

Molecular Dissection of the Hematopoietic Stem Cell Niche: The Role of Annexin II

Younghun Jung, Jingcheng (Jason) Wang, Jianhua Wang, Aaron Havens, Yanxi Sun, and Russell S. Taichman

Department of Periodontics, Prevention, and Geriatrics. University of Michigan School of Dentistry, Ann Arbor, MI

In adults the bone marrow is the sole microenvironment which supports all normal activities of hematopoiesis. Hematopoietic stem cells (HSCs) and osteoblasts (OBs) collaborate in the production of the extra cellular bone marrow matrix and cytokine synthesis, resulting in the formation of the various blood cells. Both HSCs and osteoblasts express a wide spectrum of adhesion molecules that facilitate these interactions. The changes in adhesive interactions may regulate stem cell survival and trafficking between the marrow and the circulation; additionally, the adhesion molecule(s) may have an important role in how HSCs home to bone marrow.

We turned to a novel cell blotting system in which OB proteins were isolated, run on non-denaturing discontinuous gels, and blotted with biotin-labeled cell lines (KG1a cells) that express an early hematopoietic cell phenotype (B. Seshi, *Blood* 83:2399-2409). Two major protein bands were identified that bind KG1a cells from whole osteosarcoma cell MG-63 or SaOS-2 cell lysates. Those two proteins were identified as annexin II monomer and multimer. Moreover, using laser capture and microarray analysis of molecules which are significantly expressed in endosteal OBs (which support hematopoiesis) relative to periosteal OBs (which do not) in a parallel study also identified annexin II and its binding partner p11 as potentially playing a significant role in stem cell localization.

In vitro studies with blocking antibodies confirmed that annexin II was critically involved in mediating the adhesive interactions between osteoblasts and hematopoietic cells (% changes: MG-63 47.8% and SaOS-2 56.5%, respectively). In order to further determine the role of annexin II in OB cells, we suppressed annexin II protein expression by RNA interference. For MG-63 cells, the adhesion of KG1a cells was markedly reduced in the annexin II silenced cells (% change: 85.7%). Similarly, we found a marked reduction of binding to SaOS-2 cells (% change: 57.2%). Over expression of annexin II increased expression of the protein approximately two- to three-fold in the OB cells at 3 days, which correlated with increases in binding on SaOS-2 cells (17.7%) and MG -63 cells (8.7%) probably reflecting the constitutive levels of cell surface annexin II. Next we investigated whether annexin II affected adhesion between primary human OB cells and CD34⁺ hematopoietic bone marrow cells. Here antibody to annexin II antibody blocked the adhesion of the of CD34⁺ cells (% change: 55.5%). CFU assays were performed to further verify the results. Antibody to annexin II decreased the number of recoverable cells able to form progenitor colonies in methylcellulose (% change: 56%). Immunohistochemistry confirmed that annexin II production is enhanced at endosteal sites. Together these data indicate that annexin II expressed by osteoblasts is likely to be essential for the maintenance of progenitors and adhesive capabilities *in vitro*.