論文の内容の要旨

論文題目　Elucidation of the Role of CXCR4 Signaling in Hematopoietic Stem Cell Repopulation

（造血幹細胞とCXCR4シグナル:骨髄再構築過程における役割の解明）

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Introduction:

Hematopoietic stem cells (HSCs) represent a unique cell population capable of self-renewal and multi-lineage differentiation thereby achieving the lifelong sustainment of hematopoiesis. HSC transplantation has proven to be beneficial for treating various diseases so it is therefore important to elucidate the molecular determinants leading to successful HSC engraftment. Hematopoietic reconstitution is the dynamic process following transplantation and can be divided into three major steps: 1) a homing/lodging steps where HSCs transmigrated into the cavity of bone marrow (BM); 2) a BM repopulation steps where HSCs replicated themselves while producing progenitor cells with multi-lineage differentiation potential; 3) a peripheral reconstitution steps where mature cells are released from BM to periphery (Fig1). Failure in any of these processes could impair transplantation outcomes.

Signaling through the C-X-C chemokine receptor type 4 (CXCR4) has been implicated in HSC engraftment from the observation that transplantation of mouse BM cells lacking this receptor results in poor hematopoietic reconstitution. In contrast, overexpression of CXCR4 receptor in human hematopoietic stem and progenitor cells (HSPCs) led to the enhanced engraftment in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice, suggesting the modification of this signaling in donor cells may benefit the transplanted patient. However, it remains unclear which steps specifically is CXCR4 attributable to and subsequently how it influences the fate of purified transplanted donor HSCs. In this study, we aim to

investigate the roles of CXCR4 in HSC repopulation kinetics *in vivo* by using both gain-of-function (overexpression) and loss-of-function (knockout or desensitization) strategies. In semi-quantitative analysis of gain-of-function effects, we use not only wild-type (WT) Cxcr4-overexpressing cells but also cells expressing Cxcr4 with a specific C-terminal deletion (ΔC) homologous to that found in patient with disorder termed warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome. This mutation is regarded as gain-of-function mutation because it increases the cell-surface stability of CXCR4 receptor without impairing CXCR4 signaling capabilities. By using this approach, we assessed each of the multiple steps in donor cell reconstitution in mice the received Cxcr4-modified HSC/HSPCs and dissected the stage-specific roles that Cxcr4 signaling plays in transplanted cells.

Materials and methods:

In this study HSCs were defined as $CD34^{neg/low}$ c-Kit^+ $Sca\text{-}1^+$ Lineage marker-negative. As a loss-of-function study, CXCR4 was conditionally deleted in HSCs (CXCR4-KO HSCs) by the Mx1-Cre-loxP system before transplantation. As a gain-of-function study, we generated HSC populations overexpressing either wild-type (WT)- or C-terminal truncated (ΔC)-CXCR4 by retroviral gene transfer. Control HSCs were those transduced with the mock virus in gain-of-function studies or from littermate control in loss-of function experiments.

Biological characteristics of those Cxcr4-modified HSCs were assessed *in vitro* with assays testing receptor internalization, chemotaxis, proliferation, colony formation, and cobblestone-area forming ability. The later is an indication of cells with long-term repopulating potential. *In vivo*, the homing/lodging phases (<1wk) were examined by plating recipient bone marrow cells onto methylcellulose or counting by flow cytometry. To assess short-term (2-3 weeks) and long-term (>16 weeks) reconstitution ability in test cells, a competitive repopulation assay was utilized. By looking at the chimerism of donor cells in the BM or peripheral blood (PB) of recipient mice at specific time points, we assessed how Cxcr4 signaling in the transplanted cells would contribute to the each component in hematopoietic reconstitution process.

Results:

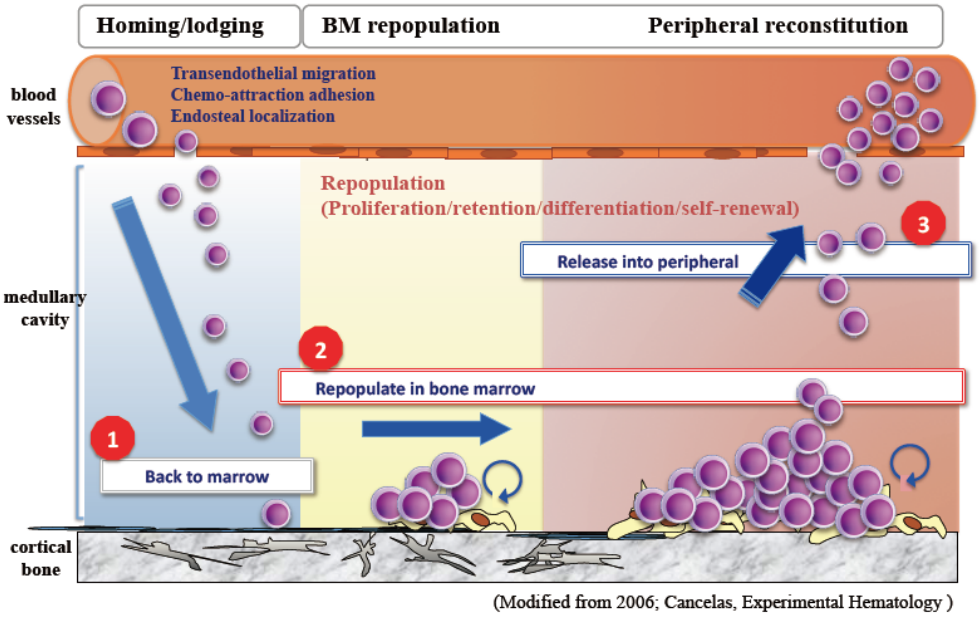
In vitro studies: Stepwise gain-of-function effect of exogenous Cxcr4 on transduced HSCs, with greater response for ΔC-Cxcr4 was observed in chemotaxis and proliferation assay. Cobblestone-area forming ability was greater in HSCs overexpressing ΔC-Cxcr4 than the Mock control and was detracted from Cxcr4-KO HSCs. Collectively, these data suggesting that augmented Cxcr4 signaling favor the survival and proliferation of HSCs *in vitro*.

In vivo studies: 1) The homing/lodging steps. It did not appear that there were any significant differences through the modification of Cxcr4 expression in the number of colony forming cells detectable in recipient BM within this phase (as earliest, 16 hours), indicating that this signaling is dispensable in donor HSC homing. 2) The BM repopulation steps. Impairment of hematopoietic repopulation in BM became evident for Cxcr4-KO HSCs between 2-3 wks. On the other hand, stepwise enhancement in donor chimerism was visible in the BM with ΔC-Cxcr4 cells, suggesting the advantage in the BM repopulation of Cxcr4-augmented HSCs. This advantage was also noticeable in the BM cells throughout developmental stages of HSCs expressing gain-of-function Cxcr4 receptor in long-term recipients (>16 weeks). More importantly, donor chimerism in long-term HSCs (LT-HSCs) population was also increased in Cxcr4-augmented group, which suggested that Cxcr4-signaling dependent donor cell expansion may occur at a stem cell level. Even though these "phenotypically-defined" LT-HSCs might not necessarily imply that functional HSC numbers were amplified. 3) The peripheral reconstitution steps. Cxcr4-KO HSCs showed poor hematopoietic reconstitution potentials which interestingly, was an observation also made with donor HSCs overexpressing WT- or ΔC-Cxcr4. Systemic injection of the Cxcr4 antagonist AMD3100 into long-term recipient increased PB donor chimerism significantly in WT- and ΔC-Cxcr4 groups, suggesting that blunted peripheral mobilization of donor cells played a causal role in poor PB reconstitution in these mice.

Mechanistic analysis revealed that altered phosphorylation kinetics of ERK and/or varied expression profile of adhesion molecules in the Cxcr4 overexpressing cells may constitute the basis for the observed positive effects.

Conclusions:

We have addressed stage-specific roles for Cxcr4 signaling in donor cell repopulation in BM for the first time using purified mouse HSC/HSPCs. With unique combinational approaches that utilized both loss-of-function and gain-of-function modification of Cxcr4 receptors, we found that Cxcr4 signaling appears unimportant for the homing/lodging of mouse HSC/HSPCs, but vital for their subsequent repopulation of BM. Cxcr4 signal enhancement likely favored BM repopulation by donor cells at a level of primitive cell populations, but was shown to be detrimental to PB reconstitution when sustained too long. Consequently, we think it important to investigate further when and how long signaling via this chemokine receptor is to be modified in order favorably to enhance HSPC engraftment in future transplantation medicine.



(Modified from 2006; Cancelas, Experimental Hematology)

Figure 1. The dynamic process of hematopoietic reconstitution after transplantation.

	Homing/ lodging (BM) <1wk	Early repopulation (BM) 2-4wks	Long-term repopulation (BM) >16wks	Long-term repopulation (PB) >16wks	HSC repopulation (*in vivo* self-renewal?)
Cxcr4-OE	→	↗	↑	↓	↑
Cxcr4-KO	→	↓	↓	↓	↓

The stage-specific roles for Cxcr4 signaling in transplanted donor cells.