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## 501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

### Septin11 and Septin 8 Play Critical and Overlapping Roles Murine Stem Cell Function

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Septins are highly conserved GTP binding proteins which regulate a variety of cellular functions including cytokinesis, polarity, cell cycle, vesicle trafficking, and exocytosis and are now considered the fourth component of the cytoskeleton. Septins are implicated in T and B cell lymphomas; and acute myeloid leukemias, where they frequently act as fusion partners with the Mixed-Lineage Leukemia (MLL) gene. Previous data in our laboratory led to the discovery of a small molecular inhibitor, DW0254, which inhibits RAS function and its downstream target RhoGTPase, Rac, including the hematopoietic-specific Rac2 GTPase, also implicated in MLL leukemias. The inhibitor leads to apoptosis of leukemia cell lines. Using biophysical methods, we discovered that DW0254 binds both the RAS chaperone, PDE6D, and Septin11, which is a member of the Septin6 family *(Sara CN, et al., Blood Cancer 2022)*. Although Septins have been reported to be involved in function of the RhoGTPase Cdc42 and hematopoietic stem progenitor cell (HSPC) polarity, the role of Septin11 in hematopoiesis has not been reported. Septin11 is a member of the Septin6 family of proteins which consists of Septin6, 8, 10, 11 and 14 with significant homology between family members. Previous data in yeast has hypothesized functional overlap within each family of Septin proteins.
To better understand the functional role of Septin11, we created lentivirus vectors tagged with a GFP reporter that contained shmiRs against *Septin11*. Culture of Septin11 knockdown (Septin11 KD) murine HSPC in IMDM (10ng/ml IL-3, 25ng/ml of SCF, TPO, Flt-3 and IL-6) resulted in an average of 13-fold decrease in cell counts compared to controls (p=0.02) and a decrease in total clonogenic activity **(Figure 1)** with defects seen in both myeloid (GM) and erythroid (BFU-E) colonies. Reduced clonogenic numbers were accompanied by a significant increase in early apoptotic cells suggesting that Septin11 is important in regulating apoptosis in HSPC.
To determine the potential role of Septin11 in more primitive hematopoietic cells, we utilized competitive repopulation assays. Transduced BoyJ HSPC (GFP+ Septin11 KD and BFP+ control, mixed 1:1) were injected into lethally irradiated C57Bl/6 mice. We observed a reduction in multilineage chimerism in the peripheral blood, bone marrow, spleen and thymus in primary transplant recipients after knockdown of Septin11 **(Table 1)**. To better define function in specific hematopoietic populations of HSPC, we performed knockdown in FACS-sorted purified cells. Engraftment studies demonstrated a significant reduction in bone marrow chimerism in primary transplant recipients after knockdown of Septin11 most clearly in LT-HSC and ST-HSC **(Table 1)** with no statistically significant change in MPP. Therefore, to better study the hematopoietic stem cell compartment, we performed secondary transplants utilizing the cells obtained from primary competitive repopulation assays. We observed an unexpected recovery in engraftment in secondary transplant recipients that was accompanied by an increase in the mRNA expression of Septin8. Since Septin8 and Septin11 are closely related we hypothesized that induction of Septin8 was subserving key functions of Septin11.
To test this hypothesis, we created a lentivirus vector that expressed both a shmiR against *Septin11* and the *Septin8* cDNA. Concurrent overexpression of Septin8 with Septin11 knockdown HSPC caused a significant increase in the total number of colonies compared to controls **(Figure 1)**. These data indicate an important role of Septin11 in murine hematopoiesis and a potential redundant role for Septin8.

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Figure 1: Murine clonogenic data.

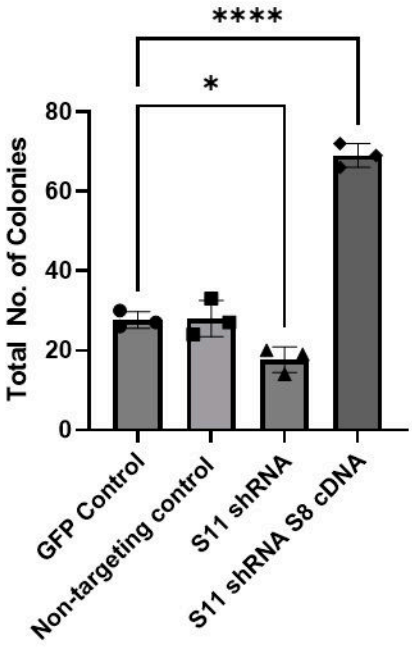


Table 1: Competitive repopulation assay and sub-population transplant data

| | Control | | Septin11 KD | | |
|---|---|---|---|---|---|
| | Chimerism | STDEV | Chimerism | STDEV | p-value |
| **Primary Competitive Repopulation Assay** | | | | | |
| HSPC BM (HSPC: Lin-Sca1+ckit+) | 73.6 | 28.8 | 17.3 | 22.5 | 0.001 |
| HSPC Spleen | 76.5 | 23.1 | 21.3 | 17.1 | 0.0003 |
| HSPC Thymus | 87.5 | 17.1 | 27.1 | 13.2 | 0.008 |
| **Secondary Competitive Repopulation Assay** | | | | | |
| HSPC BM (HSPC: Lin-Sca1+ckit+) | 88.7 | 20.3 | 88.5 | 13.2 | 0.986 |
| HSPC Spleen | 70.4 | 26.6 | 91.2 | 9.7 | 0.149 |
| HSPC Thymus | 93.6 | 7.9 | 96.4 | 3.0 | 0.493 |
| **Sub-population Transplant** | | | | | |
| MPP BM (MPP: LSK CD48+) | 58.7 | 20.3 | 48.4 | 15.7 | 0.14 |
| ST-HSC BM (ST-HSC: LSK CD48-CD150-) | 83.1 | 12.6 | 31.7 | 25.9 | <0.0001 |
| LT-HSC BM (LT-HSC: LSK CD48-CD150+) | 76.7 | 16.2 | 26.43 | 18.62 | <0.0001 |

**Figure 1**

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