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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Cohesin Haploinsufficiency Promotes Reduced Latency in Inv(16) Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is a cancer of the bone marrow that affects 20,000 adults in the United States alone, with global rates increasing over the last 30 years (Heimbruch et al. 2021, Dong et al. 2020). Approximately 15% of adult AML patients harbor the chromosomal aberrations t(8;21) or inv(16), which affect the core binding complex members RUNX1 and CBFB, respectively. Together, RUNX1 and CBFB function as a transcription factor that regulates key lineage genes and proliferation in hematopoietic cells (Pulikkan and Castilla 2018). t(8;21) and inv(16) result in the expression of the fusion proteins AML1-ETO and CBFB-SMMHC, both of which have dominant negative effects on native RUNX1 as well as novel transcriptional roles (Surapally et al. 2021).

Although both t(8;21) and inv(16) result in AMLs with similar characteristics, they differ in the number and type of co-occurring mutations (Opatz et al. 2020). More mutations per clone are observed in t(8;21) vs. inv(16) AMLs. Additionally, several studies have highlighted a difference between the rates of mutation of cohesin genes and t(8;21) vs. inv(16) (Faber et al. 2016, Opatz et al. 2020, Duployez et al. 2016, Jahn et al. 2020, Qin et al. 2022). Cohesin is comprised of the subunits SMC3, SMC1A, RAD21, and STAG2. The complex has a prominent role in hematopoietic cells in chromatin organization and DNA looping events that regulate gene expression and self-renewal (Heimbruch et al. 2021). Cohesin mutations are found in approximately 10% of AML cases (Ley et al 2013). Interestingly, cohesin mutations are found in 18-27% of t(8;21) AMLs, but in 0-4% of inv(16) AMLs (Faber et al. 2016, Opatz et al. 2020, Duployez et al. 2016, Jahn et al. 2020, Qin et al. 2022).

The low rate of co-mutation between inv(16) and cohesin genes suggests potential synthetic lethality. However, it is also possible that cohesin mutations occur at a higher rate than reported due to biases in the age, ethnicity, or treatment status of the patients included in genome/exome sequencing studies. To test if cohesin mutations and inv(16) are synthetic lethal, we used an inv(16) knock-in mouse model (*Mx1-Cre;inv(16)*) combined with a cohesin-deficient mouse (*Mx1-Cre; Smc3* $^{fl/+}$) (Kuo et al. 2006, Viny et al. 2015). We followed *inv(16);Smc3wt* and *inv(16);Smc3* $^{-/+}$ animals post-Cre activation and monitored mice for leukemic development. We found that *inv(16);Smc3* $^{-/+}$ mice developed AML with reduced latency compared to *inv(16);Smc3wt* mice, suggesting cooperativity rather than synthetic lethality.

To determine the mechanism by which *Smc3* haploinsufficiency accelerates inv(16) leukemias, we performed ATAC sequencing and single cell RNA sequencing on hematopoietic stem and progenitor cells (HSPCs). ATAC-seq revealed a large increase in open chromatin in promoter regions in *inv(16);Smc3* $^{-/+}$ vs. *inv(16);Smc3wt* HSPCs. These open regions were enriched for binding motifs of the Ets family member Fli1. Increased expression of Fli1 was observed by western blotting in *inv(16);Smc3* $^{-/+}$ HSPCs. Interestingly, CBFB-SMMHC binding sites commonly contain Ets motifs (Mandoli 2014), suggesting that the increased Fli1 expression and binding site accessibility in *inv(16);Smc3* $^{-/+}$ may drive the observed decrease in latency. scRNA-seq revealed cluster-specific differences between *inv(16);Smc3wt* and *inv(16);Smc3* $^{-/+}$ cells. In several clusters, *inv(16);Smc3* $^{-/+}$ cells showed a loss of expression of genes involved in apoptosis and differentiation. Of particular interest is *Gata2*, as loss of *Gata2* generates a more aggressive phenotype in AML (Saida et al. 2020). Therefore, *Smc3* haploinsufficiency may result in *Gata2* deregulation, thereby impacting disease latency. Currently, we have two potential explanations for the observed phenotype: Deregulated binding of Fli1 and its downstream transcriptional pathways and decreased expression of *Gata2* in sub-populations of *inv(16);Smc3* $^{-/+}$ cells. Which of these mechanisms mediates the reduced latency in *inv(16);Smc3* $^{-/+}$ animals is an area of active investigation. Collectively, our studies show that a lack of observed co-mutation does not necessarily indicate synthetic lethality. We propose that cohesin mutations may be more common in inv(16) leukemias than has been reported and suggest that a careful consideration of age, ethnicity, and treatment status may be warranted when evaluating rates of co-mutation.

Disclosures No relevant conflicts of interest to declare.

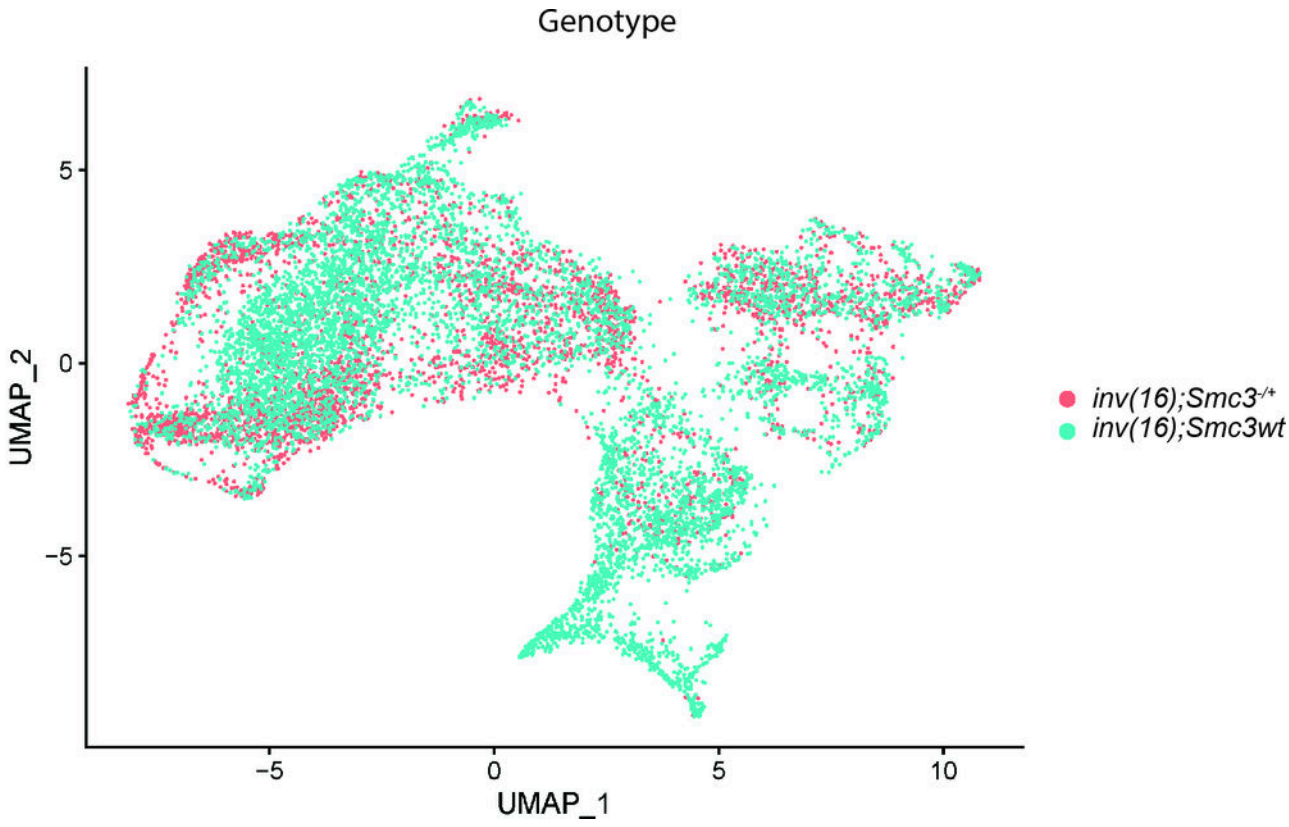

Figure 1

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