Cdc42
A Signal Coordinator in Hematopoietic Stem Cell Maintenance

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Original manuscript submitted: 04/17/07
Manuscript accepted: 05/01/07
Previously published online as a Cell Cycle E-publication: http://www.landesbioscience.com/journals/cc/abstract.php?id=4371

KEY WORDS
Rho GTPases, Cdc42, hematopoietic stem cells, cell cycle, bone marrow niche

ABSTRACT
Maintenance of a relatively quiescent cell cycle state is a distinct characteristic of adult hematopoietic stem cells (HSCs) residing in the bone marrow (BM) microenvironment. This property is considered critical for HSCs to fulfill long term self-renewal and multi-lineage differentiation potential throughout mammalian life span. To date, the mechanisms regulating the cell cycle state and the retention of HSCs in the BM microenvironment remain unclear. Cdc42, a small GTPase of the Rho family known to control various cellular functions including adhesion, migration, transcription, and growth, is shown recently in a conditional gene-targeted mouse model to coordinate HSC quiescence maintenance and BM niche residency. The study also highlights a cell-type specific role of Cdc42 in cell cycle regulation.

INTRODUCTION
Stem cells are characterized by their self-renewal ability in addition to their differentiation potential. Adult hematopoietic stem cells (HSCs) are a rare population of cells residing in a unique bone marrow (BM) microenvironment, termed niche, that undergo a complex but highly ordered hematopoiesis program throughout the life-span of mammals. Although HSCs can give rise to all blood cell lineages in a short time, the long-term HSCs remain relatively quiescent, a cell cycle state that may be critical for supporting a life-long commitment of hematopoiesis. The balance of HSCs between a quiescent state and an active cell cycle state is tightly controlled by both intrinsic and extrinsic cues. In addition to a number of intracellular molecules including cell cycle regulatory proteins and transcriptional regulators, HSC interaction with the BM niche also plays important roles in the HSC regulation. To date, however, the mechanisms coordinating the quiescent state of HSCs and their retention in the BM microenvironment remain poorly understood.

Cdc42 is a member of the Rho GTPase family that acts as an intracellular signal transducer cycling between the GTP bound, active and the GDP bound, inactive states. A variety of cell surface receptors are known to utilize Cdc42 to transduce diverse signals to specific downstream effectors in regulating cell functions including actin cytoskeleton reorganization, directional migration, gene expression, and cell cycle S-phase progression. Our previous knowledge of Cdc42 function in mammalian cells came mostly from studies using dominant negative and constitutively active mutant expression approaches. Such methods of over-expression of dominant mutant have increasingly been realized to impose non-specific effects on the functional studies of Rho GTPases, particularly in clonal cell lines. Facilitated by gene targeting technologies, definition of cell-type specific functions of individual Rho GTPases and regulators in primary cells and in physiologically relevant animal models is an emerging area of research interests. Recently, an unexpected role for Cdc42 in HSC regulation was discovered in studies of a BM-specific, inducible gene targeted mouse model. In this Perspective we discuss the cell type specific function of Cdc42 in the context of the regulation of HSC cell cycle and BM niche interaction.

HEMATOPOIETIC STEM CELLS AND THE BONE MARROW NICHE
HSCs fulfill a lifelong demand of supplying mature blood cells through a well defined hierarchy of differentiation program. It has been shown that one single HSC is capable of repopulating all blood lineages of a lethally irradiated animal. In addition to supporting blood cell development, HSCs are characterized by their self-renewal ability, a property that explains the mostly conserved HSC numbers found in animals and may...
involve asymmetric cell cycle division producing daughter HSCs and progenitors. Both the differentiation and the self-renewal of HSCs occur in the BM, which provides a uniquely suited microenvironment with balanced pro-proliferative and anti-proliferative signals in an adhesive niche. Recent genetic studies in flies, worms and mice suggested a number of features constituting a functional HSC niche: (1) the niche may be localized near the endosteum of the bone and is a physical site of HSC residency; (2) the stroma cells constituting a niche include bone-generating osteoblasts, vascular endothelial cells, and possibly other cell types from the bone marrow; and (3) the niche supplies multiple extrinsic factors that are essential for the cell fate determination of HSCs. It is in such a specialized niche that normal HSCs are maintained to perform the self-renewal and differentiation duties in a highly controlled manner.

HSC INTERACTION WITH THE NICHE AND CELL CYCLE REGULATION

The balance between a quiescent state and a proliferative state is critical in the HSC self-renewal program, as over-proliferation could lead to early exhaustion of the HSC pool and overproduction of various blood lineages contributing to diseases such as myeloid proliferative disorder, whereas over-suppression of proliferation could lead to decreased hematopoiesis causing anemia and/or neutropenia. It has been suggested that quiescent HSCs are more effective than a subpopulation of HSCs undergoing active cell cycle in providing long-term, multilineage hematopoietic reconstitution, and most long-term HSCs are considered to be quiescent and are stored in a distinct “quiescent-storage” niche. In response to stress or stimulation, HSCs can move to a “proliferative zone” in the BM to undergo asymmetric cell division or can be mobilized from the BM into the circulating blood, migrate to satellite locations in the spleen and liver, and enter into active cell cycle. The mobilized HSCs may return to the bone-marrow niches and regain their quiescent state. During this process, the mobilization of HSCs from niche is tightly coupled with their active cell cycle status, and it has remained a “chicken or egg” puzzle whether the HSC detachment and mobilization from the niche promotes the active cell cycle progression or the cell cycle state of HSCs dictates their niche residency. Alternatively, these events of HSC maintenance may happen simultaneously but are regulated through independent mechanisms.

At the molecular level, given the complex composition of the BM niche comprising of osteoblasts, endothelial cells, extracellular matrix, and signals emanating from the BM stroma cells, a complex signaling network is expected to be at work for HSC maintenance. The extrinsic signals provided by the niche include cytokines such as SCF, adhesion molecules such as integrins, developmental cues such as Wnt, Hh, Notch1, BMP, Ang and Ca^{2+}, and chemokines such as SDF-1α. Correspondingly, in order to relay these diverse signals to intracellular effectors in controlling...
Recent study of a conditional knockout mouse model, Cdc42Tg−/−15a−/−Cip1Tg−/−motoring niche. The increased HSC−31−35actin reorganization response to the chemokine SDF−1The mobilization effect of Cdc42 defective in homing, lodging, and retention in the BM endosteum. Blood, liver and spleen, and the Cdc42-deficient HSCs were massively mobilized into peripheral organs including peripheral type) and colony-forming unit stem/progenitor cells were found in cell cycle status, Cdc42 HSCs during cell cycle progression. Concomitant with the change HSCs and the associated increase in the S-phase of the short-term is due to the exit of cell cycle G HSCs. This population shift of long-term and short-term HSCs is attributable to altered BM-niche localization−related adhesion molecules including N-cadherin and β1-integrin.28,36 One particular challenge in dissecting the functional significance of these complex molecular interactions is to link the intracellular signaling components involved in cell cycle regulation to the various extra stimuli and HSC retention in the niche.

Cdc42 coordinates cell cycle regulation and niche localization of HSCs. Recent study of a conditional knockout mouse model, in which BM cells of the Mx1-Cre;Cdc42flox/flox genotype were transplanted into lethally irradiated recipient mice to achieve the BM-specific and inducible deletion of cdc42 gene, shed new light into the mechanisms of HSC regulation related to quiescence maintenance and BM niche localization.15 Deletion of Cdc42 from HSCs results in a significant increase in the number and the frequency of phenotypic short-term HSCs and a loss of long-term HSCs. This population shift of long-term and short-term HSCs is due to the exit of cell cycle G0 quiescent state by the long-term HSCs and the associated increase in the S-phase of the short-term HSCs during cell cycle progression. Concomitant with the change in cell cycle status, Cdc42−/− primitive cells (Lin−Sca1−c-Kit− genotype) and colony-forming unit stem/progenitor cells were found massively mobilized into peripheral organs including peripheral blood, liver and spleen, and the Cdc42-deficient HSCs were defective in homing, lodging, and retention in the BM endostem. The mobilization effect of Cdc42−/− HSCs is attributable to altered actin reorganization response to the chemokine SDF-1α, defective adhesion on fibronectin and stroma cells, and defective chemokinesis and transendothelial migration, activities that are intrinsic to the HSCs. As a consequence, engraftment of the Cdc42 knockout HSCs is defective in BM transplantation recipient mice. These studies demonstrate that Cdc42 is required for the maintenance of normal HSC cell cycle status, and meanwhile it is also critical for HSC retention in the BM niche (Fig. 1). Previous characterization of a Cdc42 gain-of-activity, Cdc42GAP knockout mouse model has revealed that constitutively elevated Cdc42-GTP activity causes defective actin reorganization, adhesion, and migration, but not cell cycle progression, in the HSCs, resulting in a defective engraftment.14 Thus, while a threshold level of Cdc42 activity is required for retaining HSC in the BM niche and maintaining a relatively quiescent cell cycle state of long-term HSCs, hyperactivation of Cdc42 affects only the interaction of HSCs with the BM niche.

### Table 1 Cdc42 plays cell-type specific roles as revealed by gene-targeting studies in mice

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Actin Cytoskeleton</th>
<th>Adhesion</th>
<th>Directed Migration</th>
<th>Growth</th>
<th>Cell Cycle Progression</th>
<th>Apoptosis</th>
<th>MAP Kinase Cascades</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary MEFs</td>
<td>Altered morphology; Defective filopodia formation</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Defective</td>
<td>Defective in S phase entry</td>
<td>Increased</td>
<td>Defective in JNK and ERK activation</td>
<td>40</td>
</tr>
<tr>
<td>ES cells</td>
<td>Defective actin polymerization</td>
<td>N.D.</td>
<td>N.D.</td>
<td>No gross proliferation defects</td>
<td>N.D.</td>
<td>Normal viability</td>
<td>Normal JNK, p38 and ERK activation</td>
<td>44</td>
</tr>
<tr>
<td>ES-derived fibroblastoid cell lines</td>
<td>Altered morphology; normal formation of filopodia</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>N.D.</td>
<td>Normal</td>
<td>41</td>
</tr>
<tr>
<td>Hematopoietic stem cells</td>
<td>Defective cortical actin</td>
<td>Decreased</td>
<td>Decreased</td>
<td>N.D.</td>
<td>Loss of G0 phase and increased S phase</td>
<td>Normal</td>
<td>N.D.</td>
<td>15</td>
</tr>
<tr>
<td>Keratinocytes</td>
<td>N.D</td>
<td>Decreased cell-cell contact</td>
<td>N.D.</td>
<td>N.D.</td>
<td>Increased proliferation</td>
<td>N.D.</td>
<td>N.D.</td>
<td>37</td>
</tr>
<tr>
<td>Neuronal progenitors</td>
<td>Defective actin organization</td>
<td>Defective cell-cell adhesion</td>
<td>N.D.</td>
<td>N.D.</td>
<td>Normal cell cycle length</td>
<td>N.D.</td>
<td>N.D.</td>
<td>38,39</td>
</tr>
</tbody>
</table>

N.D., not determined.

HSC behavior, HSCs express receptors for these stimuli such as c-Kit, LRP6, Patched, Jagged1, BMPR, Tie-2, Ca2+-sensing receptor, and CXCR4. Additional intrinsic molecular machineries of HSCs that have been implicated in regulating HSC cell cycle entry/progression, possibly by mediating the signal responses, involve a number of genes encoding cell cycle and transcriptional regulators, e.g., p21Cip1, p18INK4c, c-Myc, ATM and Gfi-1,31-35 as well as several niche localization-related adhesion molecules including N-cadherin and β1-integrin.28,36 These molecules have previously been implicated as potential targets of Rho GTPases and are likely to be involved in regulation of HSC behavior. HSCs express receptors for these stimuli such as c-Kit, LRP6, Patched, Jagged1, BMPR, Tie-2, Ca2+-sensing receptor, and CXCR4. Additional intrinsic molecular machineries of HSCs that have been implicated in regulating HSC cell cycle entry/progression, possibly by mediating the signal responses, involve a number of genes encoding cell cycle and transcriptional regulators, e.g., p21Cip1, p18INK4c, c-Myc, ATM and Gfi-1,31-35 as well as several niche localization-related adhesion molecules including N-cadherin and β1-integrin.28,36 One particular challenge in dissecting the functional significance of these complex molecular interactions is to link the intracellular signaling components involved in cell cycle regulation to the various extra stimuli and HSC retention in the niche.

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### MECHANISTIC IMPLICATIONS AND UNRESOLVED ISSUES

The mechanism underlying the accelerated cell cycle status and the defective niche-localization of the Cdc42−/− HSCs may be in part due to a change of expression of a number of cell cycle regulators and adhesion molecules: significantly decreased level of p21Cip1 but an increased level of c-Myc, as well as decreased β1 integrin and N-cadherin expression, were found in the Cdc42 knockout primitive HSC population.15 These molecules have previously been implicated as potential targets of Rho GTPases and are likely important for HSC quiescence state maintenance and/or BM niche retention. Based on the cohort of observations, our current working model posits Cdc42 as a signal integrator for the control of cell cycle and niche localization (Fig. 1). In the absence of Cdc42, long-term HSCs are mobilized from the more restrictive niche and move to a proliferation/differentiation promoting niche. The increased HSC

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mobilization ability, resulting from defects in actin organization as well as key adhesion molecule expressions, coupled with altered cell cycle regulator expression, propels the quiescent HSCs to enter into active cell cycle, giving rise to increased number of HSCs in the BM and the circulating blood. Both the loss of quiescence and the mobilization phenotypes of Cdc42-HSCs may contribute to the severely impaired engraftment capability, evidencing that Cdc42 deficiency leads to a loss of “stemness” of the HSCs.

Along with the finding that Cdc42 serves as a key coordinator of HSC maintenance in the BM niche, these studies of the Cdc42 conditional knockout mice raise a number of interesting questions. Accompanying the phenotypes of cell cycle activation and mobilization from the BM niche, Cdc42-HSCs display multiple defects in adhesion, migration, F-actin assembly, and transcription activation. It remains to be determined which defect or a combinatorial defects is causal for the altered HSC behaviors, and the question whether the changes in actin and/or adhesion activity and the loss of quiescence in cell cycle status are related or independent events in the Cdc42-HSCs has also yet to be answered. In the context of stem cell studies, the answers could help address a long-standing debate on whether microlocalization of HSCs in the proper BM niche might dictate the quiescent state of HSCs, or if activated cell cycle status would result in mobilization of HSCs from the niche. Other relevant questions include how Cdc42 functions in the BM stroma cells that are a part of the home environment to HSCs, as it is possible that Cdc42 not only plays a HSC intrinsic role mediating intracellular signal transduction, but also is actively involved in the remodeling of the microenvironment in BM niche.

Cdc42 is expected to serve as an integrating point of a collection of signal inputs from the HSC surface receptors. In turn, many well characterized Cdc42 effectors that comprise a group of signaling molecules with varying cell regulatory functions, including protein serine/threonine and tyrosine kinases, and scaffold proteins, may serve to mediate cell actin cytoskeleton, adhesion, polarity, transcription, and/or cell cycle progression of the HSCs (Fig. 2). Because previous studies associating these effectors with Cdc42 function were mostly based on dominant mutant expression approaches in various clonal cell lines, our knowledge on how these and likely other unknown pathways downstream of Cdc42 are involved in stem cell regulation is limited. To determine their relevance in HSC regulation is another important area of future research.

**CELL TYPE SPECIFIC ROLES OF Cdc42**

In parallel to the mouse gene targeting studies of Cdc42, the functions of several mammalian Rho GTPases in various primary cell types, including stem/progenitor cells, have begun to be appreciated in a series of gene targeting experiments. An emerging theme from these studies is that in spite of high sequence analogies among Rho GTPase family members, many Rho GTPases play functionally unique and essential roles, and these roles are often cell type specific. Studies of Cdc42 knockout models have provided data supporting such a notion (Table 1). Cdc42 is found to be critical for hair follicle progenitor cell differentiation by modulating β-catenin turnover and for the establishment of apical-basal polarity in the maintenance of self-renewing neural progenitor fate through the Par3/6-aPKC pathway. Cdc42 deletion leads to defects in cell cycle progression through the G1/S phase in primary mouse embryonic fibroblasts (MEFs) but does not seem to affect most cell functions in embryonic stem cell derived clonal fibroblastoid cells. In HSCs, Cdc42 deficiency causes cell cycle activation and loss of quiescence associated with actin structural and adhesion defects, a result that is somewhat surprising as Cdc42 is expected to be a cell growth-promoting, rather than growth-suppressing, signaling molecule. In the BM specific knockout mice, Cdc42 deficiency further causes hyper-proliferation of Gr1+/Mac1+ myeloid cells leading to a myeloid proliferative disorder but does not detectably affect the cell cycle status of Ter119+ erythroid cells (our unpublished data). In addition, Cdc42 deficiency...
does not alter the survival activity of HSCs but drastically increases the apoptotic rate in primary MEFs. Collectively, it appears that Cdc42 may play a general role in regulating actin organization and cell-extracellular matrix or cell-cell adhesion in diverse cell types which may in turn affect cell morphology and migration, whereas its role in cell cycle and cell survival regulations seems to be cell type specific. Interestingly, although BM-specific Rac1 and Rac2 double knockout mice display a similar mobilization phenotype in HSCs, the Rac1−/−;Rac2−/− HSCs show significantly decreased cell cycle and survival activities that are distinct from the Cdc42−/− HSCs. It is likely that the role of Cdc42 in maintaining HSC quiescence is unique among Rho GTPase family members.

CONCLUDING REMARKS

A number of recent studies using mouse gene targeting approaches have revealed novel functions of Rho GTPases in various animal developmental processes. They provide convincing evidence of the physiological roles and signaling pathways of individual Rho GTPases in primary cells and allow the assessment of the cell type- and developmental-stage-specific roles. The finding that Cdc42 coordinates the HSC cell cycle maintenance and BM niche interaction add to our understanding of the physiological functions of this Rho GTPase and raises a number of new questions that may bear therapeutic significance. Because loss of Cdc42 leads to mobilization and proliferation of hematopoietic stem/progenitor cells and a concomitant opening of BM niche, targeting Cdc42 activity could provide a novel strategy for HSC mobilization and engraftment. One intriguing possibility is that such a strategy might also be applicable to leukemia stem cells, in which case an effective therapy is needed to mobilize them from their niche in the BM. Since Cdc42 serves as an integrator of diverse signaling cascades, addressing how Cdc42 functionally and mechanistically contributes to specific pathways involved in HSC maintenance in the BM microenvironment links Rho GTPases with the central issues of stem cell research and should have merits in understanding the fundamental mechanisms of hematopoiesis, aging, and cancer.

References

