Network Analysis between Hematopoietic Stem Cells and Multipotent Progenitor Cells

Jun Seita[1*], Derrick J. Rossi[1], Irving L. Weissman[1]

1. Institute for Stem Cell Biology and Regenerative Medicine,
Stanford University School of Medicine, Stanford, CA U.S.A.
*E-mail: jseita@stanford.edu

In blood system, only hematopoietic stem cells (HSCs) have both self-renewal and multi-differentiation potentials, and give rise to all types of functional blood cells throughout life. Thus, regulation of HSCs fate is critical to maintain homeostasis of blood system. Although both intrinsic and extrinsic molecules are involved in regulation of HSC fate, HSCs in the steady state are largely in quiescent (G0) phase of the cell cycle, and it is presumed that extrinsic factors play key roles to trigger fate decision. In bone marrow, HSCs are believed to be in proximity to both non-hematopoietic stromal cells, as well as more committed hematopoietic cells. Recently a subset of stromal osteoblasts has been proposed to play an important role maintaining quiescence of HSCs. However, homeostatic feedback mechanisms resulting from downstream hematopoietic demand are also likely to significantly influence HSC biology, although the pathways utilized by such mechanisms are essentially unexplored. We hypothesized that the fate of HSC may be regulated by molecular interactions between HSCs and their immediate downstream multi-potent progenitor cells (MPPs).

In order to illuminate molecular interactions between HSCs and MPPs, we took a network analysis approach. Highly purified HSCs (CD34- Flk2- c-Kit+ Sca-1+ Lin-) and MPP^{flk2-}s (CD34+ Flk2- c-Kit+ Sca-1+ Lin-) were obtained by multi-color fluorescence activated cell sorting, and subjected to microarray (Affymetrix GeneChip Mouse Genome 430 2.0) analyses. GeneChip dataset of each population were normalized and analyzed individually to comprehensive lists of highly expressed genes, and from which genes encoding cell-surface or secretion proteins were identified (Figure 1a, b).

The resulting lists of genes were sorted intofunctional categories such as cytokine

receptor, cell-cell adhesion, cell-extracellular matrix (ECM) adhesion, and secretion. 16 cytokine receptors, 14 cell-cell adhesion molecules, 5 ECM receptors and 14 secretion molecules were detected in HSCs; and 13 cytokine receptors, 16 cell-cell adhesion molecules, 2 ECM receptors, and 14 secretion molecules were detected in MPP^{flk2-}. Candidate molecules that interact with these cell-surface molecules and secretion molecules were then screened for by searching protein-protein interaction databases including the KEGG database, and by comprehensive evaluation of the relevant literature (Figure 1c, d). After confirmation of expression of these genes by quantitative PCR, candidate cytokine networks (Figure 2a), cell-cell adhesion networks (Figure 2b), and cell-ECM networks (Figure 2c) for direct interaction between HSC to HSC, HSC to MPP^{flk2-}, MPP^{flk2-} to MPP^{flk2-} were assembled.

The proposed cytokine networks among HSCs and MPP^{flk2-} were tested by protein expression analysis and in vitro functional analysis. Intercellular expression of predicted cytokines (Ang1, IL-27, and PDGFD) and cell-surface expression of receptors (Tie-2, IL-27Ra, gp130, and PDGFRb) in HSCs and MPP^{flk2-} were confirmed and revealed functional cross-talk between HSCs and MPPs utilizing Ang1-Tie2 signaling. These results establish that network analysis based on GeneChip datasets of highly purified populations can be used to efficiently predict molecular networks among these populations.

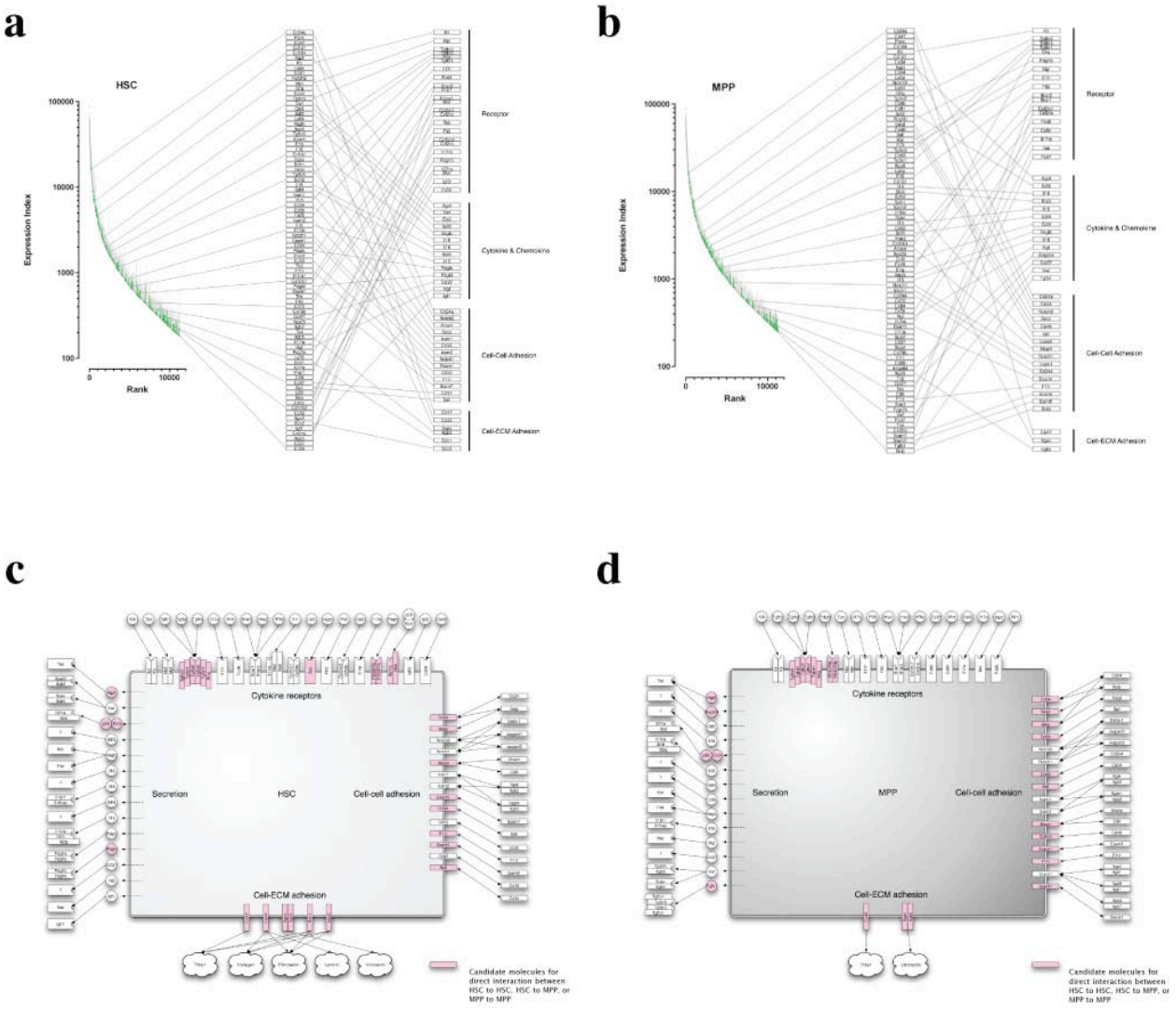

Figure 1 Network analysis based on GeneChip data of highly purified HSCs and MPP^{flk2-}

Genes encoding cell-surface or secretion proteins in top 25% percentile of Expression Index of HSCs (**a**) and their immediate progenitors MPP^{flk2-} (**b**). Predicted cytokine receptors, cell-cell adhesion molecules, cell-ECM adhesion molecules, and secretion molecules of HSCs (**c**) and MPP^{flk2-} (**d**); and the candidate molecules that interact with these cell-surface molecules and secretion molecules screened by searching protein-protein interaction databases.