Hematopoietic progenitor cells regulate their niche microenvironment through a novel mechanism of cell-cell communication

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Cellular communication within a larger microenvironment is critical for a number of physiological processes. Within the bone marrow niche, direct cell communication between hematopoietic progenitor cells (HPCs) and osteoblasts provides essential cues for their proliferation and survival. While contact-dependent communication between HPCs and osteoblasts is known to be critical, the molecular pathways that govern this interaction are largely unclear. Moreover, the downstream events occurring at the HPC/osteoblast contact site remain uncharacterized, despite their major role in signaling and remodeling within the niche microenvironment. Using live cell imaging approaches, we found that intercellular transfer is a novel mode of cell communication within the bone marrow niche microenvironment. HPCs make prolonged contact with the osteoblast surface via a specialized membrane domain enriched in prominin 1, CD63 and rhodamine PE. At the contact site, portions of the HPC specialized domain containing these molecules were taken up by the osteoblast and internalized into long-lived, SARA-positive, signaling endosomes. This resulted in the downregulation of Smad signaling by the osteoblasts and a subsequent increase in the production of stromal-derived factor-1 (SDF-1), a chemokine responsible for HPC homing to bone marrow. These findings identify a novel mechanism involving intercellular transfer to signaling endosomes for targeted regulation of signaling and remodeling events within the osteoblastic niche microenvironment.

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Through the development of a co-culture cellular niche system and the use of live-cell confocal imaging approaches, we characterized the distribution of specific plasma membrane components on the surface of HPCs. Using immunofluorescence, fluorescent protein tags, and fluorescently labeled lipids, a specialized domain of the HPC plasma membrane was identified as the cell contact site with osteoblasts. This contact domain was enriched in the stem cell marker, prominin 1, adhesion molecules such as specific tetraspanin proteins, and the lipid rhodamine PE (phosphatidyl ethanolamine, N-Rh-PE).

To better understand what controls HSPC surface polarity and ultimately whether this asymmetry is important for events occurring at the HSPC/osteoblast interface, we tested the effect of depleting cholesterol and destabilizing actin on HPC domain organization using methyl-β-cyclodextrin and cytochalasin D. After either treatment, molecules normally enriched in the specialized domain, including prominin 1 and N-Rh-PE, redistributed into punctate clusters dispersed across the plasma membrane. Hence, cholesterol and actin-based processes are both important for the polarized domain localization of HPC cell surface components.

Using live cell imaging to analyze the dynamics of the HPC/osteoblast interactions, we identified a novel mechanism for their communication. Proteins, lipids, and quantum dots could be observed transferring from the HPC contact domain to the osteoblasts in a process of intercellular transfer (Fig. 1). Through further investigation, we determined that the HPC membrane contact site was required for efficient transfer, since disruption of the membrane domain with either methyl-β-cyclodextrin or cytochalasin D resulted in a significant reduction in intercellular transfer. Furthermore, we found that the observed transfer was specific to osteoblastic cells and required direct cell contact. Therefore, this led to the hypothesis that intercellular transfer is a cell contact-dependent mechanism of cell communication within the bone marrow niche.

To address whether intercellular transfer serves as a mechanism for cell communication, we analyzed the fate of molecules transferred to osteoblasts as well the potential for downstream signaling generated from the transfer event. HPC components were internalized by osteoblasts into specific signaling endosomes positive for Smad Anchor for Receptor Activation (SARA). SARA endosomes recruit Smads, which are responsible for transforming growth factor-β (TGFβ) signaling events. Osteoblasts positive for a transfer event displayed a decrease in Smad activation and therefore, a decrease in TGFβ signaling. We know from several studies that TGFβ signaling is inhibitory to the production of the chemokine, SDF-1, which is a known regulator of the niche. These results raised the possibility that intercellular transfer may mediate changes in niche chemokine production. Indeed, we found that intercellular transfer of HPC components resulted in an increase in SDF-1 positive osteoblasts. We went on to determine that disruption of the HPC membrane domain, with either methyl-β-cyclodextrin or cytochalasin D, inhibited the increase in SDF-1 positive osteoblasts. Therefore, these data demonstrate that through a specialized mechanism of cell communication, intercellular transfer, progenitor cells can regulate their own niche environment by stimulating surrounding cells to produce key chemokines.

The intercellular transfer of proteins and lipids has come to the forefront as an important route of cell communication for immune signaling, tumor progression, pathogen infection, and now stem cell niche maintenance. Clearly, identifying the downstream signals generated from the intercellular transfer event has become an important next step. Our work has unveiled a population of signaling endosomes as downstream targets for mediating cell-cell communication. By determining how specific endosomes are targeted and under what conditions, we may further our understanding of signaling mechanisms within other intimate cellular microenvironments. Future studies will be directed at determining the molecular mechanisms of these intercellular transfer events as well as their in vivo significance. We anticipate that future work in this area will strongly impact how we think about cell communication within both normal and disease microenvironments.

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