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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

STAG2 loss Induces HSC Programs By Modulating Accessibility of AP-1 Bound Enhancers

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Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are blood disorders characterized by clonal expansion of mutant hematopoietic stem and progenitor cells (HSPCs). Mutations in genes encoding the cohesin complex, including *STAG2, RAD21, SMC3* and *SMC1A*, are frequent genetic drivers in MDS and AML. However, the mechanisms by which cohesin mutations lead to clonal expansion of HSPCs are not well understood.

Cohesin is essential for sister chromatid cohesion, DNA damage repair, maintenance of chromatin architecture, and gene regulation. To investigate the impact of cohesin mutations on chromatin accessibility, we performed ATAC-Seq in a panel of six isogenic *STAG2* wild type (WT) and knockout (KO) U937 leukemia cell lines. Among the total of 97,798 ATAC-Seq peaks, we identified significantly more *STAG2* KO (8,859) versus WT (1,622) uniquely accessible chromatin sites, which were strongly enriched for intergenic regions. Integration of ChIP-Seq profiling of cohesin binding and histone modifications demonstrated cohesin binding and an increase in the active enhancer and promoter histone mark H3K27Ac in the *STAG2* KO newly accessible chromatin, suggesting a direct role for cohesin in regulating the activity of such enhancers. Together, our data demonstrate that *STAG2 KO*-induced open chromatin may be enriched for novel *STAG2* KO-specific enhancers.

To further infer the regulatory function of these putative enhancers, we predicted binding of transcription factors (TFs) in ATAC-Seq-defined regions by performing TF footprinting using the Transcription factor Occupancy Prediction By Investigation of ATAC-seq Signal (TOBIAS) algorithm. We observed that binding of members of the AP-1 transcription family, including JUN FOS andATF, previously implicated in oncogenic transformation, was significantly enriched in the *STAG2* KO cells (Figure 1A). Conversely, *STAG2* KO cells lost CEBP TF binding in regions that lost chromatin accessibility. To identify potential regulatory targets of these TFs, we mapped TF footprints to annotated promoter regions and to enhancer-gene pairs determined by the Activity by Contact (ABC) model. We observed a strong correlation between predicted AP-1 binding and gene expression of putative regulated genes in *STAG2* KO cells, further supporting the role of these newly accessible AP-1 bound sites as regulatory elements.

To validate our findings using *in vivo* models of cohesin-mutant disease, we performed RNA-Seq and ATAC-Seq in HSPC isolated from a *Tet2/Stag2* mouse model of MDS previously developed in our lab. We confirmed an increase in accessible chromatin and demonstrated a strong enrichment of predicted Ap-1 binding in intergenic regions, in agreement with our observations in the U937 model. Gene set enrichment analysis in the *Tet2/Stag2*-mutant versus WT HSPC revealed that putative targets of Ap-1 binding were enriched for hematopoietic stem cell gene signatures (Figure 1B) . This is consistent with our observation of clonal expansion of HSPCs in this model, and suggests a mechanism by which novel Ap-1 bound enhancers may facilitate expression of key genes involved in HSPC expansion and development of MDS/AML. To explore whether our findings were applicable to other published models of *Stag2* mutant myeloid neoplasms, we performed TF footprinting using publicly available ATAC-Seq datasets generated in HSPC isolated from *Stag2* and *Runx1/Stag2* conditional knockout mice. We recapitulated increased chromatin accessibility and significant enrichment of cohesin-bound Ap-1 footprints in *Stag2* mutant over WT conditions. Together, these data further validate conservation of *STAG2 KO*-induced AP-1 bound regulatory elements in human and mouse models of MDS and AML.

In summary, we show that *STAG2* loss leads to the establishment of a novel set of accessible intergenic regions with features of enhancer activity *in vitro* and *in vivo*. We identify multiple members of the AP-1 complex with enriched predicted binding at these putative enhancers, and demonstrate that AP-1 may drive expression of HSPC signatures in models of *Stag2* mutant MDS *in vivo*. We illustrate here a potential role for the AP-1 complex in cohesin mutant myeloid malignancies, which we are currently functionally interrogating.

Disclosures No relevant conflicts of interest to declare.

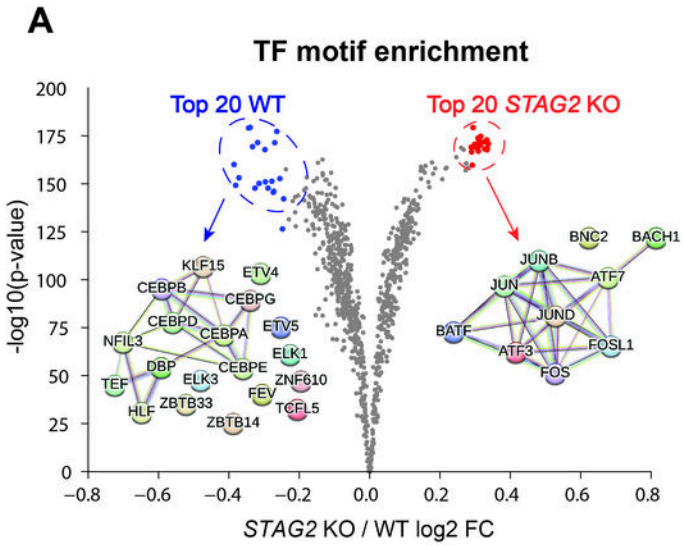

Figure 1A. Results of TOBIAS on *STAG2* KO compared to WT U937 cells and associated STRING interaction networks.

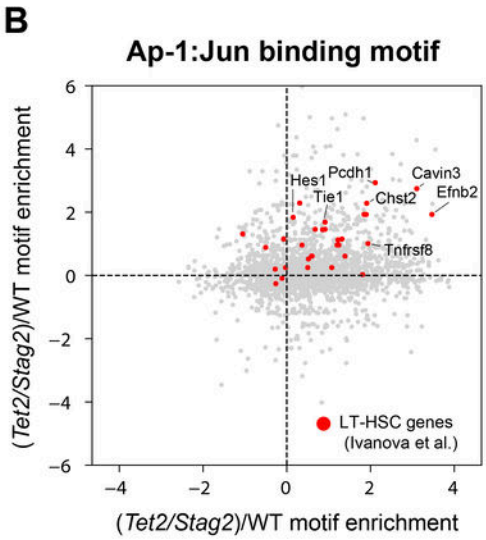

Figure 1B. TF footprint enrichment and regulated gene expression for a representative Ap-1 family motif.

Figure 1

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