2016 ACS-IRG Pilot Grant

Robert Signer, PhD

Project Title: Translational control of acute myeloid leukemia stem cells

Abstract:

Leukemia stem cells (LSCs) are self-renewing leukemia cells with the ability to propagate malignant disease, and elimination of LSCs is necessary for curative therapies. In acute myeloid leukemia (AML), failure to eradicate LSCs has left the five-year survival rate at 26%. AML is caused by various genetic lesions, including a translocation that produces the *MLL-AF9* oncogene. Mouse models of *MLL-AF9*$^{+}$ AML recapitulate the human disease, and LSCs have been immunophenotypically defined in this model, affording opportunity to interrogate LSC biology *in vivo*. Improving our molecular understanding of LSCs and uncovering mechanistic differences with hematopoietic stem cells (HSCs) is essential for discovering strategies to selectively destroy LSCs.

We discovered that HSCs have lower protein synthesis rates than other blood cells9. Low protein synthesis is necessary for HSC maintenance as modest changes in protein synthesis impair HSCs. Dysregulated protein synthesis promotes cancer development and progression. This raises the question of whether cell-type specific differences in protein synthesis can be used to identify LSCs and whether LSCs are sensitive to changes in protein synthesis. AML is marked by activation of PI3K/mTOR signaling, which promotes protein synthesis that impairs HSCs but not LSCs. Increased protein synthesis can induce proteotoxic stress, which is deleterious to HSCs. This raises the question of whether differences in the ability to cope with proteotoxic stress underlie dichotomous effects of increased protein synthesis on HSCs and LSCs. We determined that the master regulator of the proteotoxic stress response, *Hsf1*, is highly expressed by HSCs. However, HSCs also express abundant *Hsp90*, an inhibitor of Hsf1 and a chaperone that promotes folding of proteins that support cancer growth, including MLL fusion proteins. This raises the possibility that MLL-AF9 interferes with Hsp90's inhibition of Hsf1, which could increase Hsf1 activity and promote LSC survival.

Hypothesis: LSCs differ from other leukemic cells and from HSCs in terms of their protein synthesis. We propose that reducing protein synthesis impairs LSCs, but unlike HSCs, LSCs can tolerate increased protein synthesis because of increased Hsf1 activation.

Aim 1: Do protein synthesis rates distinguish LSCs from differentiated leukemia cells and HSCs?

Using a model of *MLL-AF9* driven AML, we will test if LSCs have low protein synthesis compared to differentiated leukemia cells and HSCs. This will establish if there are leukemia cell-type specific differences in protein synthesis, and reveal whether protein synthesis rates distinguish LSCs from other leukemic cells and HSCs.

Aim 2: Does reducing protein synthesis impair LSCs?

Reducing protein synthesis with a ribosomal mutation (*Rpl24*$^{Bst/+}$) impairs HSCs and delays cancer development, but whether it impairs LSCs is unknown. We will initiate AML from *Rpl24*$^{Bst/+}$ and wild-type cells and test if reduced protein synthesis impairs LSCs by performing limiting dilution transplants. These results will establish if targeting translational machinery can impair LSCs.

Aim 3: Does *Hsf1* promote LSC survival?

We will transform *Mx1-Cre*+;*Hsf1*$^{FL/FL}$ and wild-type cells with *MLL-AF9* and test if deleting *Hsf1* reduces LSC number by performing limiting dilution transplants of leukemic cells. These experiments will establish if *Hsf1* is necessary for LSCs *in vivo*.