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On how Rac controls hematopoietic stem cell activity

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Abstract

Rac GTPases form part of the family of Rho small GTPases. Rac GTPases like other Rho family GTPases, are key molecular switches controlling the transduction of external signals to cytoplasmic and nuclear effectors. The development of genetic and pharmacological tools has allowed a more precise definition of the specific roles of Rac GTPases in hematopoietic stem cells (HSCs). Our current knowledge has enabled the dissection of their specific and redundant roles. Rac GTPases are now known to be crucial in the response of HSCs response to hematopoietic microenvironment cues. This review will briefly summarize the known HSC functions which are regulated by Rac GTPases, focusing on adhesion, migration, retention, proliferation, and survival, and how Rac relates to the physiological functions of HSCs. The development of small molecule inhibitors with the ability to interfere with Rac GTPase activation offers new therapeutic strategies to manipulate the function of HSC in vivo and ex vivo.

Keywords

Hematopoietic stem cells and progenitors; Rac GTPases; bone marrow retention; engraftment

Introduction

Blood formation is initiated by hematopoietic stem cells (HSCs). HSCs and derived progenitors (HSC/Ps) accomplish the complicated mission of producing billions of blood cells every day. Minor changes of as little as an excess or a decrease of 10% in blood formation are frequently pathological, indicating that hematopoiesis requires a fine, sophisticated regulation.

HSC/P transplants are used to replace the endogenous hematopoiesis of patients in the treatment of cancer and some genetic disorders ^{1–7}. The success of these transplants depends on the number and quality of HSCs infused, a receptive host marrow and, in the case of allogeneic transplants, on the immunotolerance of the recipient for the progeny engrafted HSCs.

In adults, HSC/Ps reside in the marrow and are largely absent from the peripheral blood (PB) ⁸. The endosteal space of the adult marrow is enriched in HSC/Ps which are, located in putative “niches” where a specialized microenvironment supports and nurtures them, and allows the maintenance of an equilibrium with a minority of HSC/Ps circulating in PB.

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Conflict of Interest:

J.A.C.: no relevant conflicts.

While marrow harvests were previously utilized to collect transplantable HSCs, current clinical practice mostly applies the biological phenomenon of HSC/P mobilization from the marrow into the PB to allow leukapheresis harvest⁹ as this does not require general anesthesia and is typically associated with shorter periods of post-pancytopenia^{7,10}.

Administration of cytokines, such as granulocyte-colony stimulating factor (G-CSF)² or stem cell factor (SCF)¹¹, chemokine-receptor inhibitors (e.g. AMD3100),¹² and cytotoxic drugs (e.g. cyclophosphamide),²⁻⁶ have been used clinically to increase the number of circulating HSC/Ps. A goal of PB stem cell transplant for hemato-oncological diseases is to optimize the number of HSCs to ensure low levels of the morbidity and mortality that is associated with rescue in the myeloablative setting^{5,10}. In addition, PBSCs are ideal targets for cell and gene therapies¹³. The administration of G-CSF is currently the major method for mobilization of HSC/Ps for clinical usage. However, over 40% of patients who have undergone intensive chemotherapy, and between 10% and 20% of all patients and normal individuals, fail to mobilize sufficient numbers of HSC/Ps for successful PB stem cell transplant⁹.

HSC homing and engraftment are crucial to successful transplantation, and clinical engraftment is severely compromised when the number of donor-cells are limited. For instance, the low number of HSC/P appears to limit the use of umbilical cord blood for transplantation of adult patients, where limited HSC dose appears to be associated with delayed engraftment and unacceptably high rates of graft failure¹⁴⁻¹⁸. Another example is found in the current design of cell or gene therapy protocols, which require large amounts of HSC/Ps for ex vivo manipulation and subsequent reinfusion. The fact that chemotherapy-based protocols may be inadequate or unacceptable to mobilize stem cells in many immunodeficiencies and other non-malignant hematological diseases, makes the search for other methods of mobilization highly desirable. The current alternative is the use of cytokine-induced mobilization, but this is hampered by a high variability in the efficiency of mobilization⁹. Several investigators¹⁹⁻²¹ have suggested that mobilized PB stem cells may also contribute to the generation of non-lymphohematopoietic tissues. While controversial²²⁻²⁴, these data suggest potential additional therapeutic application of mobilized PBSCs. Thus, increasing the HSC/Ps availability, by improving stem cell mobilization, and functional modifications of the HSC/P to facilitate their homing and engraftment abilities, may provide an answer to cases of absent or poor engraftment after HSC/P transplantation.

Rac GTPases, a subfamily of Rho GTPases

Mammalian Rho GTPases are a family of small guanosine triphosphate (GTP)-binding proteins with 22 members that are involved in many important cellular functions including cell proliferation, migration, and cytoskeleton reorganization²⁵⁻²⁷. They form part of the Ras superfamily and therefore, they share considerable structure-function similarities with Ras and other Ras-related small GTPases. Similar to Ras, Rho GTPases switch from the inactive guanosine diphosphate (GDP)-bound form to the active GTP-bound form by a mechanism that is strictly regulated by the upstream guanine nucleotide exchange factors (GEFs). Once activated, the GTP-bound forms are capable of interacting with multiple downstream effector proteins in a spatial and temporal controlled manner. Conversely, GTPase-activating proteins (GAPs) stimulate the hydrolysis of bound GTP to GDP, switching Rho GTPases back to the inactive state. Rho GDP-dissociation inhibitors (GDIs) can sequester inactive GDP-bound Rho proteins in the cytosol and prevent their activation and intracellular trafficking. Further adding to the complexity of this tightly regulated mechanism, multiple Rho GEFs, Rho GAPs and Rho GDIs can activate or inactivate the

same Rho GTPase, and each Rho GTPase may activate multiple downstream effectors, initiating a network of signal transduction that impacts cell functions.

Rac in hematopoietic stem and progenitor cell (HSC/P) functions

There has been a tremendous advance in knowledge regarding the molecular pathways involved in HSC/P functions over the last few years, including trafficking to and from the marrow niche. The signals involved in HSC/P homing and mobilization include cytoskeleton rearrangements, transcription activation, survival, and cell cycle progression.

Cytoskeleton rearrangements are controlled by members of the Rho GTPase family. One of the best studied subfamilies of Rho GTPases in the context of HSC/Ps functions is Rac. Signaling associated with all homing and migration-related activities is driven through Rac GTPases. Rac integrates signals from β_1 -integrins and c-kit in HSC/P and mast cells^{28,29}. CXCL12 (SDF-1)-induced chemoattraction is mediated by Rac and is at least partly mediated by the GEF Tiam-1³⁰. Signaling of c-kit to Rac/Cdc42 is mediated through the GEF Vav^{31,32}. However, other GEFs may be crucial in Rac activation in the control of HSC/P functions. Three highly related Rac proteins are expressed in mammalian cells, Rac1, Rac2 and Rac3. Rac1 and 3 are widely expressed^{33,34}, while Rac2 expression is hematopoietic specific^{35,36}. The three Rac proteins share a high degree of protein homology. However, each appears to have overlapping as well as unique roles in HSC/P activity. Studies using dominant negative mutants lacked specificity, and their conclusions were significantly modified in in vivo studies. A summary of the phenotypes of HSC/Ps described using these dominant negative mutants and in vivo gene targeting of Rac proteins is included in Table 1. Initial studies in *Rac2*^{-/-} mice demonstrated that Rac2 is important for HSC/P adhesion and migration, and *Rac2*^{-/-} mice showed increased numbers of circulating HSC/Ps and hypermigration³⁷, suggesting an important role for Rac2 in retaining cells in the marrow hematopoietic microenvironment (HM). Rac2 activity is also important in maintaining normal Rac1 and Cdc42 function, confirming in primary cells 'the existence of a cross talk' between Rac2 and other Rho GTPases involved in regulating HSC/P migration^{37,38}. Although Rac2 is highly homologous to Rac1, Rac2 and Rac1 regulate distinct HSC/P functions. Rac1-deficient HSC/P demonstrated defective proliferative signaling via the receptor tyrosine kinase, c-kit, while loss of Rac2 activity leads to a pro-apoptotic phenotype²⁸. Double-deficient *Rac1* ^{Δ/Δ} /*Rac2*^{-/-} HSC/P showed a combination of reduced proliferation and increased apoptosis²⁸ and at the same time, *Rac1* ^{Δ/Δ} /*Rac2*^{-/-} HSC/Ps show decreased adhesion to fibronectin, despite the normal expression of CD49d ($\alpha_4\beta_1$)- and CD49e ($\alpha_5\beta_1$)-integrin adhesion molecules on these cells⁶. Since *Rac1* ^{Δ/Δ} HSC/Ps have normal adhesion to fibronectin, and *Rac2*^{-/-} HSC/Ps display an intermediate level of adhesion, it appears that Rac2 has a predominant but overlapping role with Rac1 in integrin-mediated HSC/P adhesion. In addition, *Rac1* ^{Δ/Δ} /*Rac2*^{-/-} HSC/Ps have decreased migration in response to CXCL12 compared with wild-type cells while having a significantly increased expression of CXCR4, the receptor for the CXCL12.⁶ Since Rac GTPases are key components of the signaling pathways downstream of the SCF-receptor, c-kit,⁷ the chemokine receptor CXCR4,⁸ and the β_1 -integrin-receptors for fibronectin,^{9,10} all critical mediators of HSC/P interaction with the marrow HM, it is not surprising that a combined deficiency of Rac1 and Rac2 results in massive mobilization of progenitor colony-forming unit cells (CFU-C) into the peripheral circulation and results in increased homing of CFU-C in the spleen.³⁹ CXCL12 gradients controlling homing and mobilization of HSCs control Rac activation through the incorporation of CXCR4 into membrane lipid rafts⁴⁰ and through the downstream activation of the Rac effector Wave2⁴¹.

Despite the role of Rac1 in HSC proliferation²⁸, induced deletion of Rac1 in mixed chimeric mice does not induce hematopoietic failure, indicating that Rac1 is dispensable for steady-state hematopoiesis³⁹.

The funding from the National Blood Foundation (NBF) helped me to develop an experimental strategy to analyze the specific roles of Rac proteins in vivo in two major instances. First, it helped me to analyze the mechanisms controlled by Rac in HSC activity. Through a series of innovative approaches that include the use of inducible triple transgenic animals, competitive repopulation assays, anatomical microlocalization of HSC/Ps within the marrow cavity, and limiting-dilution analysis of long-term marrow cultures, we found that Rac1 deficiency induces a defective localization in the endosteal space of the marrow cavity after transplantation³⁹. In contrast, Rac2 deficiency induces loss of retention of HSC/Ps in the marrow^{37,39} while the deletion of both Rac1 and Rac2 leads to the massive mobilization of HSC/Ps from the marrow to PB due to decreased adhesion and retention in the marrow^{28,39}. These data strongly suggest that at the molecular level HSC/P homing and retention in the marrow are not mirror image processes and that Rac1 and Rac2 have overlapping and distinct roles in HSC/P trafficking. Secondly, the NBF grant helped me to explore the translational possibilities of Rac inhibition on HSC mobilization. We were fortunate enough to have on the extraordinary collaboration of Dr. Yi Zheng (Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center) who had discovered a first-generation, rationally-designed small molecule, NSC23766, which has with inhibitory activity on Rac activation, and Dr. David Williams (Boston Children's Hospital Medical Center, Harvard Medical School), my mentor, who has extensive experience in analyzing the role of Rho family GTPases in HSC activity. Together, we discovered that in vivo Rac inhibition induced mobilization of HSCs which could then be harvested and, after drug removal, were engraftable in myeloablated syngenic animals. These data opened up the possibility of Rac targeting to improve HSC mobilization in patients resistant to mobilization-inducing conventional therapies.

The role of Rac1 in hematopoiesis during fetal development has also been examined. In an elegant study, David Williams' group found that Rac1, but not Rac2, plays a crucial role in fetal HSC/Ps trafficking. In the absence of Rac1, the number of circulating HSC/P in the blood of E10.5 embryos is severely diminished, while yolk sac definitive hematopoiesis is quantitatively normal. Intraembryonic hematopoiesis is significantly impaired in Rac1-deficient embryos as assessed by the absence of intraaortic clusters and the near complete absence of fetal liver hematopoiesis⁹. In vivo, these deficiencies correlate with a decreased migration in response to CXCL12 and impaired interaction with the HM-derived stromal cells in migration-dependent assays⁴².

Relevant Rac effector signaling pathways in HSC/Ps

PAK, POR1, and STAT family members have been implicated in Rac effector functions in primary hematopoietic cells and directly or indirectly activate specific transcriptional programs through p42/p44 and p38 extracellular signal-regulated kinases (ERKs), JNK, and Akt kinases^{28,37,39,43-45}. Our group determined that NSC23766-mediated inhibition of Rac activity translated into a significant decrease of PAK activation in vivo in marrow HSC/Ps³⁹. Wu et al. showed that Rac1 complexes with Jnk2 and β -catenin and promotes Jnk2-mediated phosphorylation of β -catenin residues Ser191 and Ser605, inducing nuclear translocation and bypassing the canonical Wnt-dependent β -catenin activation pathway⁴⁶. Interestingly, Rac and McgRacGAP (a GTP, with inhibitory activity on Rac) are also required for nuclear localization of Stat5a through direct interaction,⁴⁷ suggesting a role for Rac proteins as crucial regulators of transcription factor translocation to the nucleus and control of gene expression.

Studies performed in HSC/Ps, purified by FACS sorting as c-kit⁺ cells depleted of lineage markers, demonstrated that Rac1 and Rac2 GTPases regulate unique aspects of hematopoietic development and function⁶. Rac1^{Δ/Δ};Rac2^{-/-} HSC/Ps demonstrate reduced proliferation in response to SCF (this is also seen in the Rac1^{Δ/Δ} HSC/Ps), associated with undetectable cyclin D1 levels and with decreased ERK (p42/p44) phosphorylation. Increased apoptosis is noted in Rac1^{Δ/Δ};Rac2^{-/-} and Rac2^{-/-} HSC/Ps, associated with reduced Akt activation compared with that of wild-type cells after SCF stimulation.

Rac GTPases in Fanconi Anemia: a paradigm of BM failure syndrome

Fanconi anemia (FA) is an inherited disorder characterized by early-onset progressive and fatal marrow failure, congenital abnormalities, and predisposition towards cancer⁴⁸. The only curative therapy currently available for the fatal marrow failure is allogeneic stem cell transplantation. Unfortunately, the availability of unaffected sibling donors is low for the majority of patients, and the disease-free survival rate for allogeneic transplantation using a matched unrelated donor is not optimal, ranging from 15% to 67%^{49,50}, although there are continuing improvements in outcomes with newer stem cell transplant approaches. Since many children with FA are diagnosed before the onset of severe pancytopenia⁵¹ a possible novel experimental therapy would be the infusion of previously collected and stored autologous HSCs at the time of marrow failure. Studies which examine the feasibility of collecting HSC/Ps have shown that stem cell mobilization using G-CSF is quantitatively inferior to wild-type controls in mice⁵². FA patients require prolonged periods of daily apheresis procedures to obtain clinically relevant numbers of HSCs and even then there is inadequate mobilization in a significant portion of patients^{53,54}. Using NBF funds and other grants, we found that inhibition of Rac GTPases by in vivo administration of the small molecule NSC23766 is able to rescue part of the progenitor mobilization failure of FA-A cells in a murine model of HSC/P mobilization induced by G-CSF⁵⁵, and demonstrate proof-of-concept that HSC mobilization using Rac targeting may represent a novel method in patients with specific mobilization challenges through conventional protocols.

Conclusion

Rac GTPases appear to play crucial roles in key HSC functions related to the cytoskeleton, including adhesion, migration, retention in the marrow and engraftment. Studies using gene-targeted mice, which allow more precise studies in primary cells, have shown that Rac GTPases appear also to regulate proliferation, survival, transformation, and senescence of HSCs. Genetic and pharmacological approaches have been developed to dissect out the unique and overlapping roles of different Rac GTPases in HSC/P populations. As a result, specific molecular mechanisms of action of Rac have begun to be clarified in these cells. However, the role of specific agonist and cell-specific GEFs in the activation of Rac GTPases in HSCs has not yet been fully elucidated. In addition, the stoichiometric and functional relationships between Rac and other Rho GTPases and their downstream effectors within a highly complex network of microenvironment and humoral ligand-receptor interactions, GEF activation, and Rho GTPase dependent signaling, are yet to be completely understood.

Rho GTPases appear to be key regulators of the localization of HSCs within the HM and the interaction of HSCs with cellular niches. Further research into the roles of Rho GTPases in the microenvironment and in relation to the osteoblastic and vascular HSC niches is needed. Finally, the development of small molecule inhibitors, while difficult due to the affinity of the interactions of GEFs with Rho GTPases, offers new opportunities for therapeutic intervention at the HSC level in cell, gene, and cancer therapies.

In summary, Rac GTPases represent major molecular switches, which control HSC/P activity at different levels and have both redundant and specific roles. Specific targeting Rac GTPases may represent an excellent method to modulating HSC activity in general, and homing/mobilization in particular.

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Table 1

Summary of phenotypic features of Rac deficient HSC/P.

		Dominant negative mutants	In vivo gene targeting
Rac1	Type of mutation	<i>Rac1 T17N</i>	<i>Mx1-Cre; Vav1-Cre; Lys-Cre; Lck-Cre; CD19-Cre-Rac1^{fllox/fllox}</i>
	Functional defect	Decreased cortical actin, chemotaxis ⁵⁶	Decreased engraftment. Decreased homing. Decreased BM endosteal localization. Decreased interaction with microenvironment. Decreased proliferation. In combination with Rac2, severely increased egression from BM ^{28,39} .
Rac2	Type of mutation	<i>DN-Rac2T17N; DN-Rac2D57N (patient mutation)</i>	<i>Rac2^{-/-}</i>
	Functional defect	Increased apoptosis ⁵⁷	Loss of retention from BM. Decreased survival ^{28,37,39}
Rac3	Type of mutation	<i>Rac3T17N</i>	<i>Rac3^{-/-}</i>
	Functional defect	N/A	No major defects reported