Regulation of hematopoietic stem cell function by nitric oxide signaling

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Mechanisms controlling homing of transplanted hematopoietic stem cells (HSC) to the bone marrow (BM) and their mobilization to the blood have been extensively studied due to their clinical relevance. Nevertheless, new reports bring new insights with the potential to improve clinical BM transplantation protocols.

The groups of Broxmeyer and Huang have recently reported that short in vitro pretreatment of human cord blood (CB) $CD34^+$ enriched HSC with nitric oxide (NO) donor or with a pharmacological activator of NO, promoted their BM homing and engraftment in primary and serially transplanted immune deficient NOD/SCID mice.[1] Mechanistically, this treatment upregulated the expression and function of surface CXCR4 (the major receptor of the chemokine CXCL12), including in the most primitive human CB HSC. This NO-mediated CXCR4 up-regulation increased human $CD34^+$ HSC in vitro migration toward a gradient of CXCL12 as well as their in vivo BM homing and repopulation in primary and serially transplanted immune deficient NOD-SCID mice. A similar effect was also obtained by activation of cGMP, which is downstream to NO signaling. Limiting dilution analysis revealed that this short 16 h ex vivo NO-mediated CXCR4 up-regulation dramatically increased the frequency of primitive human $CD34^+$ SCID repopulating stem cells, showing a robust 5.8-fold increase.[1]

Nitric oxide is a short-lived, gaseous free radical, which is currently the smallest known signaling molecule. NO is mostly synthesized by a family of three NO synthase isoforms, termed NOS1-3 or neuronal (nNOS), inducible (iNOS), and endothelial (eNOS), respectively. NO signaling has many important physiological roles, including regulation of the neuronal, immune, and cardiovascular systems, also during alarm situations and infections. In mouse embryonal development blood flow induces eNOS-mediated NO generation and signaling which is essential for HSC development and initiation of hematopoiesis.[2]

NO signaling is a key player in HSC regulation. In adult mice, the coagulation receptor PAR1 is functionally expressed by HSC, including by the more primitive $EPCR^+$ long-term repopulating hematopoietic stem cells (LT-HSC). PAR1 mediates two different signaling cascades leading to opposite outcomes: pro- or anti-inflammatory, which are also pro- or anti-coagulation. In BM retained LT-HSC, thrombin/PAR1 signaling induces NO generation and signaling leading to TACE (ADAM17)-mediated EPCR shedding, CXCR4 up-regulation, BM CXCL12 secretion, and HSC mobilization. In vivo treatment of mice with NO-donors mimicked thrombin/PAR1 signaling leading to rapid CXCR4/CXCL12-mediated HSC mobilization. In contrast, aPC/EPCR/PAR1 signaling retains LT-HSC in the BM by inhibiting NO generation, reducing Cdc42 activity and enhancing VLA4 adhesion. BM retained $EPCR^+$ LT-HSC are chemotherapy resistant and the aPC/EPCR/PAR1/VLA4 axis is essential both for LT-HSC BM retention and for protecting mice from lethal chemotherapy-induced hematology failure and infections.[3]

Thus, NO mediated CXCR4 upregulation is apparently required for enhancing HSC motility in both directions: mobilization and homing. Interestingly, the HSC self-renewal agonist UM171 balances pro- and anti-inflammatory signals, with exclusive in vitro induction of human $EPCR^+$ HSC expansion. In vitro expansion of human CB $CD34^+$ HSC with UM171 led to the appearance of a small population of $EPCR^+/CD34^+$ HSC. Importantly, only $EPCR^+$ but not $EPCR^-$ human CB HSC were endowed with robust high level multi-lineage repopulation and serial reconstitution capacity in transplanted immune deficient NOD/SCID mice.[4] EPCR function protected human LT-HSC from TNF and UM171 mediated hyper inflammatory responses by preventing NO overproduction in primitive $EPCR^+$ LT-HSC, which can lead to cell-death and compromised HSC function.[4] In another study with mouse HSC, two major NO effects were observed. Increased HSC proliferation, particularly of short-term repopulating HSC, and increased terminal myeloid differentiation.[5] In addition, iNOS was found to be a negative regulator of mouse HSC migration and mobilization, due to downstream activation of hem oxygenase 1 (HO-1).[6] In vitro stimulation of human $CD34^+$ HSC obtained from adult BM, mobilized peripheral blood, and CB with NO, upregulated surface CXCR4 expression, function, and transcription, in a time and dose-dependent manner.[7]

These results have clinical relevance since currently the small numbers of human CB $CD34^+$ HSC are sufficient mostly for pediatric clinical transplantation due to the small numbers of CB HSC which are insufficient for adults. Of note, the levels of surface CXCR4 expression and in vitro migration of G-CSF-mobilized human $CD34^+$ HSC to a CXCL12 gradient correlated with their in vivo hematopoietic recovery in transplanted patients.[8] Moreover, human $CD34^+$ HSC BM homing and

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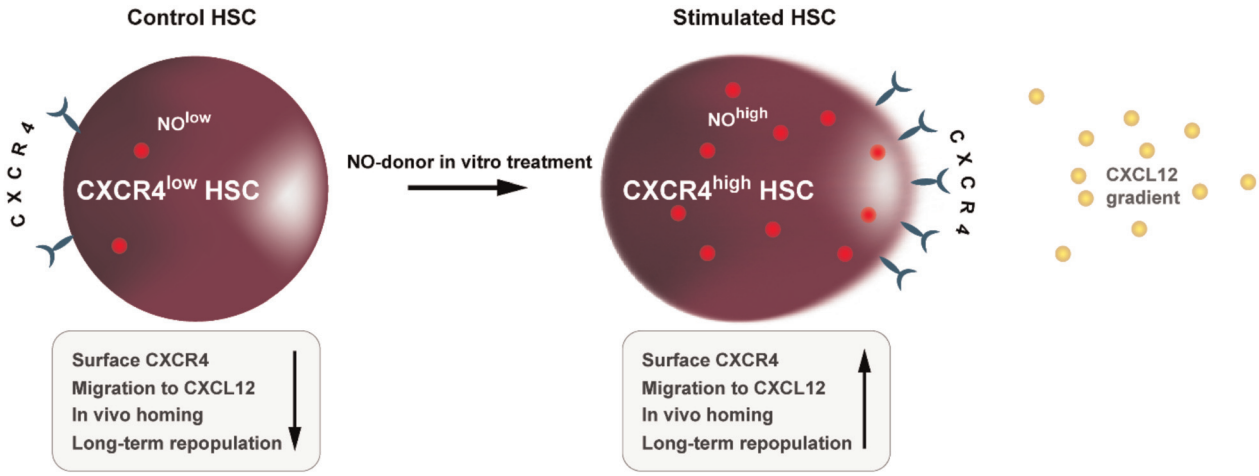

Figure 1. In vitro Nitric Oxide (NO) mediated CXCR4 upregulation enhances Human CB HSC migration to a gradient of CXCL12 in vitro, and in vivo BM homing and repopulation of transplanted immune deficient NOD/SCID mice.

engraftment in transplanted immune deficient NOD/SCID mice are also dependent on surface CXCR4 expression and signaling, confirming the clinical stem cell transplantation relevance of this functional preclinical model.[9] In line, PAR1 signaling is essential for CXCR4 mediated directional migration of both human and mouse HSC to a gradient of CXCL12 in vitro.[3,10] Importantly, the levels of PAR1 expression by mononuclear leukocytes in the blood of healthy donors during steady state correlate with their in vitro CXCR4-dependent migration to CXCL12, the yield of their G-CSF mobilized $CD34^+$ HSC and the kinetics of hematological recovery in clinically matched allogeneic transplanted patients.[10]

Taken together these studies reveal the essential roles of EPCR, PAR1, NO, and CXCR4 signaling in human HSC function, and suggest short in vitro pretreatment with thrombin, NO or its agonists in order to improve clinical CB $CXCR4^+$ HSC transplantation outcomes Figure 1.

REFERENCES

[1] Xu D, Yang M, Capitano M, et al. Pharmacological activation of nitric oxide signaling promotes human hematopoietic stem cell homing and engraftment. *Leukemia* 2020;doi: 10.1038/s41375-020-0787-z.

[2] North TE, Goessling W, Peeters M, et al. Hematopoietic stem cell development is dependent on blood flow. *Cell* 2009;137(4):736–748.

[3] Gur-Cohen S, Itkin T, Chakrabarty S, et al. PAR1 signaling regulates the retention and recruitment of EPCR-expressing bone marrow hematopoietic stem cells. *Nat Med* 2015;21(11):1307–1317.

[4] Chagraoui J, Lehnertz B, Girard S, et al. UM171 induces a homeostatic inflammatory-detoxification response supporting human HSC self-renewal. *PLoS One* 2019;14(11):e0224900.

[5] Nogueira-Pedro A, Dias CC, Regina H, et al. Nitric oxide-induced murine hematopoietic stem cell fate involves multiple signaling proteins, gene expression, and redox modulation. *Stem Cells* 2014;32(11):2949–2960.

[6] Adamiak M, Abdelbaset-Ismail A, Moore IVJB, et al. Inducible nitric oxide synthase (iNOS) is a novel negative regulator of hematopoietic stem/progenitor cell trafficking. *Stem Cell Rev Rep* 2017;13:92–103.

[7] Zhang Y, Wittner M, Bouamar H, Jarrier P, Vainchenker W, Louache F. Identification of CXCR4 as a new nitric oxide-regulated gene in human CD34+ cells. *Stem Cells* 2007;25(1):211–219.

[8] Voermans C, Kooi ML, Rodenhuis S, van der Lelie H, van der Schoot CE, Gerritsen WR. In vitro migratory capacity of CD34+ cells is related to hematopoietic recovery after autologous stem cell transplantation. *Blood* 2001;97(3):799–804.

[9] Peled A, Petit I, Kollet O, et al. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science* 1999;283(5403):845–848.

[10] Nevo N, Zuckerman T, Gur-Cohen S, et al. PAR1 expression predicts clinical G-CSF CD34(+) HSPC mobilization and repopulation potential in transplanted patients. *Hemasphere* 2019;3(5):e288.