Title: Sleeping Beauty implicates drivers of leukemic progression in mice with Trp53R270H mutation

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Myelodysplastic syndrome (MDS) is characterized by bone marrow (BM) failure and a highly variable clinical course. A catastrophic complication of MDS is the transformation to acute myeloid leukemia (AML). Notably, TP53 mutations (TP53Mut) in MDS confer the highest risk of leukemic transformation. However, some TP53Mut MDS do not progress to AML, suggesting that TP53Mut is insufficient to transform MDS to AML. Understanding the transformation mechanisms could lead to novel therapeutic strategies.

Our MDS mouse model harbors Trp53R270H (Trp53=murine TP53 gene) and deletion of genes syntenic with human chromosome 5q (del5q). To discover drivers of leukemic transformation, we used Sleeping Beauty (SB) transposon mutagenesis in our mouse model. Upon SB mutagenesis, we found that Trp53WT mice developed T-cell leukemia (n=3/10). In contrast, SB-mutagenized Trp53R270H mice developed myeloid leukemia (n=14/28) and mixed phenotype leukemia (n=7/28). These data showed a bias towards myeloid disease in SB-mutagenized Trp53R270H mice.

Next, we performed RNA sequencing to detect SB-endogenous gene fusions. In Trp53WT leukemias, the most common SB fusions involved Notch1 and Ikzf1 which are associated with T-cell leukemia. In Trp53R270H leukemias, the most common SB-fusions involved Erg and Eras, with SB-Erg detected in 17/20 of Trp53 R270H leukemias. Analysis of SB-Erg fusions suggests that SB upregulates Erg expression. Indeed, Erg levels are higher in leukemias expressing SB-Erg fusions relative to those that do not.

ERG is rarely mutated in AML, but its gene locus is commonly amplified in TP53Mut AML. ERG is known to support hematopoietic stem cell (HSC) self-renewal, and our analysis of SB-Erg leukemia transcriptomes, showed enrichment of HSC and leukemic stem cell (LSC) gene signatures. Next, we interrogated human AML RNA seq datasets (TCGA and BEAT AML) and found enrichment of similar pathways in AMLs with high ERG expression. Lastly, in a human AML single-cell RNA sequencing dataset, we found that ERG expression is highest in LSC-like cells. These findings suggest that ERG overexpression drives a stem cell-like transcriptome in AML.

In summary, we used SB-mutagenesis in Trp53/del5q MDS. In this model, Erg upregulation is associated with progression to AML and upregulation of LSC gene signatures. These data implicate ERG as a contributor to TP53Mut MDS leukemic transformation, and targeting ERG might be an effective therapeutic strategy in TP53Mut MDS.