Cell Stem Cell
Article

Hematopoietic Stem Cells Do Not Depend on N-Cadherin to Regulate Their Maintenance

Mark J. Kiel,[1] Melih Acar,[1] Glenn L. Radice,[2] and Sean J. Morrison[1,*]
[1]Howard Hughes Medical Institute, Department of Internal Medicine, and Center for Stem Cell Biology, Life Sciences Institute, University of Michigan, Ann Arbor, MI, 48109-2216, USA
[2]Center for Translational Medicine, Department of Medicine, Jefferson Medical College, Philadelphia, PA 19107, USA
*Correspondence: seanjm@umich.edu
DOI 10.1016/j.stem.2008.10.005

SUMMARY

According to the "osteoblastic niche" model, hematopoietic stem cells (HSCs) are maintained by N-cadherin-mediated homophilic adhesion to osteoblasts at the bone marrow endosteum. In contrast to this model, we cannot detect N-cadherin expression by HSCs, and most HSCs do not localize to the endosteal surface. It has nonetheless been suggested that HSCs express low levels of N-cadherin that regulate HSC maintenance. To test this, we conditionally deleted *N-cadherin* from HSCs and other hematopoietic cells in adult *Mx-1-Cre*$^{+}$*N-cadherin*$^{fl/-}$ mice. *N-cadherin* deficiency had no detectable effect on HSC maintenance or hematopoiesis. *N-cadherin* deficiency did not affect bone marrow cellularity or lineage composition, the numbers of colony-forming progenitors, the frequency of HSCs, the ability of HSCs to sustain hematopoiesis over time, or their ability to reconstitute irradiated mice in primary or secondary transplants. Loss of *N-cadherin* does not lead to HSC depletion. N-cadherin expression by HSCs is not necessary for niche function.

INTRODUCTION

Hematopoietic stem cells (HSCs) persist throughout adult life in the bone marrow, where they continuously produce new blood cells to maintain hematopoiesis. To understand the mechanisms that sustain HSCs, it is necessary to identify the niche—the specialized microenvironment in which HSCs are thought to reside (Adams and Scadden, 2006; Kiel and Morrison, 2008).

Osteoblasts are thought to contribute to HSC niches. Genetic manipulations that increase osteoblast numbers in mice also increase the number of HSCs (Calvi et al., 2003; Zhang et al., 2003). Osteoblasts have also been proposed to secrete factors that are necessary for HSC maintenance, including angiopoietin, thrombopoietin, and CXCL12 (Arai et al., 2004; Yoshihara et al., 2007), although none of these factors have yet been conditionally deleted from osteoblasts and each is also secreted by other cell types. Calcium ions from bone (due to osteoblast and osteoclast activity) also regulate HSC localization and maintenance (Adams et al., 2006). These observations raise two general possibilities for how osteoblasts could contribute to HSC maintenance. One possibility is that osteoblasts produce extracellular factors that diffuse into the marrow, directly or indirectly regulating HSC niches that are near, but not at, the endosteum. A second possibility is that osteoblasts directly promote HSC maintenance by binding HSCs, creating "osteoblastic niches" at the endosteal surface.

The possibility of bone marrow HSC niches near, but not at, the endosteum has been raised by a number of recent observations (Kiel and Morrison, 2008). We have localized HSCs within the bone marrow using SLAM family markers that give high levels of stem cell purity and found that few HSCs localize to the endosteal surface itself (Kiel et al., 2005, 2007b). Instead, most HSCs are present around sinusoids, some of which are close to the endosteum, while others are more distant. It remains uncertain whether perivascular cells directly promote HSC maintenance; however, the observation that HSCs are more likely than other hematopoietic cells to be adjacent to sinusoids (Kiel et al., 2007b) raises the possibility that there are perivascular niches. Consistent with this possibility, recent studies have suggested that perivascular cells, such as reticular cells and mesenchymal progenitors, express more CXCL12 and angiopoietin than osteoblasts (Sugiyama et al., 2006; Sacchetti et al., 2007). This raises the question of whether HSCs are maintained in direct contact with osteoblasts, or whether they are maintained in other microenvironments that are directly or indirectly influenced by factors secreted by endosteal cells.

The widely discussed osteoblastic niche model has favored the idea that HSCs are maintained in direct contact with osteoblasts (Zhang et al., 2003; Suda et al., 2005; Wilson and Trumpp, 2006; Zhang and Li, 2008). A fundamental element of this model is that HSCs adhere to the surface of osteoblasts via N-cadherin-mediated homophilic adhesion and that osteoblasts directly promote the maintenance of HSCs by mechanisms that involve cell-cell contact, including Notch and N-cadherin activation (Zhang et al., 2003; Wilson et al., 2004; Haug et al., 2008). However, genetic evidence supporting such mechanisms is lacking. Conditional deletion of *Jagged1* and/or *Notch1* (the ligand/receptor combination proposed to regulate Notch signaling between osteoblasts and HSCs) does not affect HSC maintenance or function (Mancini et al., 2005). Conditional inactivation of all canonical Notch signaling by disruption of the CSL/Rbp-J transcriptional complex also does not affect the maintenance or function of adult HSCs (Maillard et al., 2008). It remains possible that Notch could regulate HSCs through some noncanonical signaling pathway, although we are not

aware of any data that yet support this possibility. It has not been tested whether *N-cadherin* deficiency affects HSC maintenance.

Given the central role proposed for N-cadherin in the creation of osteoblastic niches, we recently tested whether HSCs express N-cadherin. We were unable to detect N-cadherin expression among highly purified HSCs by quantitative PCR, by staining with commercially available anti-N-cadherin antibodies, or in *N-cadherin:LacZ* gene trap mice (Kiel et al., 2007b). Published microarray analyses of HSCs from multiple laboratories also failed to detect *N-cadherin* expression (Ivanova et al., 2002; Kiel et al., 2005). Only bone marrow cells negative for N-cadherin staining had the capacity to give long-term multilineage reconstitution of irradiated mice (Kiel et al., 2007b), even when we used the commercially available anti-N-cadherin antibody that had been used to identify osteoblastic niches in bone marrow sections (Zhang et al., 2003; Wilson et al., 2004). This suggested that HSCs could not adhere to osteoblasts via N-cadherin and that the N-cadherin$^+$ cells imaged at the endosteum in prior studies could not have been HSCs. However, it was subsequently suggested that N-cadherin is expressed at low levels on HSCs and that it remains functionally important for their localization within a quiescent osteoblastic niche (Haug et al., 2008; Zhang and Li, 2008).

To resolve this controversy, it was suggested that *N-cadherin* should be genetically deleted from adult hematopoietic cells (Hooper et al., 2007). If N-cadherin is not expressed by HSCs, then *N-cadherin* deficiency should not affect HSC maintenance. In contrast, if HSC niches are regulated by low levels of N-cadherin expression within HSCs, then *N-cadherin* deletion should lead to the depletion of HSCs and to deficits in hematopoiesis. We have now addressed this by conditionally deleting *N-cadherin* from HSCs and other hematopoietic cells in adult *Mx-1-Cre*$^+$*N-cadherin*$^{fl/-}$ mice. Despite efficient deletion of *N-cadherin* from HSCs, these mice exhibited no detectable effects of *N-cadherin* deficiency on HSC maintenance or hematopoiesis, either acutely after *N-cadherin* deletion or several months later. Bone marrow cells from these mice exhibited normal HSC frequency and a normal capacity to reconstitute irradiated mice, even upon transplantation into secondary recipients. *N-cadherin* is therefore not required cell autonomously to regulate HSC maintenance or function. It remains possible that osteoblasts regulate HSC maintenance through other mechanisms, but HSC maintenance does not depend upon N-cadherin-mediated homophilic adhesion to osteoblasts, as proposed in current osteoblastic niche models.

RESULTS

N-cadherin Is Efficiently Deleted after pIpC Treatment

Mice with germline *N-cadherin* deficiency (*N-cadherin*$^{-/-}$) die by E11 due to severe developmental defects, including cardiovascular failure (Radice et al., 1997). Therefore, to study the effects of *N-cadherin* deficiency on adult HSCs, we mated previously described *N-cadherin*^{fl} mice (Kostetskii et al., 2005; Luo et al., 2006; Kadowaki et al., 2007; Li et al., 2008) with *Mx-1-Cre* mice (Kuhn et al., 1995) to conditionally delete *N-cadherin* from adult HSCs. Each of these strains was backcrossed for at least six generations onto a C57BL/6 background. *Mx-1-Cre* expression is activated in HSCs, other hematopoietic cells, and some other tissues by administration of polyinosine-polycytidine (pIpC) (Kuhn et al., 1995; Hock et al., 2004; Yilmaz et al., 2006b).

To test the efficiency of *N-cadherin* excision within HSCs, we cultured individual CD150$^+$CD48$^-$CD41$^-$lineage$^-$Sca-1$^+$c-kit$^+$ HSCs (1 cell/well) isolated from *Mx-1-Cre*$^+$*N-cadherin*$^{fl/-}$ mice after they had been treated with seven doses of pIpC over 14 days. These cells include more than 95% of all long-term multilineage-reconstituting cells in the bone marrow, and more than 40% of single cells within this population give long-term multilineage reconstitution after transplantation into irradiated mice (Kiel et al., 2005; Yilmaz et al., 2006a; Kiel et al., 2008). Genomic DNA was extracted from individual myeloerythroid colonies formed by these HSCs and analyzed by PCR. Of colonies cultured immediately after pIpC treatment, 53 of 54 showed complete deletion of *N-cadherin*^{fl} (Figures 1A and 1B). Of colonies that arose from HSCs cultured 1 to 3 months following pIpC treatment, 72 of 73 showed complete deletion of the *N-cadherin*^{fl} allele (Figures 1A and 1B). This indicates that at least 98% of HSCs deleted *N-cadherin* upon pIpC treatment. The rare nonrecombined cells did not appear to have a competitive advantage over *N-cadherin*-deficient HSCs, as their frequency did not increase with time after pIpC treatment.

To ensure the accurate detection of *N-cadherin* excision, we sequenced the PCR products corresponding to the wild-type, floxed, and deleted *N-cadherin* alleles and confirmed 100% identity to the appropriate regions of the *N-cadherin* gene (data not shown). Moreover, we designed independent PCR primers that specifically amplified a region of *N-cadherin* (the first exon) that is deleted upon *Cre*-mediated excision and confirmed excision of the floxed region of *N-cadherin* in hematopoietic colonies from pIpC-treated *Mx-1-Cre*$^+$*N-cadherin*$^{fl/-}$ mice (Figure S1). pIpC treatment of *Mx-1-Cre*$^+$*N-cadherin*$^{fl/-}$ mice therefore leads to *N-cadherin* deletion.

N-cadherin Deficiency Does Not Alter Hematopoiesis

If *N-cadherin* is critical for HSC maintenance, then *N-cadherin* deficiency should lead to defects in hematopoiesis. We therefore tested whether acute or chronic loss of *N-cadherin* affected hematopoiesis in *Mx-1-Cre*$^+$*N-cadherin*$^{fl/-}$ mice. To test this, we harvested peripheral blood and bone marrow from *Mx-1-Cre*$^+$*N-cadherin*$^{fl/-}$ mice or littermate controls (mice lacking *Cre* or bearing at least one wild-type allele of *N-cadherin*) either immediately following pIpC treatment (2 weeks pIpC; Figures 1C–1G) or 1–3 months following 2 weeks of pIpC administration (Figures 1H–1L). We did not detect any effect of *N-cadherin* deletion on white blood cell (WBC), erythrocyte (RBC), or platelet (Plts) concentration in the blood of *Mx-1-Cre*$^+$*N-cadherin*$^{fl/-}$ mice as compared to littermate controls at either time point (Figures 1C and 1H). We also did not detect any effect of *N-cadherin* deficiency on bone marrow cellularity (Figures 1D and 1I) or composition with respect to the erythroid (Ter119$^+$ cells), myeloid (Mac-1$^+$Gr-1$^+$), B (B220$^+$), or T cell (CD3$^+$) lineages (Figures 1E and 1J). Finally, we did not detect any effect of *N-cadherin* deficiency on the frequency (Figures 1F and 1K) or types (Figures 1G and 1L) of colony-forming progenitors in the bone marrow. These results indicate that *N-cadherin* is not required within HSCs for hematopoiesis.