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ORAL ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Forward Genetic Screen Implicates Drivers of Leukemic Progression in a Novel Model of *Trp53* ^{R270H} myelodysplastic Syndrome

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Myelodysplastic syndrome (MDS) is characterized by bone marrow failure and a highly variable clinical course. The most catastrophic complication of MDS is transformation to secondary acute myeloid leukemia (sAML). Notably, mutations in *TP53* confer the single highest risk of transformation to sAML and death. However, some patients with *TP53* mutated MDS do not develop sAML, suggesting that additional genetic events cooperate with *TP53* mutations to transform MDS to sAML. Understanding the mechanisms of transformation of MDS to sAML could provide targets for therapeutic intervention.

To model the genetics of MDS, we crossed mice bearing *Trp53* ^{R270H} (*Trp53* is the murine *TP53* gene) and deletion of genes syntenic with human chromosome 5q (del(5q)). To discover how additional mutations contribute to disease progression, we utilized *Sleeping Beauty (SB)* transposon mutagenesis in *Trp53* ^{R270H}/del(5q) mice. SB transposase mobilized *SB* mutagenic T2/Onc transposons which randomly insert within the genome. T2/Onc transposons are designed to induce gain or loss of function alterations depending on the site and orientation of insertion with respect to targeted genes. We used the *Mx1-Cre* transgene to activate *SB* transposase and T2/Onc transposition in hematopoietic progenitors. *Trp53* ^{R270H} anddel(5q)(or cytogenetically normal, CN) mice were crossed to *SB* mice to generate donor mice of the following genotypes: *Trp53* ^{R270H}/del(5q)/ *SB, Trp53* ^{R270H}/CN /*SB*, *Trp53* ^{WT}/del(5q)/ *SB*, *Trp53* ^{WT}/CN/ *SB* mice, and mice without *SB* transposition, (no transposition, NT: *Trp53* ^{R270H}/del(5q) /NT). Bone marrow cells were transplanted into recipients, and *SB* insertional mutagenesis was activated using pI-pC to activate Cre. Mice receiving *Trp53* ^{WT}/CN/ *SB* bone marrow developed more frequent T-cell leukemia (n=3/10) than myeloid leukemia (n=1/10). In contrast, mice receiving *Trp53* ^{R270H}*/del(5q)/SB* and *Trp53* ^{R270H}/CN /*SB* bone marrow developed predominantly myeloid leukemia (n=14/28) more commonly than T-cell leukemia (1/28). Mixed phenotype leukemia was seen in 7/28 of these mice. Together, these data demonstrate a strong bias towards myeloid disease in *SB*-mutagenized *Trp53* ^{R270H} bone marrow.

To identify genes with *SB* insertions, we performed RNA sequencing to detect *SB T2/Onc* transposon-endogenous genefusion transcripts. Among *Trp53* ^{WT}/CN/ *SB* leukemias, the most common recurrent *SB* fusions involved *Notch1* and *Ikzf1* as has previously reported for *SB*-associated T-cell leukemias . Among *Trp53* ^{R270H}*/del(5q)/SB* and *Trp53* ^{R270H}/CN /*SB* leukemias, the most common recurrent *SB*-fusions involved *Erg, Eras* and *Il2rb* with *Erg* fusions detected 85% of *Trp53* ^{R270H} leukemias (n=17/20). *SB* inserted upstream of *Erg* promoter indicating that these fusions likely upregulate expression of *Erg*. Indeed, *Erg* levels are significantly higher in leukemias that express *SB-Erg* fusions relative to leukemias that do not ($p<0.0023$).

ERG is not recurrently mutated in human AML, but the *ERG* gene locus is commonly amplified, especially *TP53* mutant AML. ERG is known to support normal hematopoietic stem cell self-renewal. Notably, *Erg*-insertions were also detected in a model of MDS expressing stabilized cyclin E with SB-mediated progression to erythroleukemia (Loeb 2019). Using gene set enrichment analysis, we found that hematopoietic stem cell and leukemic stem cell signatures are enriched in *Erg-SB* fusion leukemias. In our analyses of two independent data sets (TCGA and BEAT AML), stem cell signatures are also among the most highly enriched pathways in human AMLs expressing high *ERG* levels. Furthermore, in a human AML single cell RNA sequencing

dataset (van Galen 2019), we found that *ERG* expression is highest in AML cells with the most immature stem and progenitor-like features. Together, these findings implicate a role for ERG as a driver of progression of MDS to AML by enhancing aberrant self-renewal.

In summary, we present a novel murine model of *Trp53/del(5q)* MDS. In this model, *Erg* upregulation is associated with progression to AML and upregulation of leukemia stem cell gene expression profiles. These data implicate ERG as a major contributor to progression of MDS to secondary AML in the setting of mutant p53. Understanding the mechanisms of disease progression and self-renewal in myeloid malignancies with p53 mutations is critical to define effective therapeutic strategies in these rapidly fatal, treatment resistant diseases.

Disclosures No relevant conflicts of interest to declare.

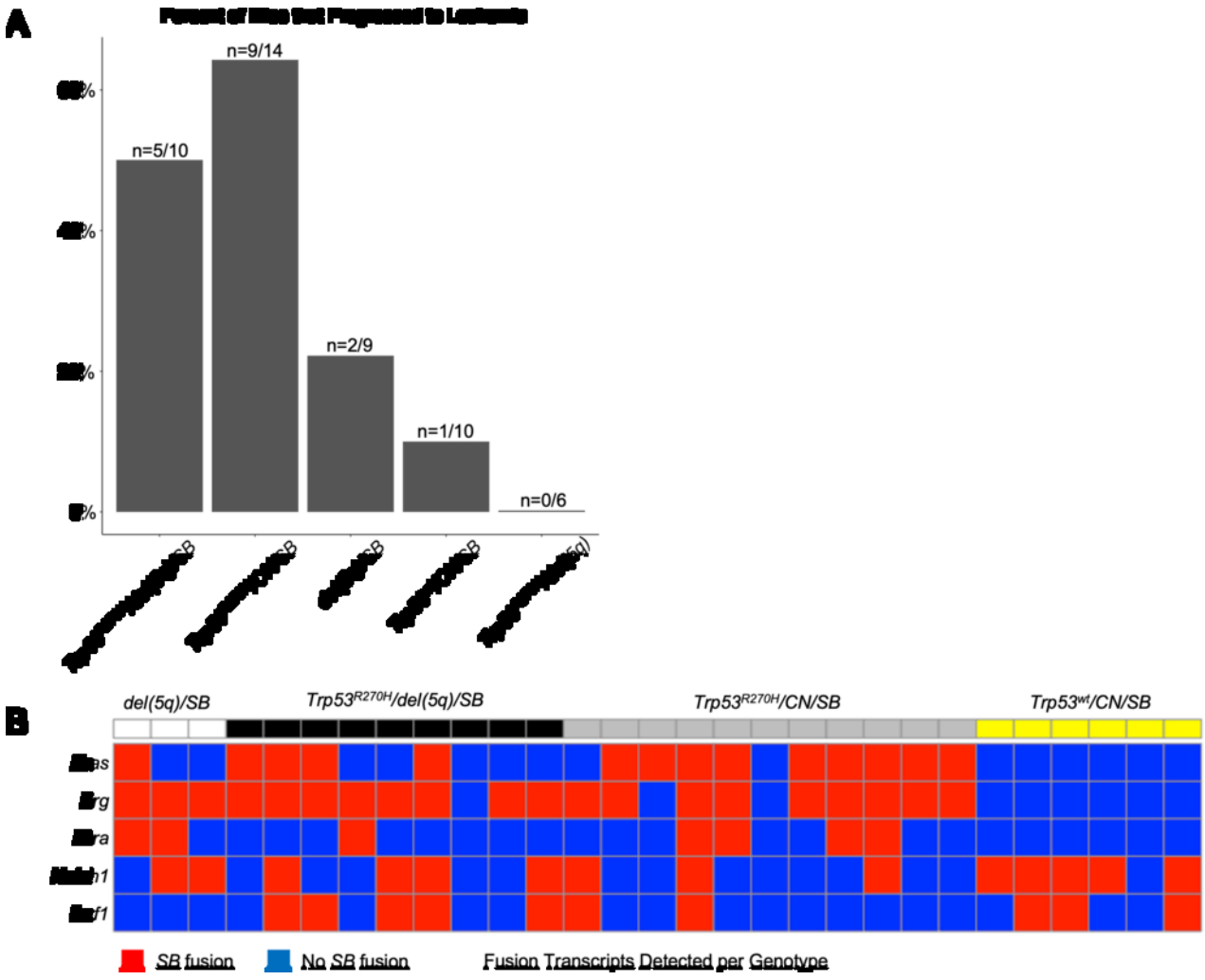

Figure 1

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