M & I
Microbiology
and Immunology
University of North Carolina at Chapel Hill

DISSERTATION SEMINAR

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"G protein-coupled receptor kinase 3 (GRK3) regulates G protein-coupled receptors on murine bone marrow niche mesenchymal stem cells and hematopoietic stem-progenitor cells."

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3:30 p.m.
6004 Marsico Hall

Dissertation Advisor: Dr. Teresa Tarrant

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ABSTRACT

Jaime Marie Brozowski: G protein-coupled receptor kinase 3 (GRK3) regulates G protein-coupled receptors on murine bone marrow niche mesenchymal stem cells and hematopoietic stem-progenitor cells
(Under the direction of Teresa K. Tarrant)

The bone marrow microenvironment, termed *niche*, supports hematopoietic cell development and thus, is vital for establishment of the immune system. Within the niche reside bone marrow mesenchymal stem cells (BmMSCs) that surround the hematopoietic stem-progenitor cells (HSPCs) to support their development, maintenance, and function; however, the intracellular regulatory mechanisms of BmMSCs and HSPCs are still being defined. The goal of this dissertation work is to provide further insight into the regulatory mechanisms that modulate functionality of BmMSCs and HSPCs. Our data suggest *G protein-coupled receptor kinase 3* (GRK3) functions as a negative regulator of G protein-coupled receptors (GPCRs) on BmMSCs and HSPCs.

BmMSCs isolated from GRK3-deficient (*Grk3-/-*) mice have enhanced proliferation and osteogenic differentiation *ex vivo* compared to wildtype (WT) BmMSCs. *Grk3-/-* BmMSC cultures also have higher levels of CXCL12, an essential chemokine for HSPC development, and interestingly, *Grk3-/-* mice have increased hematopoietic cell numbers isolated from the bone marrow. Both *Grk3-/-* BmMSC proliferation and osteogenic differentiation were reduced to WT level upon reduction of sphingosine-1-phosphate (S1P), and *Grk3-/-* BmMSCs have sustained ERK1/2 signaling upon stimulation of sphingosine-1- phosphate receptor (S1PR) with S1P in comparison to WT BmMSCs. In addition, we report GRK3 recruits β-arrestin, a protein necessary for receptor internalization, to the C-terminus of S1PR1, and we demonstrate

BmMSCs lacking GRK3 regulation have impaired S1PR1 internalization. Our findings suggest GRK3 regulates GPCR S1PR on BmMSCs.

Grk3-/- mice have increased bone marrow lineage negative (Lin^-) $Sca1^+$ $c\text{-}Kit^+$ (LSK) HSPC and oligopotent progenitor numbers, as well as increased total leukocytes in the peripheral blood. Since increased stem cell numbers and function potentiate cellular engraftment and hematopoiesis, we tested whether GRK3 deficiency enhances hematopoietic cell function *in vivo* after short-term transplantation, termed *colony forming unit-spleen* (CFU-S) assay. Transplanted *Grk3-/-* LSK HSPCs or *Grk3-/-* whole bone marrow increases colony counts on the explanted spleen in comparison to WT controls, suggesting hematopoiesis of *Grk3-/-* HSPC is enhanced. Further, both *Grk3-/-* hematopoietic myeloid granulocytic and monocytic (CFU-GM) and lymphoid (CFU-Pre-B) colony counts increased *ex vivo* upon CXCR4 ligand stimulation (CXCL12), and *Grk3-/-* myeloid colony counts reduced to WT levels with CXCR4 antagonist treatment (AMD3100). Taken together, *in vivo* and *ex vivo* CFU data suggest GRK3 regulates bone marrow HSPC numbers, and this is, at least in part, mediated through CXCL12/CXCR4 stimulation.

Herein, we describe a newly elucidated pathway of regulation on two niche cells, BmMSCs and HSPCs. Specifically, our data suggest GRK3 functions as a negative regulator of GPCRs on both BmMSCs and HSPCs and can modulate stem cell function.