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Previews

$G\alpha_s$ Uncouples Hematopoietic Stem Cell Homing and Mobilization

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Defects of hematopoietic stem cell adhesion or migration generally lead to reduced homing to, and enhanced mobilization from, the bone marrow. In a recent publication in *Nature*, Adams et al. (2009) demonstrate that the guanine-nucleotide-binding stimulatory α subunit ($G\alpha_s$) can, unexpectedly, promote both phenomena.

Hematopoietic stem cells (HSCs) are permanent travelers. They move sequentially during development from the aorta-gonad-mesonephros to the fetal liver and the bone marrow (BM), and continue to traffic between the BM and extramedullary tissues throughout life. Little is known, however, regarding the pathways that direct the migration of HSCs out of the fetal liver and subsequently promote their engagement in the BM microenvironment during late gestation. Homing assays conducted after transplantation into lethally irradiated recipients have revealed, however, that the mechanisms that regulate HSC homing to the BM are not simply the mirror image of those that direct HSC egress from the BM microenvironment. These two migratory phenomena have important clinical implications, since homing is critical for successful engraftment after transplantation, whereas mobilization (e.g., induced by granulocyte colony-stimulating factor, G-CSF) allows the harvest of peripheral blood HSCs for future transplantation procedures.

At the molecular level, the G protein-coupled receptor (GPCR) CXCR4 is at the nexus of these trafficking pathways, promoting both homing and retention in the BM. For example, the inhibition of CXCR4 markedly alters homing and engraftment, whereas HSCs deficient in *Cxcr4* exhibit spontaneous progenitor mobilization from the BM (reviewed in Papayannopoulou and Scadden, 2008). GPCR signaling is coupled to heterotrimeric guanine nucleotide-binding (or G) protein complexes, composed of α and βγ subunits, which transduce signals to downstream effectors. In homeostasis, GDP is bound to Gα, and Gα, β, and γ combine to form a heterotrimer. The agonist binding induces a conformational change allowing GDP to be exchanged for GTP, which then leads to the dissociation of the G proteins, releasing α from the remaining βγ dimer. Whereas the cAMP-generating adenylyl cyclase is activated by $G\alpha_s$ proteins (containing a stimulatory α subunit), it is inhibited by $G\alpha_i$ proteins, containing an inhibitory α subunit (Figure 1). CXCR4 is generally thought to signal through $G\alpha_i$ proteins because its function is sensitive to pertussis toxin (PT). Although variable, some reports have observed only partial inhibition of hematopoietic stem and progenitor cell (HSPC) homing after exposure to PT (Kollet et al., 2001; Papayannopoulou et al., 2003), arguing that additional Gα proteins may also participate in homing regulation.

The recent work from Dr. David Scadden's group (Adams et al., 2009) sheds new light into these questions, demonstrating that $G\alpha_s$ deficiency in HSPCs prevents homing in the fetal and adult BM and, most notably, also blocks mobilization into the bloodstream. In their studies, Adams et al. generated chimeric mice to circumvent the early embryonic lethality of $G\alpha_s^{-/-}$ mice. $G\alpha_s$-deficient HSCs engrafted normally in the fetal liver of chimeric mice but could not engraft in the fetal BM. To deplete $G\alpha_s$ in adult mice, the authors crossed a strain in which the first exon of the $G\alpha_s$ gene was flanked by *loxP* sites ($G\alpha_s^{fl/fl}$) with a *Mx1-Cre* line in which *Cre* expression is induced after poly(I:C) administration. BM mononuclear cells from poly(I:C)-treated $G\alpha_s^{fl/fl}$ *Mx1-Cre* animals transplanted into lethally irradiated wild-type recipient mice exhibited deficient rolling on BM endothelial cells and impaired homing to spleen and BM. Interestingly, this deficiency was specific for HSPCs and was not observed when lymphocytes were examined in rolling assays. Although most of the adhesion and homing signals that have been proposed to regulate HSPC migration have traditionally correlated with results from similar studies of leukocytes, the current study argues that distinct signaling mechanisms control the trafficking of HSPCs versus mature hematopoietic lineages.

Surprisingly, in addition to the observed homing defect, Adams et al. (2009) found that endogenous, $G\alpha_s$-deficient HSPCs could not be mobilized after G-CSF treatment. As the authors point out, it is possible that the concomitant deficits in HSC homing and egress explain the unchanged numbers of circulating and BM HSPCs observed in the absence of $G\alpha_s$. Alternatively, $G\alpha_s$ deficiency might induce compensatory mechanisms or receptor desensitization that might prevent HSPC mobilization induced by G-CSF. Whereas $G\alpha_s$ deficiency significantly affected HSPC trafficking, it did not alter cell cycle, apoptosis, or differentiation capacity, as measured by colony-forming units in culture (CFU-C) assays. These results contrast sharply with previous studies in mice lacking downstream Rac GTPases in which HSPC homing and migration are defective but

mobilization is dramatically increased (Cancelas et al., 2005). The dual defects of both homing and mobilization in the absence of $G\alpha_s$ suggest that these two phenomena can be uncoupled, arguing for the participation of other contributing GPCRs (Figure 1). Such promigratory receptors may be of clinical interest, since the authors demonstrate that pharmacological stimulation with cholera toxin, which irreversibly activates $G\alpha_s$ in a selective manner by inhibiting its intrinsic GTPase activity, increased HSC homing and engraftment.

The interesting discoveries by Adams et al. (2009) highlight numerous avenues for future study, most notably the task of identifying upstream receptors that mediate $G\alpha_s$'s effects, as well as its downstream targets. Given the large number of $G\alpha_s$-coupled receptors and downstream pathways, the task will likely be challenging. One putative receptor class is the adrenergic receptors (Adr), since homing and mobilization are influenced by Adrβ2, which is expressed by HSCs (Katayama et al., 2006; Spiegel et al., 2007). However, *Adrβ2*$^{-/-}$ mice do not exhibit severe trafficking defects, suggesting the presence of redundancy with other pathways and/or alternate signaling mechanisms. $G\alpha_s$-coupled prostaglandin E2 receptors represent appealing alternative candidates, since they mediate HSC proliferation (North et al., 2007), and have recently been implicated in HSC homing (Hoggatt et al., 2009). On the downstream side of the equation, the opposing actions of $G\alpha_s$ and $G\alpha_i$ on adenylyl cyclase activity raise the possibility that cAMP levels may act as a biological rheostat governing HSC migration decisions (Figure 1). It is also notable that the $G\alpha_s$ gene has multiple imprinted promoters that result in maternally, paternally, or biallelically expressed gene products, leading to sex- and tissue-specific phenotypes (Weinstein et al., 2007). If the candidate receptors outlined above are not, in the end, implicated as the source of $G\alpha_s$ in this setting, alternate studies using gender-based comparative screening of $G\alpha_s$ partners may provide further insight about the receptor(s) mediating the observed HSC migration phenotype.

Although reductions in CXCL12/CXCR4 function have correlated tightly with the efficiency of HSC mobilization, very few biological processes, if any, are composed of a single regulatory arm. The studies by Adams et al. (2009) raise the prospect of at least one other group of GPCRs whose activation promotes both homing and mobilization.

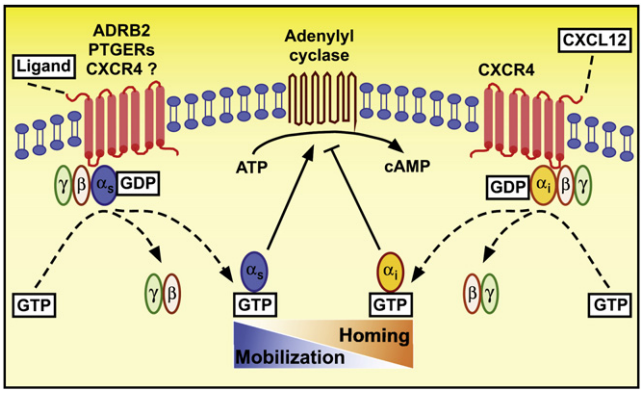

Figure 1. Uncoupling of HSC Homing and Mobilization through G Protein-Coupled Receptors

Upon ligand engagement, GDP is exchanged for GTP, leading to the dissociation of the G proteins into α and βγ dimers that signal through multiple downstream pathways (data not shown). High-energy $G\alpha_s$ induces the synthesis of cAMP, whereas $G\alpha_i$ inhibits it. CXCR4 signaling through $G\alpha_i$ promotes HSC homing and BM retention and, on the other hand, reductions in CXCR4 activity induce mobilization. The study of Adams et al. (2009) raises the hypothesis that CXCR4 also associates with $G\alpha_s$. In addition, their data suggest that the activation of other $G\alpha_s$-containing GPCRs, possibly the β_2-adrenergic receptor (ADRB2) and/or prostaglandin E receptors (PTGERs), induce HSPC mobilization. Thus, HSPC decision making about mobilization or retention/homing may result from the integration of a tightly controlled balance of $G\alpha_s$ and $G\alpha_i$ activities.

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