation to low oxygen tension. An initial study concluded that Hif-1α is essential for HSC maintenance, whereby Hif-1α–deficient HSCs lost their ability to self-renew in serial transplantation assays. In another study, we demonstrated that Hif-2α is dispensable for cell-autonomous HSC maintenance, both under steady-state conditions and following transplantation. Given these unexpected findings, we set out to revisit the role of Hif-1α in cell-autonomous HSC functions. Here we demonstrate that inducible acute deletion of Hif-1α has no impact on HSC survival. Notably, unstressed HSCs lacking Hif-1α efficiently self-renew and sustain long-term multilineage hematopoiesis upon serial transplantation. Finally, Hif-1α–deficient HSCs recover normally after hematopoietic injury induced by serial administration of 5-fluorouracil. We therefore conclude that despite the hypoxic nature of the bone marrow microenvironment, Hif-1α is dispensable for cell-autonomous HSC maintenance.

(Blood. 2016;127(23):2841-2846)

Introduction

Hematopoietic stem cells (HSCs) reside in hypoxic bone marrow (BM) niches where they self-renew and sustain life-long multilineage hematopoiesis.1-3 Cellular responses to hypoxia are predominantly mediated by the hypoxia-inducible factors, Hif-1 and Hif-2, which facilitate the transcription of hypoxia-responsive genes. Several studies used a conditional gene knockout strategy to determine the role of Hif-1α and Hif-2α in HSC functions.4,5 An initial study concluded that inducible Hif-1α deletion from the mouse hematopoietic system results in the progressive loss of HSCs upon serial transplantation,4 indicating that Hif-1α is required for HSC maintenance.4 We demonstrated that constitutive or inducible hematopoiesis-specific Hif-2α deletion did not affect HSC maintenance.5 Surprisingly, codelletion of Hif-1α and Hif-2α had no impact on HSC numbers, steady-state hematopoiesis, or reconstitution upon transplantation.5 These unexpected findings gave rise to the hypothesis that Hif-1α may not be as essential for HSC maintenance as previously suggested.5 Here, using serial transplantation assays, we demonstrate that unstressed HSCs do not critically require Hif-1α to survive and self-renew.

Materials and methods

Mice

Hif-1α50, Mcl-Cre, and Vav-iCre mice have been described previously6-8 and were of C57BL/6 genetic background. Sex-matched 8- to 12-week-old mice were used. Animal experiments were authorized by the UK Home Office.

FACS analysis

Analysis and sorting was done using BD LSRII Fortessa cell analyzer and BD FACSariaII cell sorter. Antibodies (see supplemental Table 1 on the Blood Web site) were used as described previously.9,11 Representative gating is shown in supplemental Figure 1.

Transplantation assays

A total of 500 000 CD45.2+ test donor unfractionated BM cells were injected intravenously into lethally irradiated (10 Gy) CD45.1+/ CD45.2+ congenic recipients alongside 500 000 CD45.1+ competitor unfractionated BM cells. Conditional gene deletion was achieved by administration of 6 intraperitoneal polyinosinic-polycytidylic acid (pI-pC) injections over 10 days, every other day (GE Healthcare; 0.3 mg per dose). For Lin− Sca-1−c-Kit+ (LSK) cell transplantation assays,


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Figure 1. Hematopoiesis-specific deletion of Hif-1α does not affect HSC survival and maintenance and their ability to respond to 5-FU–induced stress. (A) Experimental design. A total of 500,000 donor-derived (CD45.2⁺) unfractionated BM cells from untreated Hif-1αΔ/Δ;Mx1-Cre and control (without Mx1-Cre) mice were transplanted into lethally irradiated syngeneic CD45.1⁺/CD45.2⁺ recipient mice (together with 500,000 CD45.1⁺ competitor BM cells). Two independent donors were used per genotype. Eight weeks posttransplantation, the mice received 6 sequential doses of pIpC over a period of 10 days (every alternate day) and were analyzed 2 weeks after last dose of pIpC. CD45.2⁺ LSK cells from the primary recipients were serially transplanted into secondary and tertiary recipients. (B) Percentage of CD45.2⁺ donor-derived cells in PB before and after pIpC treatment. (C) CD45.2⁺ cells in PB of primary recipients treated with pIpC and PCR controls. (D) CD45.2⁺ cells in PB of primary recipients treated with pIpC. (E) Percentage of CD45.2⁺ LSK cells in BM of primary recipients treated with pIpC and PCR controls. (F) CD45.2⁺ LSK cells in BM of primary recipients treated with pIpC. (G) CD45.2⁺ LSK cells in BM of secondary recipients treated with pIpC and PCR controls. (H) CD45.2⁺ LSK cells in BM of secondary recipients treated with pIpC. (I) CD45.2⁺ LSK cells in BM of tertiary recipients treated with pIpC and PCR controls. (J) CD45.2⁺ LSK cells in BM of tertiary recipients treated with pIpC. (K) Percentage of CD45.2⁺ donor-derived cells in PB after transplantation of CD45.2⁺ LSK cells from primary recipients into secondary recipients. (L) Percentage of CD45.2⁺ donor-derived cells in PB after transplantation of CD45.2⁺ LSK cells from primary recipients into tertiary recipients. (M) Recipients with long-term multilineage reconstitution. (N) CD45.2⁺ cells in BM of tertiary recipients treated with pIpC and PCR controls. (O) CD45.2⁺ cells in BM of tertiary recipients treated with pIpC. (P) Recipients with long-term multilineage reconstitution. (Q) Surviving animals (%). (R) Total BM cellularity (%). (S) LSK cells (×10⁶). (T) HSCs (×10⁴). (U) LK cells (×10⁵).
3000 LSK cells sorted from donor BM were transplanted together with 200,000 CD45.1+ unfractonated BM cells.

**Statistical analysis**

Statistical significance was determined using Mann-Whitney or Mantel-Cox tests.

**Results and discussion**

We acutely deleted Hif-1α specifically within the hematopoietic system using Mxl-Cre^fl/fl^ (Figure 1A). Given that Mxl-Cre recombines within the hematopoietic system, BM microenvironment, and extramedullary tissues,7,13 we first transplanted unfractonated BM from untreated CD45.2^Hif-1α^fl/fl^/Mxl-Cre and control (Hif-1α^+/+^) without Mxl-Cre or Hif-1α^fl/fl^ without Mxl-Cre mice into wild-type (WT) lethally irradiated syngeneic recipients (Figure 1A). Following reconstitution (Figure 1B), recipients received pcP 8 weeks after transplantation and were analyzed 2 weeks later. Peripheral blood (PB) analyses of the pcP-treated recipients revealed that deletion of Hif-1α (Figure 1C) had no impact on CD45.2^+^ donor-derived chimerism (Figure 1D) and multilineage hematopoiesis in the BM (Figure 1E) and spleen (supplemental Figure 2). The contribution of donor-derived cells to Lin^−^c-Kit^+^ (LK), LSK, LSKCD48^CD150^+ HSC, LSKCD48^CD150^+ multi-potent progenitor (MPP), LSKCD48^CD150^− hematopoietic progenitor cell-1 (HPC-1), and LSKCD48^CD150^− HPC-2 compartments was also comparable (Figure 1F-G). Therefore, acute deletion of Hif-1α has no immediate impact on HSC survival and multilineage hematopoiesis.

To test the self-renewal capacity of Hif-1α-deficient (Hif-1α^Δ/Δ^) HSCs, we performed competitive serial transplantation assays with sorted CD45.2^+^ LSK cells from primary recipients 2 weeks after pcP treatment (Figure 1A). LSK cells from primary recipients efficiently reconstituted short- and long-term hematopoiesis in secondary recipients (Figure 1H). Sixteen weeks after transplantation, sustained Hif-1α deletion was confirmed (Figure 1I), and Hif-1α^Δ/Δ^ LSK cells efficiently contributed to all differentiated lineages in the BM, spleen (Figure 1J; supplemental Figure 3), and LK, LSK, HSC, MPP, and HPC BM compartments of the secondary recipient mice (Figure 1K-L). Finally, sorted Hif-1α^Δ/Δ^ LSK cells from secondary recipients (16 weeks after secondary transplantation) successfully reconstituted long-term multilineage hematopoiesis in tertiary recipients (Figure 1M-N). Complete Hif-1α deletion was maintained for the duration of these experiments (Figure 1O).

Therefore, self-renewing long-term HSCs do not critically require Hif-1α to maintain their pool upon the stress of serial transplantation.

To test the cell-autonomous role of Hif-1α in HSC stress responses, we used Hif-1α^fl/fl^/Vav-iCre mice in which Hif-1α is conditionally deleted specifically from hematopoietic cells using a codon-improved Cre (iCre).5,8 Hif-1α^fl/fl^/Vav-iCre and control mice received 3 doses of 5-fluouracil (5-FU) and were analyzed 10 days after the last 5-FU administration (Figure 1P). We also analyzed untreated Hif-1α^fl/fl^/Vav-iCre and control mice that did not receive 5-FU. Although 5-FU-treated mice had decreased survival compared with untreated mice, we observed no differences in survival between 5-FU–treated Hif-1α^fl/fl^/Vav-iCre and control mice (Figure 1Q). The 5-FU–treated Hif-1α^fl/fl^/Vav-iCre and control mice had comparable total BM cellularity and BM LSK, HSC, and LK cell numbers (Figure 1R); thus, Hif-1α is not essential for cell-autonomous HSC maintenance following serial 5-FU administration.

We next determined the long-term consequences of Hif-1α deletion from the hematopoietic system. We transplanted unfractonated BM cells from untreated Hif-1α^fl/fl^/Mxl-Cre or control mice, administered pcP, and analyzed the primary recipients 32 weeks after pcP treatment (Figure 2A). PB and BM analyses indicated that loss of Hif-1α did not affect long-term multilineage hematopoiesis (Figure 2B-C) or donor-derived chimerism in the stem and progenitor cell compartments of the BM (Figure 2D-E) and spleens (data not shown) of the recipient mice 32 weeks after pcP treatment. Moreover, LSK cells of both genotypes equally reconstituted long-term multilineage hematopoiesis in secondary recipients (Figure 2F-G) and evenly contributed to the stem and progenitor cell compartments of the recipients (Figure 2H). Efficient Hif-1α deletion was confirmed by polymerase chain reaction (PCR) on genomic DNA from donor-derived cells (Figure 2I). Given that donor-derived HSCs in this experiment have undergone long-term stress and are very rare in secondary recipients, to perform tertiary transplantation assays instead of retransplanting purified LSK cells, we transplanted unfractonated BM cells harvested from secondary recipients 16 weeks posttransplantation. BM cells of both genotypes equally generated multilineage hematopoiesis in tertiary recipients (Figure 2J-K). Efficient Hif-1α gene deletion was maintained for the duration of the serial transplantation experiment (Figure 2L). Thus, HSCs with chronic Hif-1α deficiency sustain normal steady-state multilineage hematopoiesis and display normal regenerative capacity upon serial transplantation.

Finally, we set out to delete Hif-1α more broadly from the BM using Mxl-Cre, which, in addition to hematopoietic cells, also recombines in the BM microenvironment.13 Hif-1α^fl/fl^/Mxl-Cre and control mice received 6 doses of pcP and were analyzed 30 days after the last
injection (supplemental Figure 4A). pIpC-treated Hif-1αfl/fl;Mx1-Cre and control mice had comparable numbers of nucleated BM cells, LSK cells, and HSC, MPP, HPC-1, and HPC-2 populations (supplemental Figure 4B). We next treated Hif-1αfl/fl;Mx1-Cre and control mice with 6 doses of pIpC followed by 2 doses of 5-FU (supplemental Figure 4C). We found that a combined stress of pIpC treatment and subsequent 5-FU administration resulted in substantially decreased survival of these mice (supplemental Figure 4D). Importantly, however, there was no difference in survival of pIpC- and 5-FU-treated Hif-1αfl/fl;Mx1-Cre and control mice; therefore, Mx1-Cre–mediated deletion of Hif-1α had no impact on HSC numbers or their ability to respond to 5-FU.

HSCs are constantly exposed to local hypoxia within the BM and the low oxygen tension has been proposed to protect HSCs. Takubo et al found a defect in HSC self-renewal upon Hif-1α deletion and suggested that Hif-1α is an important mediator of HSC functions. Here we set out to acutely induce deletion of Hif-1α in the hematopoietic system and determine its short- and long-term impact on HSC maintenance. We found that inactivation of Hif-1α did not compromise survival of HSCs 2 weeks after gene ablation and unstrained Hif-1αlox/lox HSCs self-renewed equally efficiently compared with control counterparts upon serial transplantation. Furthermore, deletion of Hif-1α had no long-term consequences for maintenance of unstrained HSCs within 32 weeks after pIpC administration and did not compromise their ability to repopulate recipient mice upon serial transplantation. Different conclusions from our study and that of Takubo et al may result from discrepancies in experimental designs. Although they serially transplanted LSK cells from pIpC-treated Hif-1αlox/lox;Mx1-Cre mice, we conducted our serial transplantation assays using Hif-1αlox/lox;Mx1-Cre cells sorted from pIpC-treated WT chimeric recipient mice harboring Hif-1αlox/lox;Mx1-Cre BM cells. Given that Mx1-Cre recombines also within the BM microenvironment and that Hif-1α is required for BM mesenchymal progenitor cell functions, Hif-1α deletion from the BM microenvironment may have affected HSC functions, thus contributing to the phenotypes observed by Takubo et al.

Finally, to test the cell-autonomous role of Hif-1α in ageing, Takubo et al transplanted Hif-1αlox/lox;Mx1-Cre BM cells into recipient mice and administered pIpC 4 months later. Although
they did not observe any differences 4 months after pIpC treatment, 11 months after pIpC treatment, they found that the level of repopulation by Hif-1α−deficient BM cells was decreased. This raises a possibility that the cell-autonomous Hif-1α deficiency causes HSC defects only when exposed to an ageing BM microenvironment.

In closing, our data presented here, taken together with our previous findings, indicate that Hif-1 and Hif-2 are not essential for HSC functions. Specifically, we conclude that despite the hypoxic nature of the BM microenvironment, self-renewing HSCs do not critically require intrinsic Hif-1α to maintain their pool and sustain long-term multilineage hematopoiesis.

Acknowledgments

K.R.K. is a Cancer Research UK Senior Fellow. K.R.K.’s laboratory is supported by grants from Cancer Research UK, Bloodwise, Edinburgh Cancer Research UK Centre Development Fund, The Wellcome Trust ISSF award, Medical Research Council, and the Kay Kendall Leukaemia Fund. T.L.H. was supported by Cancer Research UK.

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