Cell Stem Cell
Article

Contractile Forces Sustain and Polarize Hematopoiesis from Stem and Progenitor Cells

Jae-Won Shin,[1,2] Amnon Buxboim,[1] Kyle R. Spinler,[1] Joe Swift,[1] David A. Christian,[3] Christopher A. Hunter,[3] Catherine Léon,[4] Christian Gachet,[4] P.C. Dave P. Dingal,[1] Irena L. Ivanovska,[1] Florian Rehfeldt,[1] Joel Anne Chasis,[5,6] and Dennis E. Discher[1,2,*]

[1]Biophysical Engineering Lab, University of Pennsylvania, Philadelphia, PA 19104, USA
[2]Cell and Molecular Biology and Pharmacology Graduate Groups, University of Pennsylvania, Philadelphia, PA 19104, USA
[3]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
[4]UMR S949, Inserm, Université de Strasbourg, Établissement Français du Sang, Strasbourg, 67000, France
[5]Life Sciences Division, University of California, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[6]Division of Hematology/Oncology, University of California, San Francisco, San Francisco, CA 94143, USA
*Correspondence: discher@seas.upenn.edu
http://dx.doi.org/10.1016/j.stem.2013.10.009

SUMMARY

Self-renewal and differentiation of stem cells depend on asymmetric division and polarized motility processes that in other cell types are modulated by nonmuscle myosin-II (MII) forces and matrix mechanics. Here, mass spectrometry-calibrated intracellular flow cytometry of human hematopoiesis reveals MIIB to be a major isoform that is strongly polarized in hematopoietic stem cells and progenitors (HSC/Ps) and thereby downregulated in differentiated cells via asymmetric division. MIIA is constitutive and activated by dephosphorylation during cytokine-triggered differentiation of cells grown on stiff, endosteum-like matrix, but not soft, marrow-like matrix. In vivo, MIIB is required for generation of blood, while MIIA is required for sustained HSC/P engraftment. Reversible inhibition of both isoforms in culture with blebbistatin enriches for long-term hematopoietic multilineage reconstituting cells by 5-fold or more as assessed in vivo. Megakaryocytes also become more polyploid, producing 4-fold more platelets. MII is thus a multifunctional node in polarized division and niche sensing.

INTRODUCTION

Stem cells must be able to self-renew and also give rise to diverse cell types by asymmetric division in appropriate microenvironments (Knoblich, 2010). This differential segregation of cell fate determinants produces progenitors that expand symmetrically to generate tissue. Hematopoietic stem cells (HSCs, as a subset of $CD34^+$ cells) exemplify these key properties of stem cells in that they are often quiescent in niches of the bone marrow (BM), but they and/or their daughter cells polarize and divide asymmetrically in suitable niches to generate progenitors that further divide and specialize to terminally differentiated erythroid, megakaryocyte, and white cell lineages. A number of models for marrow and soluble signal regulation of HSC maintenance and differentiation have been described (Trumpp et al., 2010), but many physical aspects of hematopoiesis remain unclear. Many cell types apply forces to the matrix that they adhere to, and the flexibility of extracellular matrix is already known to modulate differentiation of marrow-derived mesenchymal stem cells (MSCs) (Engler et al., 2006) as well as the expansion of adult HSCs and progenitors (HSC/Ps) (Holst et al., 2010). In both of these latter studies, myosin-II (MII) inhibition revealed a key role for actomyosin forces in adhesion and sensing of matrix. However, cell contractile forces contribute to many processes in stem cell and progenitor maintenance and division with likely relationships to differentiation.

Cytokinesis is driven by nonmuscle MII in a cell's cortex, and the asymmetry of stem cell division in *C. elegans* is also established by MII (Ou et al., 2010). Differentiation in embryogenesis indeed requires active MII (Conti et al., 2004), and while inhibition of MII in adherent embryonic stem cells (ESCs) increases survival in culture by preserving intercellular contacts (Chen et al., 2010), inhibition can also lead to multinucleated cells (Canman et al., 2003). Actomyosin forces generally stabilize the plasma membrane with an active cortical tension or rigidity (Merkel et al., 2000), but these forces also drive cell rounding in cytokinesis (Sedzinski et al., 2011) and can change dramatically in differentiation (of MSCs) (Engler et al., 2006). Indeed, while it has been known for many years that as granulocytes differentiate they become soft to better traffic from marrow through the endothelial barrier and into the circulation (Lichtman, 1970), any MII changes in such cells leaving the marrow or in other hematopoietic cells is currently unknown.

Mammals express three isoforms of MII: A (*MYH9*), B (*MYH10*), and C (*MYH14*), and each is regulated transcriptionally as well as posttranslationally. MIIA is found in most tissues (Ma et al., 2010) including blood (Maupin et al., 1994) and is essential to embryonic differentiation (Conti et al., 2004). MIIB is particularly enriched in brain and cardiac tissues, and it is often polarized to the rear of migrating cells (Vicente-Manzanares et al., 2008; Raab et al., 2012). Recent studies have revealed roles for MIIB in hematopoiesis, specifically in megakaryocyte (MK) differentiation (Lordier et al., 2012) and in the asymmetric process of erythroid enucleation (Ubukawa et al., 2012). MIIB myofilaments are known to attach more strongly to and detach

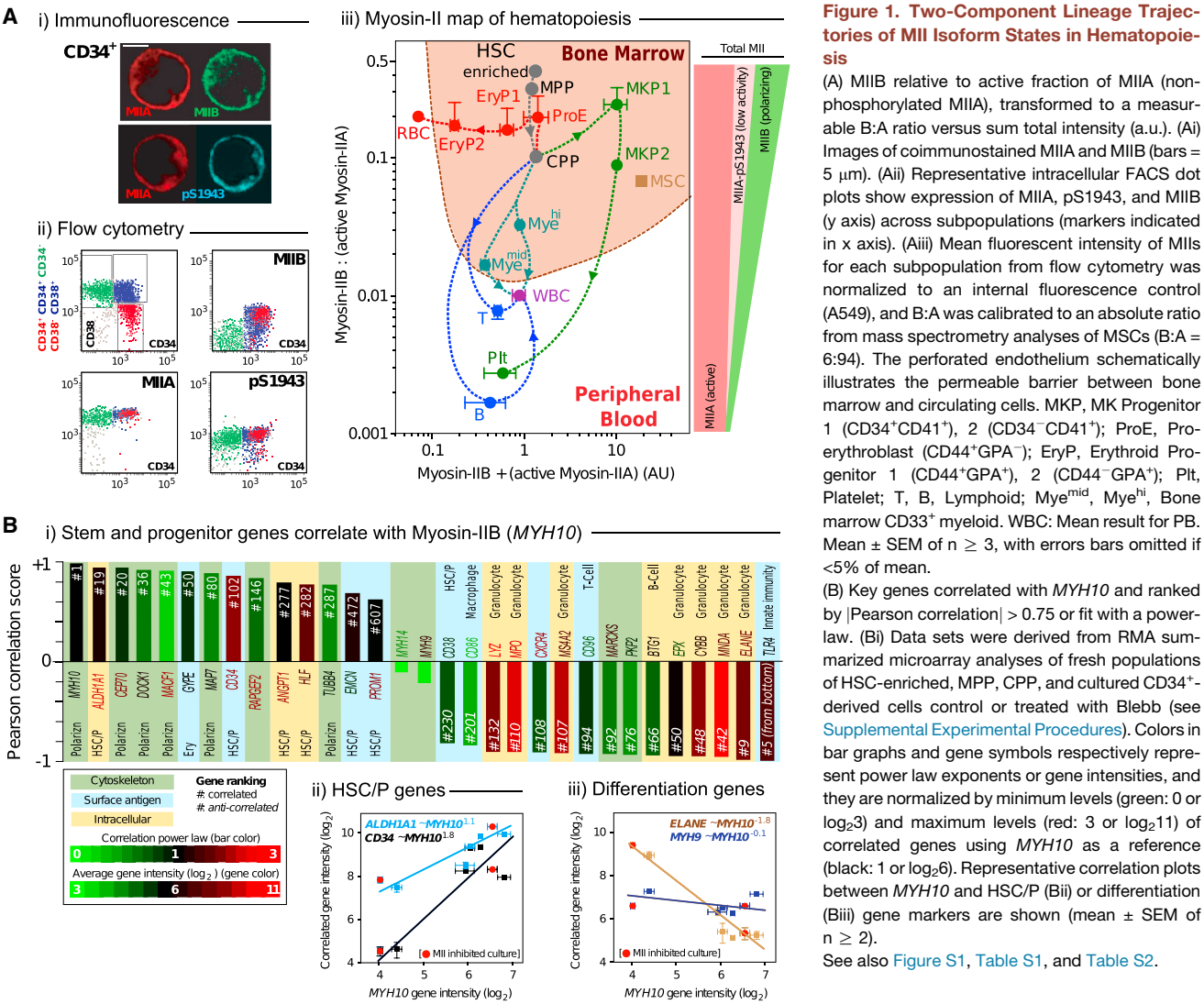

Figure 1. Two-Component Lineage Trajectories of MII Isoform States in Hematopoiesis

(A) MIIB relative to active fraction of MIIA (non-phosphorylated MIIA), transformed to a measurable B:A ratio versus sum total intensity (a.u.). (Ai) Images of coimmunostained MIIA and MIIB (bars = 5 μm). (Aii) Representative intracellular FACS dot plots show expression of MIIA, pS1943, and MIIB (y axis) across subpopulations (markers indicated in x axis). (Aiii) Mean fluorescent intensity of MIIs for each subpopulation from flow cytometry was normalized to an internal fluorescence control (A549), and B:A was calibrated to an absolute ratio from mass spectrometry analyses of MSCs (B:A = 6:94). The perforated endothelium schematically illustrates the permeable barrier between bone marrow and circulating cells. MKP, MK Progenitor 1 ($CD34^+CD41^+$), 2 ($CD34^-CD41^+$); ProE, Proerythroblast ($CD44^+GPA^-$); EryP, Erythroid Progenitor 1 ($CD44^+GPA^+$), 2 ($CD44^-GPA^+$); Plt, Platelet; T, B, Lymphoid; Mye^{mid}, Mye^{hi}, Bone marrow $CD33^+$ myeloid. WBC: Mean result for PB. Mean ± SEM of $n \geq 3$, with errors bars omitted if <5% of mean.

(B) Key genes correlated with *MYH10* and ranked by |Pearson correlation| > 0.75 or fit with a power-law. (Bi) Data sets were derived from RMA summarized microarray analyses of fresh populations of HSC-enriched, MPP, CPP, and cultured $CD34^+$-derived cells control or treated with Blebb (see Supplemental Experimental Procedures). Colors in bar graphs and gene symbols respectively represent power law exponents or gene intensities, and they are normalized by minimum levels (green: 0 or $\log_2 3$) and maximum levels (red: 3 or $\log_2 11$) of correlated genes using *MYH10* as a reference (black: 1 or $\log_2 6$). Representative correlation plots between *MYH10* and HSC/P (Bii) or differentiation (Biii) gene markers are shown (mean ± SEM of $n \geq 2$).

See also Figure S1, Table S1, and Table S2.

more slowly from F-actin than MIIA, resulting in higher force generation per MIIB (Wang et al., 2003). However, MIIB in human ESCs or stem cells in general has unknown functions. MIIC appears restricted to epithelial cells (Ma et al., 2010) and serves here as a useful negative control in expression analyses. Here, we reveal critical roles of MIIA and MIIB in adult hematopoiesis and use that understanding to enrich highly heterogeneous HSC/Ps for long-term hematopoietic stem cells.

RESULTS

MII Isoforms Switch from B-and-A to Only A in Human Adult Hematopoiesis

Immunofluorescence of human $CD34^+$ cells reveals cortical MIIB as well as MIIA (Figure 1Ai), but flow cytometry and immunoblots show that myosin levels vary with surface markers and also across differentiated lineages (Figure 1Aii, Figures S1A and S1B, available online). Mass spectrometry-calibrated intracellular flow (MS-IF) cytometry (Figure S1C, Supplemental Experimental Procedures) was developed to quantify absolute isoform stoichiometry, which is not possible by antibody methods alone due to differential sensitivities of antibodies to isoforms. MS-IF cytometry of diverse hematopoietic cell types reveals that MIIB is no more than ~30% of total MII across cell types and has a large dynamic range of ~5,000-fold compared to ~80-fold for MIIA (Table S1). However, MS also revealed MIIA phosphorylation at S1943 (pS1943), which deactivates MIIA through myofilament disassembly (Dulyaninova et al., 2007), and so a phosphospecific antibody was used to estimate the pS1943 stoichiometry of MIIA through a calibration scheme using mutant GFP-MIIA (see Supplemental Experimental Procedures). Based on this, ~50%–60% of MIIA is phosphorylated as pS1943 in the three key HSC/P subpopulations of $CD34^+$ cells (Figure S1D) per standard surface markers (Majeti et al., 2007; Novershtern et al., 2011):

{"HSC enriched" cells: $CD34^+$, $CD38^-$, $CD90^+$, $CD45\text{-}RA^-$, $CD133^+$}
{Multipotent progenitor ("MPP"): $CD34^+$, $CD38^-$, $CD90^-$, $CD45\text{-}RA^-$, $CD133^+$},