Progress towards improving homing and engraftment of hematopoietic stem cells for clinical transplantation

Xinxin Huang and Hal E. Broxmeyer

Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana, USA

Correspondence to: Xinxin Huang, Ph.D.

Department of Microbiology and Immunology

Indiana University School of Medicine

950 West Walnut Street, R2-302

Indianapolis, IN 46202-5181 USA

Phone: 317-278-9941

Fax: 317-274-7592

E-mail: xinxhuan@iupui.edu

This is the author's manuscript of the article published in final edited form as:

Huang, X., & Broxmeyer, H. E. (2019). Progress towards improving homing and engraftment of hematopoietic stem cells for clinical transplantation. Current Opinion in Hematology, 26(4), 266–272. https://doi.org/10.1097/MOH.0000000000000510

Purpose of review

Hematopoietic cell transplantation (HCT) is a life-saving treatment for a variety of hematological and non-hematological disorders. Successful clinical outcomes after transplantation rely on adequate hematopoietic stem cell (HSC) numbers, and the homing and subsequent short- and long-term engraftment of these cells in the bone marrow. Enhancing the homing capability of HSCs has the potential for high impact on improving HCT and patient survival.

Recent findings

There are a number of ways to enhance HSC engraftment. Neutralizing negative epigenetic regulation by histone deacetylase 5 (HDAC5) increases surface CXCR4 expression and promotes human HSC homing and engraftment in immune deficient (NSG) mice. Short-term treatment of cells with glucocorticoids, pharmacological stabilization of hypoxia inducible factor (HIF)-1α, increasing membrane lipid raft aggregation, and inhibition of Dipeptidyl peptidase 4 (DPP4) facilitates HSC homing and engraftment. Added to these procedures, modulating the mitochondria permeability transition pore (MPTP) to mitigate ambient air induced Extra Physiological Oxygen Stress/Shock (EPHOSS) by hypoxic harvest and processing, or using cyclosporine A during air collection increases functional HSC numbers and improves HSC engraftment.

Summary

A Better understanding of the regulation of human HSC homing mediated by various signaling pathways will facilitate development of more efficient means to enhance HCT efficacy.

Keywords

HSC, homing, HDAC5, glucocorticoid, EPHOSS

INTRODUCTION

Each day, over 100 billion new blood cells are produced by the human body. These new blood cells, containing more than ten different mature cell types, are derived from a rare population of hematopoietic stem cells (HSCs) throughout a lifetime[1-3]. HSCs are the most well-characterized adult stem cell type, and there has been an enormous boost in our understandings of cellular and molecular properties of HSCs, with many studies documenting various pathways involved in the balance between HSC self-renewal and differentiation[3]. Therapeutically, HSCs are the only stem cells routinely used successfully in clinical practice, and allogeneic hematopoietic cell transplantation (HCT) remains the only curative treatment strategy for many malignant and non-malignant blood cell disorders[4,5]. Upon transplantation, HSCs can provide the recipient a new hematopoietic and immune system, and thus they have been recognized as relevant target cells for gene therapy[6■,7].

Homing is an initial critical step for HSC transplantations, wherein intravenously administered HSC containing cell population migrate after infusion into recipients from peripheral blood to the bone marrow (BM) microenvironment[8]. BM niche provides a unique environment of matrix supports and signals that balance HSCs proliferation and differentiation, and HSC homing to BM is necessary for reconstituting the whole hematopoietic system [9■,10,11]. Successful clinical outcomes after HCT rely on adequate HSC number and their homing and subsequent short- and long-term engraftment in the BM. Therefore, developing better strategies to enhance HSC homing efficacy has the potential to improve HCT and patient survival, especially when the numbers of HSCs are limited, as seen in poorly mobilized peripheral blood (mPB) or umbilical cord blood (CB) [12-14]. The interaction between CXCL12/stromal cell-derived factor

(SDF)-1 and its receptor CXCR4 play an important role in directing HSC homing, CXCL12/CXCR4 interactions are involved in chemotaxis (directed cell movement of immature hematopoietic cells)[15] and their intracellular signaling has been considered as a promising target for improving HSC transplantations[16,17]. Recent studies from our group and others have identified new approaches to potentially improve HSC homing and engraftment, including enhancement of CXCL12/CXCR4 interactions, stabilization of HIF-1α and mitigating EPHOSS. We discuss these emerging findings, with an emphasis on unique and overlapping themes.

EPIGENETIC REGULATION OF HSC HOMING

The word “epigenetic” refers to a heritable alteration in gene activity by mechanisms other than changes of the genetic code itself[18,19]. Epigenetic control of gene expression is very important for animal development and human health[20,21]. Dysregulation of epigenetic mechanisms have been associated with many diseases, including cancer, heart diseases, and neuropsychiatric disorders[22,23,24■]. Epigenetic regulation involves DNA methylation, histone modifications, and RNA associated silencing. These different epigenetic mechanisms can function coordinately via interactions and cross-talk to form layers of regulation[25,26,27■,28]. The histones around which DNA is wrapped are subject to a series of modifications including acetylation, methylation, ubiquitination and phosphorylation[29]. These histone modifications are usually located on the histone tails and can directly affect chromatin structure, which further defines active or repressed gene expression states.

In order to gain an understanding of epigenetic regulation of HSC homing, we screened a chemical compound library containing various epigenetic enzyme inhibitors to evaluate their

effects on human HSC surface expression of CXCR4. We found that treatment of histone deacetylase inhibitors resulted in dramatic increases in surface expression of CXCR4 [30■■]. We further demonstrated that inhibition of histone deacetylase led to increased HSC chemotaxis (directed cell movement) towards CXCL12, leading to enhanced homing and long term engraftment of human HSCs in an NSG mouse model. Protein acetylation, especially histone acetylation, plays a crucial role in the regulation of protein function and gene transcription. Histone deacetylases (HDACs) are erasers of acetylation from lysine residues, while histone acetyltransferases (HATs) are responsible for adding an acetyl functional group back[31]. The balance between HDACs and HATs controls many physiological processes[32]. Indeed, we found that p300 HAT inhibitors, C646 and EML425, both showed suppressive effects of HDAC inhibitors on surface CXCR4 expression, suggesting that the balance between acetylation and deacetylation is important for the regulation of CXCR4 expression[30■■]. In mammals, HDACs comprise 18 genes that are grouped into five subfamilies (class I, IIa, IIb, III, IV) based on sequence similarity[33]. To further reveal the mechanisms of HSC homing regulation by HDACs, shRNA corresponding to individual HDACs or specific HDAC inhibitors, were used and their effects on surface CXCR4 expression were examined. Surprisingly, we found that only HDAC5 inhibition resulted in strong upregulation of CXCR4 expression on the cell membrane[30■■]. Consistently, HDAC5 inhibition promoted HSC chemotaxis, homing and long-term engraftment similar to that of pan HDAC inhibitors. HDAC5 belongs to class IIa HDACs, which can shuttle between the cytoplasm and nucleus, assemble into multiprotein complexes and which are responsive to various environmental stimuli. Thus, regulation of HDAC5 provides a mechanism for linking extracellular signaling with HSC homing to the BM environment. We further found

that HDAC5 inhibition increased histone acetylation at the CXCR4 promoter and acetylated p65 levels in the nucleus, which is important for CXCR4 transcription. Inhibition of Nuclear Factor-κB (NF-κB) signaling suppressed CXCR4 upregulation and enhanced HSC homing after HDCA5 inhibition, suggesting involvement of NF-κB signaling in HSC homing.

GLUCOCORTICOID REGULATION OF HSC HOMING

We also identified glucocorticoids as significant enhancers of CXCR4 surface expression and HSC chemotaxis[13,34■■]. Glucocorticoids are a class of steroid hormones secreted by the adrenal cortex[35]. Glucocorticoids bind to the glucocorticoid receptor to exert their biological effects[36]. We found that in human HSCs, the activated glucocorticoid receptor translocated into the nucleus and bound to glucocorticoid response elements in the promoter region of CXCR4, followed by the recruitment of SRC1/p300 histone acetyltransferase complex, which promoted histone H4 acetylation to facilitate CXCR4 transcription. Similar to effects of HDAC5 inhibition, this was associated with increased CXCR4 expression on the surface of HSC and resulted in enhanced human HSC homing and long-term engraftment in recipient NSG mice. Both HDAC5 inhibition and glucocorticoid treatment involved elevated histone acetylation at the CXCR4 promoter region. It will be interesting to see if there is any cross talk between HDAC5 and glucocorticoid, and if combination of such treatment resulted in an enhanced effect on homing/engrafting capability of HSCs.

STABILIZATION OF HIF-1α PROMOTES HSC HOMING

HIF-1α is a DNA binding transcriptional factor and functions as a critical mediator of cellular

response to hypoxia [37■,38,39]. Thus HIF-1α plays an important role during animal development, energy metabolism, cell survival and tumor angiogenesis. HSCs reside in a hypoxic BM microenvironment that supports stabilization of HIF-1α. It has been shown that HIF-1α regulates HSC activity and quiescence[40-42]. It has been reported that pharmacological stabilization of HIF-1α facilitates HSC homing and engraftment[43]. Pulse treatment with 16-16 dimethyl prostaglandin E2 (dmPGE2) or dimethyloxalylglycine (DMOG) led to significant increases in HIF-1α protein level. This resulted in upregulation of CXCR4 transcription by HIF-1α binding with hypoxia response elements -1.3kb from the transcription start site at the CXCR4 promoter region. Consequently, both dmPGE2 and DMOG treatment resulted in enhanced HSC chemotaxis, homing and engraftment due to better responsiveness to BM CXCL12 gradients[43,44]. Furthermore, it was demonstrated that HIF-1α is required for dmPGE2 mediated CXCR4 upregulation, enhanced HSC migration and homing. Recent work from another group reported that caffeic acid phenethyl ester (CAPE) administration promotes HSC homing and engraftment by inducing expression of HIF-1α [45]. CAPE treatment upregulated protein levels of HIF-1α and CXCL12 in BM endothelial cells. The HIF-1α inhibitor PX-478 suppressed CAPE-mediated enhanced HSC homing, further adding evidence for the importance of HIF-1α upregulation during HSC homing and engraftment.

INCREASING MEMBRANE LIPID RAFT AGGREGATION ENHANCES HSC HOMING

Cell membranes are composed of lipid bilayers containing many peripheral and integral membrane proteins. Lipid rafts are specialized membrane microdomains enriched in cholesterol and glycosphingolipids, and have been identified as playing a primary role in membrane signaling

transduction[46,47]. Incorporation of CXCR4 into lipid rafts is essential for optimal association with downstream signaling molecules. Studies from our laboratory found that short-term mild heating (39°C) resulted in increased membrane lipid raft aggregation, leading to elevated CXCR4 aggregation and colocalization with lipid rafts[48]. Increased co-localization of CXCR4 with lipid rafts resulted in enhanced interaction between CXCR4 and RAC1, thus leading to enhanced RAC1 activation and responsiveness of HSC towards BM CXCL12 gradients. Consequently, mild heating promoted human HSC homing and engraftment in an NSG mouse model. This suggested that mild heat treatment may be a simple and expensive approach to enhance human HCT in patients. However, other potential consequences of short-term mild heating of cells must be assessed to make sure that there are no side effects associated with such cell treatments.

INHIBITION OF DPP4 PROMOTES HSC HOMING AND ENGRAFTMENT

Dipeptidyl peptidase 4 (DPP4) is a 110-kDa cell surface serine protease expressed on the surface of HSCs and functions to selectively cleave the N-terminal penultimate Alanine or Proline amino acids[49]. The enzymatic activity of DPP4 is important for regulation of cellular functions and modulation of certain disease states[50,51]. Studies from our laboratory have demonstrated the roles of DPP4 in HSC mobilization induced by G-CSF[52,53], as well as HSC homing and engraftment by modulating CXCL12[54]. DPP4 cleaves the N-terminal dipeptide of CXCL12, generating a truncated form of CXCL12 that could not activate CXCR4. To suppress this unwanted effect, blocking the enzymatic activity of DPP4 served as a practical strategy to promote CXCL12/CXCR4 interaction and enhance the responsiveness of HSCs to CXCL12 gradients. Short term pretreatment of human CB $CD34^+$ cells or donor mouse BM cells with Diprotin A, a

DPP4 inhibitor, led to enhanced homing and engraftment in sublethally irradiated NSG mouse recipients[55] and lethally irradiated mouse recipients[54]. Sitagliptin is an FDA approved DPP4 inhibitor for the treatment of type II diabetes, and Sitagliptin administration to recipients has been shown in the clinical trials to enhance engraftment of single cord blood transplantation in patients[56,57]. This promising clinical strategy is now waiting for clinical verification by others.

MITIGATING EPHOSS TO ENHANCE COLLECTION OF HSCS

HSCs reside in a hypoxic BM niche *in vivo*[58-60]. Our studies demonstrated a pernicious effect of collecting and processing of BM and CB HSCs in ambient air that involved a phenomenon termed Extra Physiological Oxygen Stress/Shock (EPHOSS)[61,62]. This irreversible EPHOSS phenomenon is mediated by a p53-cyclophilin D-mitochondria permeability transition pore (MPTP) axis, with links to HIF-1α and the hypoxamir mir-210. Collection and processing of mouse BM and human CB HSCs under hypoxia conditions, such that the collected cells were never exposed to ambient air oxygen tension, mitigated EPHOSS and resulted in 2-5 fold increases in recovery of long-term repopulating HSCs compared to that of ambient air collection. Alternatively, HSCs could be protected from EPHOSS by modulating the MPTP opening via Cyclophilin D inhibition, genetically or by using the small molecule inhibitor cyclosporine A. This resulted in increased recovery of long-term repopulating HSCs. Thus, there are greater numbers of HSCs residing in mouse BM and human CB than previously reported, suggesting that HCT could be improved if EPHOSS is mitigated during the collection and processing of the cells. In an effort to identify other approaches to mimic “hypoxia harvest”, we found that collection and processing of mouse BM in the presence of specific combinations of

anti-oxidants and epigenetic enzyme inhibitors could also enhance recovery of HSCs[63]. Efforts to identify other means to suppress the phenomenon of EPHOSS are currently ongoing in our laboratory.

CONCLUSION

Successful clinical outcomes after HCT rely on adequate HSC numbers and the homing and subsequent short- and long-term engraftment of these cells in the BM. Enhancing the homing capability of HSCs could have a great impact on improving HCT procedures and patient survival. A better understanding of the molecular mechanisms regulating HSC homing and engraftment should facilitate development of more efficient means to enhance HCT in the future. In this review, we summarized current knowledge of the regulation of HSC homing and engraftment (Fig.1). These regulations form several different layers, and range from the cell membrane (DPP4, lipid rafts) to the cytoplasm (MPTP) and inside the nucleus (HDAC5, glucocorticoid receptor, HIF-1α). Among all these means, the effects of dmPGE2 and DPP4 inhibitor on HSC therapy have been tested in the clinic [56,57,64]. Importantly, combinations of DPP4 inhibition and dmPGE2 treatment has been shown to have synergistic effects on HSC engraftment in murine models[65]. Thus, a combination of DPP4 inhibition and dmPGE2 treatment represents a potentially promising way worthy of further testing in clinical settings in the future.

It remains to be seen if combinations of treatment to enhance CXCR4 expression can further increase homing and engrafting capacity of HSCs, and if not, which procedure might be best used in the clinical setting. It also remains to be seen if the increased number of HSCs obtained after collection/processing under hypoxic conditions to mitigate EPHOSS effects, can further enhance

engrafting capacity, since hypoxia collected/processed HSCs expressed lower CXCR4 surface protein than that of ambient air collected HSCs. In addition, *ex-vivo* expansion of HSCs is currently being studied as another means to enhance HCT[66-71], of interest is to integrate collection of cells in hypoxia with *ex-vivo* expansion and homing modulators (Fig.2). Recently, functional human HSCs have been successfully generated from pluripotent stem cells and endothelial cells[72,73]. It would also be of interest to determine if engraftment of these reprogrammed HSCs can be enhanced by modulating mechanisms regulating HSC homing as discussed in this review article.

KEY POINTS

- Inhibiting HDAC5 increases surface CXCR4 expression and promotes human HSC homing and engraftment by increasing acetylation levels of histone and p65.
- Glucocorticoid pretreatment of human HSCs and HPCs enhances their homing and engraftment capability in NSG mice.
- Pharmacological stabilization of HIF-1α by dmPGE2 and DMOG facilitates HSC homing and engraftment.
- Modulating MPTP to mitigate EPHOSS by hypoxic harvest and cyclosporine A increases functional HSC numbers and thus improves HSC engraftment, but enhance CXCR4 expression in HSCs might further enhance their engrafting capacity.
- Enhancing homing of cells collected by mitigating EPHOSS or after *ex-vivo* expansion may result in further increased engraftment.

Acknowledgements

None.

Financial support and sponsorship

The work was supported by US Public Health Service Grants from NIH to H.E.B. (R35HL139599, R01HL056416, R01HL112669, R01DK109188, and U54 DK106846).

Conflicts of interest

The authors have no conflicts of interest to declare.

Figure legned

Figure 1. Model for the molecule mechanisms regulating HSC homing and engraftment. The regulation of HSC homing and engraftment form several different layers, and range from the cell membrane (DPP4, lipid rafts) to the cytoplasm (MPTP) and inside the nucleus (HDAC5, glucocorticoid receptor, HIF-1α).

Figure 2. Multiple strategies involving enhanced homing of cells collected by EPHOSS mitigation and *ex-vivo* expansion to increase the efficacy of HCT. Shown are examples of such studies by our group that may be incorporated into future experimental models eventually leading to evaluation in a clinical setting. [ ], designates references.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest
■■ of outstanding interest

1. Carroll D, St Clair DK: **Hematopoietic stem cells: Normal versus malignant.** *Antioxid Redox Signal* (2018) **29**(16):1612-1632.

2. Doulatov S, Notta F, Laurenti E, Dick JE: **Hematopoiesis: A human perspective.** *Cell Stem Cell* (2012) **10**(2):120-136.

3. Ng AP, Alexander WS: **Haematopoietic stem cells: Past, present and future.** *Cell Death Discov* (2017) **3**:17002.

4. Ratajczak MZ, Suszynska M: **Emerging strategies to enhance homing and engraftment of hematopoietic stem cells.** *Stem Cell Rev* (2016) **12**(1):121-128.

5. Calvi LM, Link DC: **The hematopoietic stem cell niche in homeostasis and disease.** *Blood* (2015) **126**(22):2443-2451.

6. Laurenti E, Gottgens B: **From haematopoietic stem cells to complex differentiation landscapes.** *Nature* (2018) **553**(7689):418-426.

■ An excellent review on our understanding of hematopoietic stem cells and development of new therapies for hematopoietic diseases.

7. Morgan RA, Gray D, Lomova A, Kohn DB: **Hematopoietic stem cell gene therapy: Progress and lessons learned.** *Cell Stem Cell* (2017) **21**(5):574-590.

8. Lapidot T, Dar A, Kollet O: **How do stem cells find their way home?** *Blood* (2005) **106**(6):1901-1910.

9. Szade K, Gulati GS, Chan CKF, Kao KS, Miyanishi M, Marjon KD, Sinha R, George BM, Chen JY, Weissman IL: **Where hematopoietic stem cells live: The bone marrow niche.** *Antioxid Redox Signal* (2018) **29**(2):191-204.

■ An excellent review on celluluar architecture and function of the hematopoietic stem cell niche.

10. Crane GM, Jeffery E, Morrison SJ: **Adult haematopoietic stem cell niches.** *Nat Rev Immunol* (2017) **17**(9):573-590.

11. Yu VW, Scadden DT: **Heterogeneity of the bone marrow niche.** *Curr Opin Hematol* (2016) **23**(4):331-338.

12. Broxmeyer HE: **Enhancing the efficacy of engraftment of cord blood for hematopoietic cell transplantation.** *Transfus Apher Sci* (2016) **54**(3):364-372.

13. Guo B, Huang X, Broxmeyer HE: **Enhancing human cord blood hematopoietic stem cell engraftment by targeting nuclear hormone receptors.** *Curr Opin Hematol* (2018)

25(4):245-252.

14. Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D, Arny M, Thomas L, Boyse EA: **Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells.** *Proc Natl Acad Sci U S A* (1989) **86**(10):3828-3832.

15. Kim CH, Broxmeyer HE: **In vitro behavior of hematopoietic progenitor cells under the influence of chemoattractants: Stromal cell-derived factor-1, steel factor, and the bone marrow environment.** *Blood* (1998) **91**(1):100-110.

16. Ratajczak MZ, Adamiak M, Plonka M, Abdel-Latif A, Ratajczak J: **Mobilization of hematopoietic stem cells as a result of innate immunity-mediated sterile inflammation in the bone marrow microenvironment-the involvement of extracellular nucleotides and purinergic signaling.** *Leukemia* (2018) **32**(5):1116-1123.

17. Nagasawa T: **Cxcl12/sdf-1 and cxcr4.** *Front Immunol* (2015) **6**:301.

18. Heard E, Martienssen RA: **Transgenerational epigenetic inheritance: Myths and mechanisms.** *Cell* (2014) **157**(1):95-109.

19. Deichmann U: **Epigenetics: The origins and evolution of a fashionable topic.** *Dev Biol* (2016) **416**(1):249-254.

20. Canovas S, Ross PJ: **Epigenetics in preimplantation mammalian development.** *Theriogenology* (2016) **86**(1):69-79.

21. Nuhrenberg T, Gilsbach R, Preissl S, Schnick T, Hein L: **Epigenetics in cardiac development, function, and disease.** *Cell Tissue Res* (2014) **356**(3):585-600.

22. Perduca V, Omichessan H, Baglietto L, Severi G: **Mutational and epigenetic signatures in cancer tissue linked to environmental exposures and lifestyle.** *Curr Opin Oncol* (2018) **30**(1):61-67.

23. Handy DE, Castro R, Loscalzo J: **Epigenetic modifications: Basic mechanisms and role in cardiovascular disease.** *Circulation* (2011) **123**(19):2145-2156.

24. Cheung P, Vallania F, Warsinske HC, Donato M, Schaffert S, Chang SE, Dvorak M, Dekker CL, Davis MM, Utz PJ, Khatri P *et al*: **Single-cell chromatin modification profiling reveals increased epigenetic variations with aging.** *Cell* (2018) **173**(6):1385-1397 e1314.

■ This article employs a highly multiplexed mass cytometry analysis and provides chromatin modification profile at single cell level.

25. Huang X, Broxmeyer HE: **M(6)a reader suppression bolsters hsc expansion.** *Cell Res* (2018) **28**(9):875-876.

26. Holoch D, Moazed D: **Rna-mediated epigenetic regulation of gene expression.** *Nat Rev Genet* (2015) **16**(2):71-84.

27. Zhou H, Wang B, Sun H, Xu X, Wang Y: **Epigenetic regulations in neural stem cells and neurological diseases.** *Stem Cells Int* (2018) **2018**:6087143.

■ An excellent review on epigenetic regulations in neuscience field.

28. Sasidharan Nair V, El Salhat H, Taha RZ, John A, Ali BR, Elkord E: **DNA methylation and repressive h3k9 and h3k27 trimethylation in the promoter regions of pd-1, ctla-4, tim-3, lag-3, tigit, and pd-l1 genes in human primary breast cancer.** *Clin Epigenetics* (2018) **10**:78.

29. Van HT, Santos MA: **Histone modifications and the DNA double-strand break response.** *Cell Cycle* (2018) **17**(21-22):2399-2410.

30. Huang X, Guo B, Liu S, Wan J, Broxmeyer HE: **Neutralizing negative epigenetic regulation by hdac5 enhances human haematopoietic stem cell homing and engraftment.** *Nat Commun* (2018) **9**(1):2741.

■■ This article has identified for the first time that HDAC5 inhibiton resulted in enhanced human hematopoietic stem cell homing and engraftment by increasing acetylation levels of hisone and p65.

31. Yang XJ, Seto E: **Hats and hdacs: From structure, function and regulation to novel strategies for therapy and prevention.** *Oncogene* (2007) **26**(37):5310-5318.

32. Peserico A, Simone C: **Physical and functional hat/hdac interplay regulates protein acetylation balance.** *J Biomed Biotechnol* (2011) **2011**(371832.

33. Delcuve GP, Khan DH, Davie JR: **Roles of histone deacetylases in epigenetic regulation: Emerging paradigms from studies with inhibitors.** *Clin Epigenetics* (2012) **4**(1):5.

34. Guo B, Huang X, Cooper S, Broxmeyer HE: **Glucocorticoid hormone-induced chromatin remodeling enhances human hematopoietic stem cell homing and engraftment.** *Nat Med* (2017) **23**(4):424-428.

■■ This article has identified for the first time that glucocorticoid pretreatment resulted in enhanced human hematopoietic stem cell homing and engraftment by regulating chromatin remodeling.

35. Blum A, Maser E: **Enzymology and molecular biology of glucocorticoid metabolism in humans.** *Prog Nucleic Acid Res Mol Biol* (2003) **75**(173-216.

36. Jing D, Huang Y, Liu X, Sia KCS, Zhang JC, Tai X, Wang M, Toscan CE, McCalmont H, Evans K, Mayoh C *et al*: **Lymphocyte-specific chromatin accessibility pre-determines glucocorticoid resistance in acute lymphoblastic leukemia.** *Cancer Cell* (2018) **34**(6):906-921 e908.

37. Huang X, Trinh T, Aljoufi A, Broxmeyer HE: **Hypoxia signaling pathway in stem cell regulation: Good and evil.** *Current Stem Cell Reports* (2018) **4**(2):149-157.

■ An excellent review on hypoxia and HIF-1α in stem cell regulation.

38. Galbraith MD, Allen MA, Bensard CL, Wang X, Schwinn MK, Qin B, Long HW, Daniels DL, Hahn WC, Dowell RD, Espinosa JM: **Hif1a employs cdk8-mediator to stimulate rnapii elongation in**

response to hypoxia. *Cell* (2013) **153**(6):1327-1339.

39. Wang H, Flach H, Onizawa M, Wei L, McManus MT, Weiss A: **Negative regulation of hif1a expression and th17 differentiation by the hypoxia-regulated microrna mir-210.** *Nat Immunol* (2014) **15**(4):393-401.

40. Forristal CE, Winkler IG, Nowlan B, Barbier V, Walkinshaw G, Levesque JP: **Pharmacologic stabilization of hif-1alpha increases hematopoietic stem cell quiescence in vivo and accelerates blood recovery after severe irradiation.** *Blood* (2013) **121**(5):759-769.

41. Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda E, Kubota Y, Shima H, Johnson RS, Hirao A, Suematsu M, Suda T: **Regulation of the hif-1alpha level is essential for hematopoietic stem cells.** *Cell Stem Cell* (2010) **7**(3):391-402.

42. Imanirad P, Solaimani Kartalaei P, Crisan M, Vink C, Yamada-Inagawa T, de Pater E, Kurek D, Kaimakis P, van der Linden R, Speck N, Dzierzak E: **Hif1alpha is a regulator of hematopoietic progenitor and stem cell development in hypoxic sites of the mouse embryo.** *Stem Cell Res* (2014) **12**(1):24-35.

43. Speth JM, Hoggatt J, Singh P, Pelus LM: **Pharmacologic increase in hif1alpha enhances hematopoietic stem and progenitor homing and engraftment.** *Blood* (2014) **123**(2):203-207.

44. Hoggatt J, Singh P, Sampath J, Pelus LM: **Prostaglandin e2 enhances hematopoietic stem cell homing, survival, and proliferation.** *Blood* (2009) **113**(22):5444-5455.

45. Chen X, Han Y, Zhang B, Liu Y, Wang S, Liao T, Deng Z, Fan Z, Zhang J, He L, Yue W *et al*: **Caffeic acid phenethyl ester promotes haematopoietic stem/progenitor cell homing and engraftment.** *Stem Cell Res Ther* (2017) **8**(1):255.

46. Ando J, Kinoshita M, Cui J, Yamakoshi H, Dodo K, Fujita K, Murata M, Sodeoka M: **Sphingomyelin distribution in lipid rafts of artificial monolayer membranes visualized by raman microscopy.** *Proc Natl Acad Sci U S A* (2015) **112**(15):4558-4563.

47. Simons K, Ehehalt R: **Cholesterol, lipid rafts, and disease.** *J Clin Invest* (2002) **110**(5):597-603.

48. Capitano ML, Hangoc G, Cooper S, Broxmeyer HE: **Mild heat treatment primes human cd34(+) cord blood cells for migration toward sdf-1alpha and enhances engraftment in an nsg mouse model.** *Stem Cells* (2015) **33**(6):1975-1984.

49. O'Leary H, Ou X, Broxmeyer HE: **The role of dipeptidyl peptidase 4 in hematopoiesis and transplantation.** *Curr Opin Hematol* (2013) **20**(4):314-319.

50. Ou X, O'Leary HA, Broxmeyer HE: **Implications of dpp4 modification of proteins that regulate stem/progenitor and more mature cell types.** *Blood* (2013) **122**(2):161-169.

51. Broxmeyer HE, Hoggatt J, O'Leary HA, Mantel C, Chitteti BR, Cooper S, Messina-Graham S, Hangoc G, Farag S, Rohrabaugh SL, Ou X *et al*: **Dipeptidylpeptidase 4 negatively regulates colony-stimulating factor activity and stress hematopoiesis.** *Nat Med* (2012) **18**(12):1786-1796.

52. Christopherson KW, 2nd, Cooper S, Broxmeyer HE: **Cell surface peptidase cd26/dppiv mediates g-csf mobilization of mouse progenitor cells.** *Blood* (2003) **101**(12):4680-4686.

53. Christopherson KW, Cooper S, Hangoc G, Broxmeyer HE: **Cd26 is essential for normal g-csf-induced progenitor cell mobilization as determined by cd26-/- mice.** *Exp Hematol* (2003) **31**(11):1126-1134.

54. Christopherson KW, 2nd, Hangoc G, Mantel CR, Broxmeyer HE: **Modulation of hematopoietic stem cell homing and engraftment by cd26.** *Science* (2004) **305**(5686):1000-1003.

55. Campbell TB, Hangoc G, Liu Y, Pollok K, Broxmeyer HE: **Inhibition of cd26 in human cord blood cd34+ cells enhances their engraftment of nonobese diabetic/severe combined immunodeficiency mice.** *Stem Cells Dev* (2007) **16**(3):347-354.

56. Farag SS, Srivastava S, Messina-Graham S, Schwartz J, Robertson MJ, Abonour R, Cornetta K, Wood L, Secrest A, Strother RM, Jones DR *et al*: **In vivo dpp-4 inhibition to enhance engraftment of single-unit cord blood transplants in adults with hematological malignancies.** *Stem Cells Dev* (2013) **22**(7):1007-1015.

57. Farag SS, Nelson R, Cairo MS, O'Leary HA, Zhang S, Huntley C, Delgado D, Schwartz J, Zaid MA, Abonour R, Robertson M *et al*: **High-dose sitagliptin for systemic inhibition of dipeptidylpeptidase-4 to enhance engraftment of single cord umbilical cord blood transplantation.** *Oncotarget* (2017) **8**(66):110350-110357.

58. Zhang CC, Sadek HA: **Hypoxia and metabolic properties of hematopoietic stem cells.** *Antioxid Redox Signal* (2014) **20**(12):1891-1901.

59. Testa U, Labbaye C, Castelli G, Pelosi E: **Oxidative stress and hypoxia in normal and leukemic stem cells.** *Exp Hematol* (2016) **44**(7):540-560.

60. Spencer JA, Ferraro F, Roussakis E, Klein A, Wu J, Runnels JM, Zaher W, Mortensen LJ, Alt C, Turcotte R, Yusuf R *et al*: **Direct measurement of local oxygen concentration in the bone marrow of live animals.** *Nature* (2014) **508**(7495):269-273.

61. Mantel CR, O'Leary HA, Chitteti BR, Huang X, Cooper S, Hangoc G, Brustovetsky N, Srour EF, Lee MR, Messina-Graham S, Haas DM *et al*: **Enhancing hematopoietic stem cell transplantation efficacy by mitigating oxygen shock.** *Cell* (2015) **161**(7):1553-1565.

62. Broxmeyer HE, O'Leary HA, Huang X, Mantel C: **The importance of hypoxia and extra physiologic oxygen shock/stress for collection and processing of stem and progenitor cells to understand true physiology/pathology of these cells ex vivo.** *Curr Opin Hematol* (2015) **22**(4):273-278.

63. Cai Q, Capitano M, Huang X, Guo B, Cooper S, Broxmeyer HE: **Combinations of antioxidants and/or of epigenetic enzyme inhibitors allow for enhanced collection of mouse bone marrow hematopoietic stem cells in ambient air.** *Blood Cells Mol Dis* (2018) **71**(23-28.

64. Cutler C, Multani P, Robbins D, Kim HT, Le T, Hoggatt J, Pelus LM, Desponts C, Chen YB, Rezner B, Armand P *et al*: **Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation.** *Blood* (2013) **122**(17):3074-3081.

65. Broxmeyer HE, Pelus LM: **Inhibition of dpp4/cd26 and dmpge(2) treatment enhances engraftment of mouse bone marrow hematopoietic stem cells.** *Blood Cells Mol Dis* (2014) **53**(1-2):34-38.

66. Boitano AE, Wang J, Romeo R, Bouchez LC, Parker AE, Sutton SE, Walker JR, Flaveny CA, Perdew GH, Denison MS, Schultz PG *et al*: **Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells.** *Science* (2010) **329**(5997):1345-1348.

67. Fares I, Chagraoui J, Gareau Y, Gingras S, Ruel R, Mayotte N, Csaszar E, Knapp DJ, Miller P, Ngom M, Imren S *et al*: **Cord blood expansion. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal.** *Science* (2014) **345**(6203):1509-1512.

68. Wagner JE, Jr., Brunstein CG, Boitano AE, DeFor TE, McKenna D, Sumstad D, Blazar BR, Tolar J, Le C, Jones J, Cooke MP *et al*: **Phase i/ii trial of stemregenin-1 expanded umbilical cord blood hematopoietic stem cells supports testing as a stand-alone graft.** *Cell Stem Cell* (2016) **18**(1):144-155.

69. Huang X, Lee MR, Cooper S, Hangoc G, Hong KS, Chung HM, Broxmeyer HE: **Activation of oct4 enhances ex vivo expansion of human cord blood hematopoietic stem and progenitor cells by regulating hoxb4 expression.** *Leukemia* (2016) **30**(1):144-153.

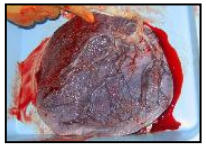
70. Nikiforow S, Ritz J: **Dramatic expansion of hscs: New possibilities for hsc transplants?** *Cell Stem Cell* (2016) **18**(1):10-12.

71. Guo B, Huang X, Lee MR, Lee SA, Broxmeyer HE: **Antagonism of ppar-gamma signaling expands human hematopoietic stem and progenitor cells by enhancing glycolysis.** *Nat Med* (2018) **24**(3):360-367.

72. Sandler VM, Lis R, Liu Y, Kedem A, James D, Elemento O, Butler JM, Scandura JM, Rafii S: **Reprogramming human endothelial cells to haematopoietic cells requires vascular induction.** *Nature* (2014) **511**(7509):312-318.

73. Sugimura R, Jha DK, Han A, Soria-Valles C, da Rocha EL, Lu YF, Goettel JA, Serrao E, Rowe RG, Malleshaiah M, Wong I *et al*: **Haematopoietic stem and progenitor cells from human pluripotent stem cells.** *Nature* (2017) **545**(7655):432-438.

Cord Blood (CB) Collection and Transplantation



Collection/Processing of Cord Blood
Hypoxic Collection

Hypoxic Chamber

Collection in hypoxia (3% O_2) or in air in presence of cyclosporine A or combinations of antioxidant +/- epigenetic enzyme inhibitors [61-63]

⇧HSC numbers

⇧Engraftment

***Ex-vivo* Expansion**
4-7 days culture

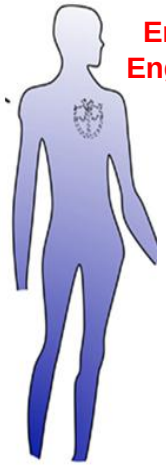
HSC expansion *Ex Vivo*
(SCF, TPO, FL) plus:

DPP4 Inhibition [49,51]
OCT4 [69]
PPARγ antagonists [71]

(⇧HSC numbers)

Enhanced engraftment

GRAFT RECIPIENT



Enhanced Engraftment

Homing
Ex-vivo culture
4-16 hours

Enhanced Homing/Engraftment

Enhanced Homing

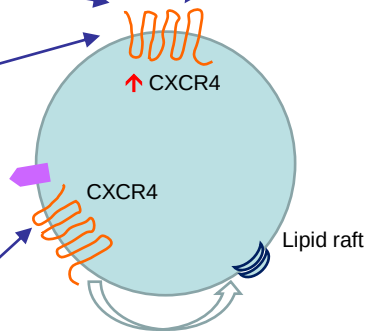
•Glucocorticoids (e.g. Flonase, Dexamethasone, Cortisol, Medrol) [34■■]

PGE2 [44]

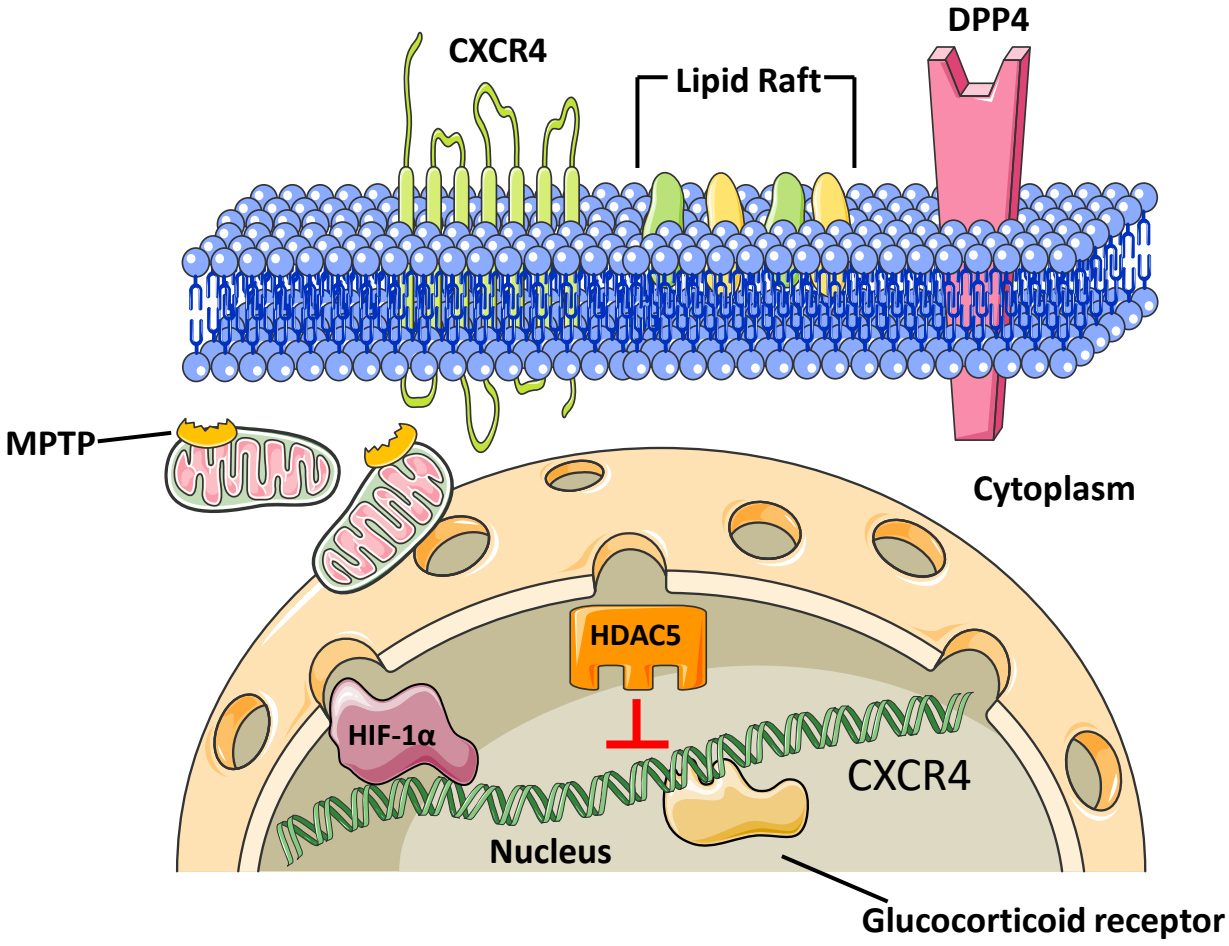
HDAC5 Inhibitor [30■■]

DPP4/CD26 Inhibition (e.g. sitagliptin (Januvia®) [49-57]

⇧SDF-1



Short term Mild heat (39^0C)exposure [48]


CXCR4
Lipid Raft
DPP4
MPTP
Cytoplasm
HDAC5
HIF-1α
CXCR4
Nucleus
Glucocorticoid receptor