HEMATOPOIESIS AND STEM CELLS

Pharmacologic increase in HIF1α enhances hematopoietic stem and progenitor homing and engraftment

Jennifer M. Speth,[1] Jonathan Hoggatt,[1,2] Pratibha Singh,[1] and Louis M. Pelus[1]

[1]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN; and [2]Department of Stem Cell and Regenerative Biology, Harvard University/Harvard Stem Cell Institute, Cambridge, MA

Key Points

- $dmPGE_2$ stabilizes the transcription factor HIF1α in stem and progenitor cells.
- Pharmacologic stabilization of HIF1α increases CXCR4 and enhances stem and progenitor homing and engraftment.

Hematopoietic stem cell (HSC) transplantation is a lifesaving therapy for a number of immunologic disorders. For effective transplant, HSCs must traffic from the peripheral blood to supportive bone marrow niches. We previously showed that HSC trafficking can be enhanced by ex vivo treatment of hematopoietic grafts with 16-16 dimethyl prostaglandin E_2 ($dmPGE_2$). While exploring regulatory molecules involved in $dmPGE_2$ enhancement, we found that transiently increasing the transcription factor hypoxia-inducible factor 1-α (HIF1α) is required for $dmPGE_2$-enhanced CXCR4 upregulation and enhanced migration and homing of stem and progenitor cells and that pharmacologic manipulation of HIF1α is also capable of enhancing homing and engraftment. We also now identify the specific hypoxia response element required for CXCR4 upregulation. These data define a precise mechanism through which ex vivo pulse treatment with $dmPGE_2$ enhances the function of hematopoietic stem and progenitor cells; these data also define a role for hypoxia and HIF1α in enhancement of hematopoietic transplantation. (*Blood*. 2014;123(2):203-207)

Introduction

Hematopoietic stem cell (HSC) transplantation is a curative treatment of immunologic malignancies, inherited metabolic diseases, and congenital immunodeficiencies, and is an attractive method for gene therapy. Transplantation success is partly dictated by the quality and number of donor cells transplanted, and is dependent on their ability to home to bone marrow (BM) niches, self-renew and differentiate. Some sources of HSCs display reduced engraftment efficiency because of inadequate number, and/or poor homing. Identifying strategies to enhance homing and expansion of HSCs can improve transplant efficiency, particularly when HSC number is limited. It is known that prostaglandin E_2 (PGE_2) can stimulate hematopoietic stem and progenitor cell (HSPC) proliferation.[1,2] Recently, the long-acting PGE_2 analog 16-16 dimethyl PGE_2 ($dmPGE_2$) was identified in a zebrafish chemical screen as a regulator of hematopoiesis, and ex vivo exposure to $dmPGE_2$ was shown to increase engraftment in murine and nonhuman primate models.[3,4] This strategy has now progressed to a phase 1 clinical trial.[5] We previously demonstrated that part of the mechanisms of action for PGE_2 were the result of increases in homing, survival, and proliferation of murine and human HSCs.[6] PGE_2 enhances HSC homing primarily by increasing CXCR4 expression on HSCs; however, the mechanism(s) whereby PGE_2 modulates CXCR4 and HSC homing has not been defined.

HSCs have been reported to lie in hypoxic BM niches[7-10] that support stabilization of hypoxia-inducible factor 1 α (HIF1α) within HSCs. HSCs that reside in hypoxic niches have greater hematopoietic-repopulating ability,[11] although recent evidence suggests that HSCs may inherently maintain hypoxic status independently from their specific BM localization.[12] HIF1α dose-dependently regulates HSC activity,[9] and intracellular oxygenation status plays a role in HSC quiescence and expansion.[9,13,14] Given that HIF1α and hypoxia regulate CXCR4 transcription in some cancer cell lines[15-20] and PGE_2 can stabilize HIF1α in prostate cancer cells,[21-23] we hypothesized that PGE_2 may increase CXCR4 and HSC engraftment through effects on HIF1α. Herein, we demonstrate that PGE_2 stabilizes HIF1α protein and transcriptional activity, which is required for enhanced HSPC homing, and identify a pharmacologic target for ex vivo enhancement of HSC function.

Materials and methods

Mice

Mice were bred in-house or were purchased from Jackson Laboratory (Bar Harbor, ME) and maintained in the Indiana University School of Medicine animal facility. Conditional HIF1α knockout (KO) mice were generated by breeding $HIF1\alpha^{Flox/Flox}$ and tamoxifen-Cre mice, then crossing hemizygous floxed pups with homozygous $HIF1\alpha^{Flox/Flox}$ mice. Resulting Cre^{+} $HIF1\alpha^{Flox/Flox}$ mice were used. Experiments were approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine. Additional materials and methods are provided in the supplemental Methods (available on the *Blood* Web site).

Submitted July 18, 2013; accepted October 23, 2013. Prepublished online as *Blood* First Edition paper, October 28, 2013; DOI 10.1182/blood-2013-07-516336.

The online version of this article contains a data supplement.

There is an Inside *Blood* commentary on this article in this issue.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

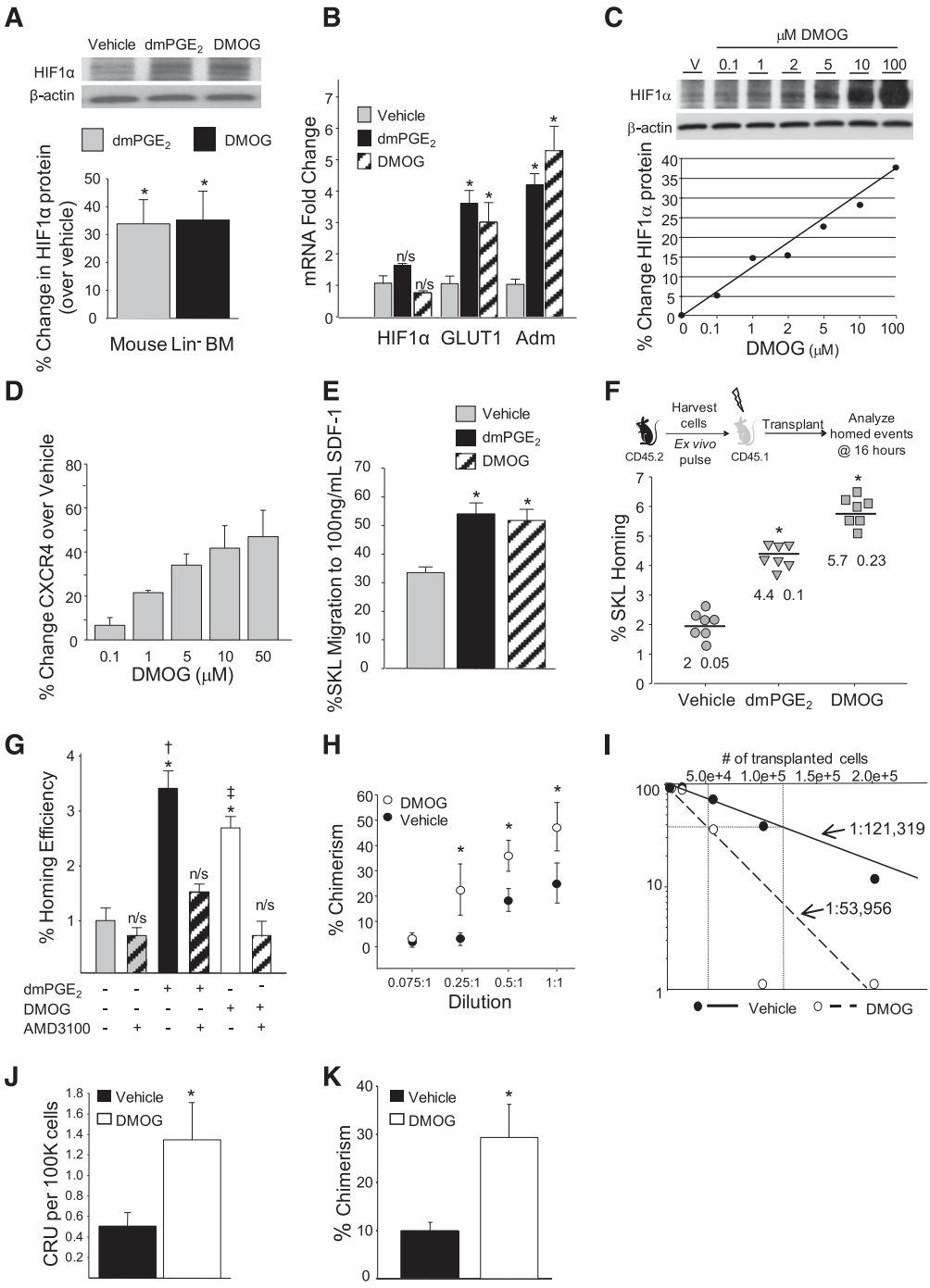

Figure 1. PGE_2 increases HIF1α protein and downstream responsive genes. (A) (Top) Representative blot of HIF1α protein 4 hours after treatment with vehicle, 1 μM of $dmPGE_2$, or 5 μM of DMOG. (Bottom) Densitometry analysis of HIF1α protein expression in mouse $lineage^{neg}$ BMCs treated with vehicle, $dmPGE_2$, or DMOG. Data are expressed as X ± standard error of the mean (SEM) percent change in protein levels over vehicle control from 3 separate experiments. (B) Expression of HIF1 responsive genes in mouse $lineage^{neg}$ BMCs after treatment with $dmPGE_2$ and DMOG as determined by quantitative reverse-transcription polymerase chain reaction. Data are X ± SEM, N = 3 experiments. *$P < .05$. (C) (Top) Dose-response blot of HIF1α protein 4 hours after treatment with vehicle or DMOG. (Bottom) Densitometry analysis of HIF1α protein expression in mouse $lineage^{neg}$ BMC. (D) Dose response of CXCR4 on SKL cells treated with vehicle or DMOG. $Lineage^{neg}$ BMCs were treated with DMOG for 2 hours at 37°C, washed, and incubated in RPMI with 10% heat-inactivated fetal calf serum for 16 hours. CXCR4 expression on LSK-gated cells was analyzed by flow cytometry. (E) In vitro transwell migration of murine SKL cells. One million $lineage^{neg}$ BMCs were treated with vehicle, $dmPGE_2$, or DMOG. Cells were assayed for the ability to migrate to 100 ng/mL recombinant murine SDF-1 for 4 hours at 37°C. Data are expressed as X ± SEM, N = 9. (F) BMCs from CD45.1 mice were treated with vehicle, 1 μM of $dmPGE_2$, or 5 μM of DMOG and 1×10^6 treated $lineage^{neg}$ cells transplanted into lethally irradiated CD45.2 mice. At 16 hours later, BM was analyzed for homed SKL cells. Data are represented as X ± SEM from 2 separate experiments (N = 4-5 mice/group/experiment, each assayed individually). (G) Similar homing experiment with or without the addition of AMD3100 10 minutes before transplantation. Data are represented as X ± SEM from 2 separate experiments (N = 4-5 mice/group/experiment, each assayed individually) *$P < .05$. (H) Percent contribution (chimerism) and (I) frequency analysis for DMOG determined by Poisson statistics using LCALC software. Vehicle P_0= 121 319 and DMOG P_0= 53 956. (J) Competitive repopulating units of DMOG and vehicle-treated cells in peripheral blood 6 months after transplant. Data are represented as X ± SEM from 2 pooled experiments (N = 5 mice/group/experiment, each assayed individually). (K) Secondary transplant percent chimerism at 6 months after transplant (N = 6 mice/group each assayed individually). *$P < .05$.

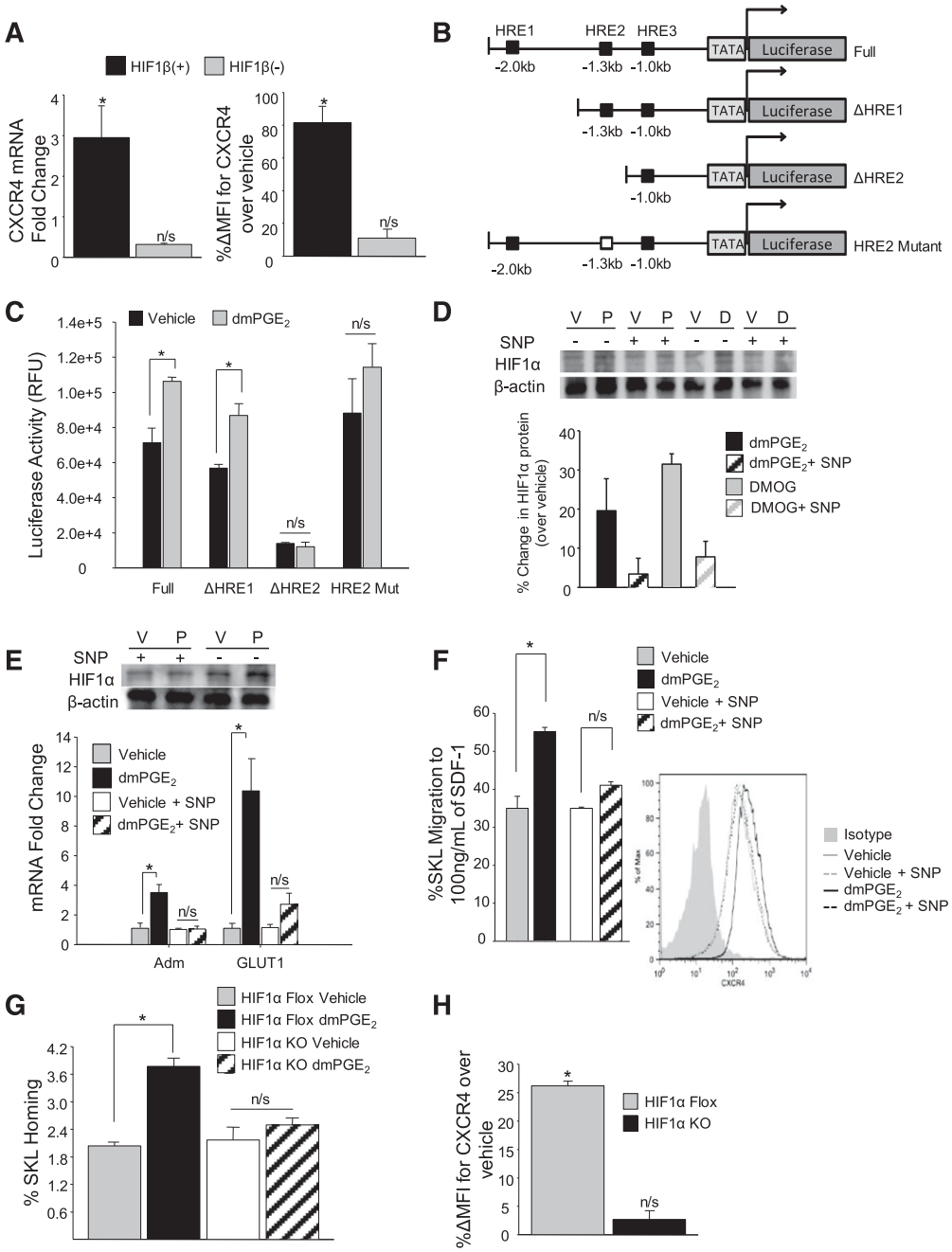

Figure 2. HIF1α transcriptional activity is necessary for PGE$_2$-induced CXCR4 upregulation. (A) (Left) CXCR4 cell surface expression (X ± SEM; N = 3 experiments) on HIF1β (−) and HIF1β (+) cells 24 hours after treatment with dmPGE$_2$. CXCR4 cell surface expression was determined by flow cytometry. Data are expressed as percent change in mean fluorescence intensity of CXCR4 over vehicle. *$P < .05$. (Right) CXCR4 cell surface expression (X ± SEM; N = 3 experiments) in HIF1β (−) and HIF1β (+) cells 2 hours after treatment with vehicle or dmPGE$_2$ determined by quantitative reverse-transcription polymerase chain reaction. (B) Schematic of pGL2b luciferase reporter constructs containing various regions of the murine CXCR4 promoter. (C) In vitro Luciferase reporter assay. 293T cells were transfected with either full-length CXCR4 promoter constructs containing all HREs (Full), truncated constructs containing 1 HRE (ΔHRE1) or 2 HREs (ΔHRE2), or a mutated 1.3 kb of HRE (HRE 2 Mut). After 24 hours, cells were split equally and treated with either vehicle or dmPGE$_2$ for 16 hours at 37°C. Luciferase activity was measured using the Firefly Luciferase assay kit (Promega). Data are represented as X ± SEM from 2 separate experiments (N = 6). *$P < .05$. (D) (Top) Representative blot of HIF1α protein 4 hours after treatment with vehicle, dmPGE$_2$, or DMOG, with or without the addition of SNP. (Bottom) Densitometry analysis of HIF1α protein expression in mouse lineage^{neg} BMCs treated with vehicle, dmPGE$_2$, or DMOG, with or without the addition of SNP. Data are expressed as X ± standard deviation percent change in protein levels over vehicle control from 2 separate experiments. (E) Expression of HIF1 responsive genes after treatment with vehicle or dmPGE$_2$ with or without SNP. Data are expressed as X ± SEM, N = 3 experiments. (F) (Left) In vitro transwell migration of murine SKL cells to 100 ng/mL of SDF-1. Lineage^{neg} cells were treated with vehicle or 1 μM of dmPGE$_2$ with or without 100 μM of SNP for 2 hours at 37°C. Data are expressed as mean ± SEM, N = 3 experiments. *$P < .05$. (Right) Representative fluorescence-activated cell sorter histogram showing CXCR4 expression on SKL cells compared with isotype control. (G) In vivo homing of HIF1α KO cells. BMCs from conditional HIF1α KO or floxed control (CD45.2) mice were treated with vehicle or dmPGE$_2$, and 1 × 10^6 treated lineage^{neg} cells were transplanted into lethally irradiated BoyJ (CD45.1) mice. At 16 hours later, BM was analyzed for homed SKL cells. Data are represented as X ± SEM from 1 experiment (N = 4-5 mice/group/experiment, each assayed individually). (H) CXCR4 expression (X ± SEM) on HIF1α KO and HIF1α Floxed SKL cells after treatment with dmPGE$_2$. N = 3 mice/group, each assayed individually. *$P < .05$.

Results and discussion

We previously demonstrated that short-term exposure to PGE_2 upregulates HSPC CXCR4 and enhances their migration to stromal cell-derived factor 1 (SDF-1) and BM homing in vivo. However, the mechanisms whereby PGE_2 modulates HSPC CXCR4 and homing are unknown. In prostate cancer cells and renal tubular cells,[21,22] PGE_2 stabilizes HIF1α protein without affecting messenger RNA (mRNA), and inhibiting PGE_2 biosynthesis reduces HIF1α and HIF-responsive genes.[23] In HEK cells and in microglial cells, HIF1α upregulates CXCR4 by interacting with hypoxia response elements (HREs) within the CXCR4 promoter.[18,19] We hypothesized that the enhancing effect of PGE_2 on HSPC CXCR4, migration, and homing could result from HIF1α stabilization and enhanced HIF1α transcriptional activity and that pharmacologic manipulation of HIF1α may be an adjunct or alternative strategy to enhance HSC engraftment. We first determined whether PGE_2 stabilizes HIF1α in primary BM cells (BMCs), compared with the prolyl hydroxylase domain inhibitor dimethyloxalylglycine (DMOG), a known HIF1α stabilizer.[24] Pulse treatment of lineage^{neg} BMCs with 1 μM of $dmPGE_2$ or 5 μM of DMOG significantly increased HIF1α protein expression by ~35% (Figure 1A), with no effect on HIF1α mRNA (Figure 1B). Concomitantly, mRNA levels of 2 downstream HIF1 responsive genes adrenomedullin and glucose transporter-1 were significantly increased after PGE_2 and DMOG treatment (Figure 1B), confirming that HIF1α stabilization led to increased HIF1α transcriptional activity.

Given the similar effects of $dmPGE_2$ and DMOG on HIF1α, we investigated if DMOG similarly affects HSPC function. Pulse exposure of lineage^{neg} BMC dose-dependently increased HIF1α protein expression (Figure 1C) with a concomitant dose-response increase in CXCR4 levels on Sca-1$^+$ c-kit$^+$ lineage^{neg} (SKL) cells (Figure 1D). Functionally, DMOG pulse exposure also enhanced SKL migration to SDF-1 comparable to $dmPGE_2$ (Figure 1E). In a congenic in vivo homing model, treatment of BMCs with DMOG enhanced SKL cell homing equivalent to $dmPGE_2$ (Figure 1F). As we reported for PGE_2,[6] enhanced homing of SKL cells by DMOG was completely blocked by the selective CXCR4 antagonist AMD3100 (Figure 1G). In a limiting dilution, head-to-head congenic transplant model, DMOG treatment of BMC before transplantation significantly increased peripheral blood chimerism at 6 months after transplantation compared with vehicle-treated cells (Figure1H), with no differences in lineage reconstitution between recipient, vehicle, and DMOG-treated cells (data not shown). Enhanced chimerism as a result of DMOG treatment stemmed from a twofold increase in HSC frequency (Figure 1I) and competitive repopulating units (Figure 1J). Enhanced chimerism was maintained in secondary transplants (Figure 1K). That the enhancing effects of $dmPGE_2$ on HSPC homing and engraftment can be duplicated by DMOG suggests that PGE_2 may mediate its effect on HSPC CXCR4 through stabilization of HIF1α.

Under normoxic conditions, HIF1α is ubiquitinated and targeted for proteasomal degradation.[25-27] In the absence of oxygen, HIF1α is stabilized and translocated to the nucleus by HIF1β, where the translocated HIF complex interacts with HREs within the promoter of responsive genes.[28] To further define a HIF1α requirement for PGE_2-induced CXCR4 upregulation, we used mutant mouse hepatoma cells lacking or reconstituted with the HIF1β nuclear translocator.[29] In mutant hepatoma cells stably transfected with HIF1β, $dmPGE_2$ increased CXCR4 mRNA approximately threefold, and CXCR4 surface expression approximately twofold, whereas $dmPGE_2$ failed to increase CXCR4 in HIF1β (−) mutant cells (Figure 2A). The CXCR4 promoter contains 3 potential HREs, with the HRE located −1.3 kb from the transcriptional start site being necessary for hypoxia-induced CXCR4 upregulation in HEK cells.[18] Using a series of luciferase CXCR4 promoter vectors containing specific combinations of HREs (Figure 2B), a significant increase in luciferase activity was observed in $dmPGE_2$-treated cells transfected with the vector containing the −1.3 kb of HRE. However, when the −1.3 kb of HRE was either absent (ΔHRE2) or mutated (HRE2 Mut), no change in luciferase activity (Figure 2C) was observed after $dmPGE_2$ treatment, indicating that the −1.3 kb of HRE is required for CXCR4 regulation by both hypoxia and PGE_2. Taken together, these data indicate that HIF1α transcriptional activity is required for PGE_2-induced gene transcription of CXCR4 and results from HIF1α nuclear translocation and binding to the −1.3 kb of HRE within the CXCR4 promoter, although we cannot rule out other regulatory elements between HRE1 and HRE2 that may be contributing to CXCR4 regulation in response to hypoxia.

To support the hypothesis that HIF1α is required for transcriptional upregulation of CXCR4 by PGE_2, we used pharmacologic and genetic approaches to determine whether the effects of PGE_2 on increasing CXCR4 expression and migration to SDF-1 in primary HSPCs were mediated by HIF1α stabilization. Using sodium nitroprusside (SNP), a compound that inhibits HIF1α protein accumulation,[30,31] we saw an inhibition in both DMOG and $dmPGE_2$-mediated HIF1α stabilization (Figure 2D), as well as $dmPGE_2$-mediated upregulation of HIF-responsive genes (Figure 2E). SNP also blocked the increase in migration to SDF-1 and CXCR4 expression, normally seen with PGE_2 (Figure 2F). Conditional deletion of HIF1α resulted in loss of PGE_2-induced enhanced HSPC homing in HIF1α KO cells (Figure 2G), and no increase in CXCR4 expression after $dmPGE_2$ treatment was observed (Figure 2H), further supporting the observation that HIF1α is required for PGE_2-induced CXCR4 upregulation and enhanced homing.

In summary, we provide new mechanistic insight into the effects of PGE_2 on HSC homing and engraftment and define a new ex vivo pharmacologic strategy to enhance hematopoietic transplantation. This finding that HIF1α stabilization by DMOG improves HSC homing and engraftment, along with recent evidence that human CD34$^+$ cells cultured in hypoxic conditions exhibit elevated levels of CXCR4[32] and that in vivo administration of DMOG enhances HSC recovery after total body irradiation,[33] suggests several potential therapeutic strategies targeting HIF1α to improve HSC engraftment and repopulation.

Acknowledgments

The authors would like to thank Dr Mircea Ivan for providing HIF1β mutant cell lines, Dr Wilhelm Krek for providing the Luciferase Reporter vectors, as well as Anthony Sinn and Kacie Peterman from the Melvin and Bren Simon Cancer Center in Vivo Therapeutics Core for irradiation assistance and mouse transplants.

This study was supported by National Institutes of Health (NIH) grant HL096305 (L.M.P.). J.H. was supported by NIH training grants DK07519, HL07910, and HL087735. J.M.S. was supported by NIH training grant DK07519. Flow cytometry was performed in the Flow Cytometry Resource Facility of the Indiana University Simon

Cancer Center (NCI P30 CA082709). Additional core support was provided by a Center of Excellence in Hematology grant P01 DK090948.

Authorship

Contribution: J.M.S. designed and performed experiments, performed data analysis and interpretation, and wrote the manuscript; J.H. designed research and wrote the manuscript; P.S. performed experiments; and L.M.P. designed experiments, analyzed data, and wrote the manuscript.

Conflict-of-interest disclosure: J.H. and L.M.P. have received consulting fees from Fate Therapeutics. The remaining authors declare no competing financial interests.

Correspondence: Louis M. Pelus, Department of Microbiology and Immunology, Indiana University School of Medicine, 950 West Walnut St, Indianapolis, IN 46202; e-mail: lpelus@iupui.edu.

References

1. Verma DS, Spitzer G, Zander AR, McCredie KB, Dicke KA. Prostaglandin E1-mediated augmentation of human granulocyte-macrophage progenitor cell growth in vitro. *Leuk Res.* 1981; 5(1):65-71.
2. Pelus LM. Association between colony forming units-granulocyte macrophage expression of Ia-like (HLA-DR) antigen and control of granulocyte and macrophage production. A new role for prostaglandin E. *J Clin Invest.* 1982;70(3): 568-578.
3. North TE, Goessling W, Walkley CR, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature.* 2007;447(7147):1007-1011.
4. Goessling W, Allen RS, Guan X, et al. Prostaglandin E2 enhances human cord blood stem cell xenotransplants and shows long-term safety in preclinical nonhuman primate transplant models. *Cell Stem Cell.* 2011;8(4):445-458.
5. Cutler C, Multani P, Robbins D, et al. Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation. *Blood.* 2013;122(17):3074-3081.
6. Hoggatt J, Singh P, Sampath J, Pelus LM. Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood.* 2009;113(22):5444-5455.
7. Parmar K, Mauch P, Vergilio JA, Sackstein R, Down JD. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. *Proc Natl Acad Sci USA.* 2007;104(13): 5431-5436.
8. Simsek T, Kocabas F, Zheng J, et al. The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. *Cell Stem Cell.* 2010;7(3):380-390.
9. Takubo K, Goda N, Yamada W, et al. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. *Cell Stem Cell.* 2010; 7(3):391-402.
10. Wang LD, Wagers AJ. Dynamic niches in the origination and differentiation of haematopoietic stem cells. *Nat Rev Mol Cell Biol.* 2011;12(10): 643-655.
11. Winkler IG, Barbier V, Wadley R, Zannettino AC, Williams S, Lévesque JP. Positioning of bone marrow hematopoietic and stromal cells relative to blood flow in vivo: serially reconstituting hematopoietic stem cells reside in distinct nonperfused niches. *Blood.* 2010;116(3):375-385.
12. Nombela-Arrieta C, Pivarnik G, Winkel B, et al. Quantitative imaging of haematopoietic stem and progenitor cell localization and hypoxic status in the bone marrow microenvironment. *Nat Cell Biol.* 2013;15(5):533-543.
13. Eliasson P, Rehn M, Hammar P, et al. Hypoxia mediates low cell-cycle activity and increases the proportion of long-term-reconstituting hematopoietic stem cells during in vitro culture. *Exp Hematol.* 2010;38(4):301-310, e2.
14. Harris JM, Esain V, Frechette GM, et al. Glucose metabolism impacts the spatiotemporal onset and magnitude of HSC induction in vivo. *Blood.* 2013; 121(13):2483-2493.
15. Ishikawa T, Nakashiro K, Klosek SK, et al. Hypoxia enhances CXCR4 expression by activating HIF-1 in oral squamous cell carcinoma. *Oncol Rep.* 2009;21(3):707-712.
16. Pore N, Maity A. The chemokine receptor CXCR4: a homing device for hypoxic cancer cells? *Cancer Biol Ther.* 2006;5(11):1563-1565.
17. Schioppa T, Uranchimeg B, Saccani A, et al. Regulation of the chemokine receptor CXCR4 by hypoxia. *J Exp Med.* 2003;198(9):1391-1402.
18. Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W. Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. *Nature.* 2003;425(6955): 307-311.
19. Wang X, Li C, Chen Y, et al. Hypoxia enhances CXCR4 expression favoring microglia migration via HIF-1alpha activation. *Biochem Biophys Res Commun.* 2008;371(2):283-288.
20. Zagzag D, Lukyanov Y, Lan L, et al. Hypoxia-inducible factor 1 and VEGF upregulate CXCR4 in glioblastoma: implications for angiogenesis and glioma cell invasion. *Lab Invest.* 2006;86(12): 1221-1232.
21. Fernández-Martínez AB, Jiménez MI, Manzano VM, Lucio-Cazaña FJ. Intracrine prostaglandin E(2) signalling regulates hypoxia-inducible factor-1α expression through retinoic acid receptor-β. *Int J Biochem Cell Biol.* 2012;44(12):2185-2193.
22. Liu XH, Kirschenbaum A, Lu M, et al. Prostaglandin E2 induces hypoxia-inducible factor-1alpha stabilization and nuclear localization in a human prostate cancer cell line. *J Biol Chem.* 2002;277(51):50081-50086.
23. Palayoor ST, Tofilon PJ, Coleman CN. Ibuprofen-mediated reduction of hypoxia-inducible factors HIF-1alpha and HIF-2alpha in prostate cancer cells. *Clin Cancer Res.* 2003;9(8):3150-3157.
24. Ivan M, Haberberger T, Gervasi DC, et al. Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. *Proc Natl Acad Sci USA.* 2002;99(21):13459-13464.
25. Kamura T, Sato S, Iwai K, Czyzyk-Krzeska M, Conaway RC, Conaway JW. Activation of HIF1alpha ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. *Proc Natl Acad Sci USA.* 2000;97(19): 10430-10435.
26. Maxwell PH, Wiesener MS, Chang GW, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature.* 1999;399(6733):271-275.
27. Salceda S, Caro J. Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. *J Biol Chem.* 1997;272(36):22642-22647.
28. Kaluz S, Kaluzová M, Stanbridge EJ. Regulation of gene expression by hypoxia: integration of the HIF-transduced hypoxic signal at the hypoxia-responsive element. *Clin Chim Acta.* 2008; 395(1-2):6-13.
29. Watson AJ, Weir-Brown KI, Bannister RM, et al. Mechanism of action of a repressor of dioxin-dependent induction of Cyp1a1 gene transcription. *Mol Cell Biol.* 1992;12(5): 2115-2123.
30. Takabuchi S, Hirota K, Nishi K, et al. The inhibitory effect of sodium nitroprusside on HIF-1 activation is not dependent on nitric oxide-soluble guanylyl cyclase pathway. *Biochem Biophys Res Commun.* 2004;324(1):417-423.
31. Sogawa K, Numayama-Tsuruta K, Ema M, Abe M, Abe H, Fujii-Kuriyama Y. Inhibition of hypoxia-inducible factor 1 activity by nitric oxide donors in hypoxia. *Proc Natl Acad Sci USA.* 1998;95(13): 7368-7373.
32. Roy S, Tripathy M, Mathur N, Jain A, Mukhopadhyay A. Hypoxia improves expansion potential of human cord blood-derived hematopoietic stem cells and marrow repopulation efficiency. *Eur J Haematol.* 2012; 88(5):396-405.
33. Forristal CE, Winkler IG, Nowlan B, Barbier V, Walkinshaw G, Levesque JP. Pharmacologic stabilization of HIF-1α increases hematopoietic stem cell quiescence in vivo and accelerates blood recovery after severe irradiation. *Blood.* 2013;121(5):759-769.

blood

2014 123: 203-207
doi:10.1182/blood-2013-07-516336 originally published online October 28, 2013

Pharmacologic increase in HIF1α enhances hematopoietic stem and progenitor homing and engraftment

Jennifer M. Speth, Jonathan Hoggatt, Pratibha Singh and Louis M. Pelus

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.