**Vav3/Rac2 are Novel Targets for Therapeutic Intervention in High-risk Lymphoblastic Leukemia**

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The BCR-ABL oncogene, the product of the Philadelphia chromosome (Ph) translocation, encodes a consititutively active tyrosine kinase (TK) fusion protein that is both necessary and sufficient to induce leukemia. Currently, the primary treatment for CML and Ph+ B-ALL patients are TK inhibitors. However, in blast phase CML prognosis remains poor due to imatinib resistance and epigenetic silencing of downstream genes controlling cell cycle progression. Targeted intervention to impair a novel nuclear complex, Bmi1/Vav3/Rac2, downstream of the BCR-ABL mutation combined with TK inhibitors may provide a novel approach for multitarget therapeutic intervention in high-risk leukemia. Bmi1 is a polycomb repression complex (PRC) protein and is required for enzymatic activity of the PRC1 complex. It represses transcription of CDKN2A, a tumor suppressor gene encoding p16, and induces self-renewal activity of leukemic progenitors by increasing expression of stem-cell-associated genes including *Cbx5*. Bmi1 was found by the Cancelas lab to be co-immunoprecipitated with the guanine exchange factor Vav3 in nuclear extracts. Vav3 activates Rac2, a RhoGTPase involved in functions such as the organization of the actin cytoskeleton, cell migration and adhesion, control of gene expression, and the activation of proliferation and survival pathways.We examined the mechanism for activation of Bmi1 through the known Vav3/Rac2 interaction. This activity is dependent on a novel nuclear activity of Vav3/Rac2 which activates Bmi1.

To assess the effect of Vav3 on Bmi1 activation, we performed western blots for pBmi1and total Bmi1on nuclear extracts of tumor cells from wild type (WT) and Vav3 knockout mice expressing the BCR-ABL oncogene. We then performed qRT-PCR measuring transcription of the cell cycle inhibitor *Cdkn2a,* a gene crucial in leukemic transformation, and the PRC1 gene *Cbx5,* recently implicated in self-renewal acquisition of leukemic initiating cells,in WT and Vav3 deficient EGFP+EYFP+B220+ sorted splenocytes and solid tumors from leukemic mice. Each of the two conditions was further subdivided into a retrovirally-transduced Bmi1 overexpressing sample and a wild type Bmi1 expression sample. A colony forming unit assay was conducted using WT and Rac2 deficient pro-B cells isolated from mouse bone marrow and virally transduced to express P190 BCR-ABL and to overexpress or not Bmi1.

Vav3 deficient tumor cells showed markedly decreased levels of phosphorylated (active) Bmi1 as compared to WT BCR-ABL cells. We noted a two-fold increase in *Cdkn2a* expression in Vav3 deficient cells as compared to WT cells and Vav3 deficient cells overexpressing Bmi1 overexpression indicating that the repression of *Cdkn2*a expression depends on Vav3 activity that can be restored by overexpression by Bmi1 A four-fold decrease in *Cbx5* expression was noted in Vav3 deficient cells as compared to WT and Vav3 deficient Bmi1-overexressing samples, further indicating a negative regulatory role of Vav3 in the transcriptional regulation of crucial leukemogenic players. Furthermore, functional analysis of clonal leukemic B-cell progenitors showed a four-fold increase in the number of progenitors overexpressing Bmi1 which was prevented by deletion of Rac2. This data phenocopies previous data, generated in our laboratory, that demonstrate the same effect in Vav3 deficient leukemic cells.

In summary, we have demonstrated that Vav3/Rac2 control transcriptional expression of Cdkn2a and Cbx5 expression and in vitro B lymphoid leukemogenesis at the progenitor level. Overexpression of Bmi1 restores leukemogenic levels of *Cdkn2a* and *Cbx5* but does not restore the negative effect of Vav3/Rac2 deficiency on leukemic progenitor proliferation. These data confirm the role of Vav3/Rac2 activation in leukemogenesis and demonstrate that they control an array of transformation signaling pathways including leukemogenic Bmi1-dependent repression of *Cdkn2a* and expression of *Cbx5*.