

## Live cell imaging of interactions between hematopoietic progenitor cells and osteoblastic cells

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Hematopoietic stem cells localize to a complex niche within the bone marrow where they interact specifically with the bone forming osteoblasts. Despite knowledge of the importance of osteoblast contact for hematopoietic stem cell maintenance, how hematopoietic progenitor cells find osteoblasts within this microenvironment and what role the ensuing interaction between these cells plays in hematopoietic stem cell renewal and differentiation is not well understood. Here, we investigate the cellular and molecular interactions that occur between hematopoietic progenitor and osteoblastic cells using live-cell imaging techniques. KG1a and primary CD34+ hematopoietic progenitor cells were used in co-cultured experiments with osteoblastic cells. Through microscopy techniques, we observed a dynamic, amoeboid motility by the progenitor cells that was directed to the osteoblastic cells. The progenitor cells formed a specialized uropod domain that was enriched in lipid raft molecules (GPI-GFP and cholera toxin B subunit) and served as the contact point between the progenitor and osteoblastic cells. Additionally, stem cell markers CD34 and CD133 were found to coalesce at the uropod contact site along with quantum dot nanocrystals. The quantum dots localized specifically at the cell surface of the progenitor cell uropod and could be disrupted by cholesterol sequestration. Remarkably, the quantum dot loaded uropod appeared to transfer and be taken up by the osteoblasts and preliminary investigation suggests that CD34 and CD133 may also transfer from the progenitor to the osteoblastic cells. This molecule exchange is reminiscent of the T-cell/antigen presenting cell interaction and may implicate a potential signaling mechanism mediated by the release and /or up-take of specific proteins within the hematopoietic/osteoblastic niche. Future studies will be directed at determining the mechanism of this molecule transfer and the potential downstream functions of this cell-cell interaction.