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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Synergy of Stag2-Cohesin Loss Results in Expansion of Npm1c-Mutant Hematopoietic Stem and Progenitor Cells

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STAG2 is a member of cohesin complex that is recurrently mutated in >10 cancers and is essential in maintaining the integrity of the 3D genome partitioning structure known as topologically structural domains (TADs). Our previous work has demonstrated that depletion of various cohesin factors, including Stag2, leads to increased hematopoietic stem and progenitor population (HSPC) self-renewal and myeloid-biased differentiation. Loss of Stag2 leads to impaired sub-TADs and affects key hematopoietic transcription factors, such as PU.1, to access and engage their target genes. Yet, the extent and cooperative transformational role of Stag2-cohesin with leukemia driver mutations, such as Npm1c, remains unexplored.
To determine whether Stag2-cohesin regulates leukemic 3D chromatin organization, we generated dual Stag2 $^{\Delta}$ Npm1 $^{c/+}$ murine models with tamoxifen inducible UbcCreER ^{T2}. After 4 weeks, HSPCs (LSK cells; Lin-Sca1+Kit+) are increased in Stag2 $^{\Delta}$ Npm1 $^{c/+}$ double mutant mice (**Figure 1A**). Within LSK, Stag2 $^{\Delta}$ Npm1 $^{c/+}$ but not Npm1 $^{c/+}$ has marked expansion of the myeloid bias MPP2 (LSK+Flk2-Cd150+Cd48+) and MPP3 (LSK+Flk2-Cd150-Cd48+) compartment. Interestingly, Stag2 $^{\Delta}$ single mutant exhibit LSK and MPP3 expansion at 16 weeks, suggesting the early expansion of HSPC compartment is the result of Stag2 $^{\Delta}$Npm1 $^{c/+}$ synergistic acceleration. No difference in serial replating of LSK and GMP (LSK-Cd34+FcyRII+) cells were observed between Npm1 $^{c/+}$ and Stag2 $^{\Delta}$ Npm1 $^{c/+}$. However, Stag2 $^{\Delta}$ Npm1 $^{c/+}$ LSK cells had impaired reconstitution capacity and myeloid biased output in primary and secondary transplantation. To determine if the blockage is specific to Stag2-cohesin loss, we generated the Smc3/Npm1c double mutant mice, where both Stag1-cohesin and Stag2-cohesin complexes were partially depleted upon heterozygous deletion of Smc3. Interestingly, there were no changes in LSK or MPP3 population in Smc3 $^{+/-}$ Npm1 $^{c/+}$ mice, suggesting it is specific to Stag2-cohesin loss which obstructs differentiation in Npm1 $^{c/+}$ HSPC.
Mechanistically, we performed bulk ATAC-seq on sorted HSC ^{LT} (LSK+Flk2-Cd150+Cd48-) and MPP3 population, as poor long-term reconstitution suggests functional alteration in HSC ^{LT}. Comparing to WT, both Stag2 $^{\Delta}$ and Stag2 $^{\Delta}$ Npm1 $^{c/+}$ HSC ^{LT} have increased chromatin accessibility, while only Stag2 $^{\Delta}$ Npm1 $^{c/+}$ MPP3 have persistent opening of chromatin. Motif analysis of increased accessible peaks identified RUNX2, GATA3 and MEIS1 are among the increased accessible region in Stag2 $^{\Delta}$ Npm1 $^{c/+}$ MPP3, which reflects the increased myeloid output in the transplantation. Recently, various group have suggested that Npm1c directly binds to chromatin and drives the overexpression of genes, especially at HOX clusters and MEIS1. This suggests a possible synergistic mechanism which Stag2-cohesin regulates chromatin accessibility of key TF loci, which influences normal and leukemic stem and progenitor differentiation. To comprehensively profile the LSK population, we performed scRNAseq at 4 weeks post mutation activation, results showed a marked immune pathway activation in Stag2 $^{\Delta}$ Npm1 $^{c/+}$ LSK cells. To inter cell fate transition, we employed CellDancer, an RNA velocity based algorithm and found that Stag2 $^{\Delta}$ Npm1 $^{c/+}$ MPP cells have altered differentiation trajectory and delayed kinetics at MPP3 stages, suggesting a differentiation block (**Figure 1B**). In conjunction with our results, we postulate that Stag2-cohesin regulates TF interactions with regulatory elements during cell fate transitions. We are currently investigating the direct interaction of Stag2-cohesin with regulatory elements in Npm1 $^{c/+}$ cells and compare to WT and Stag2 $^{\Delta}$ Npm1 $^{c/+}$ cells. We will investigate the 3D genome organization of Npm1 $^{c/+}$ and Stag2 $^{\Delta}$ Npm1 $^{c/+}$ cells to determine whether Stag2 loss induces the phenotypic changes via Ctcf-dependent or independent manner.

Disclosures Viny: *Arima Genomics:* Membership on an entity's Board of Directors or advisory committees.

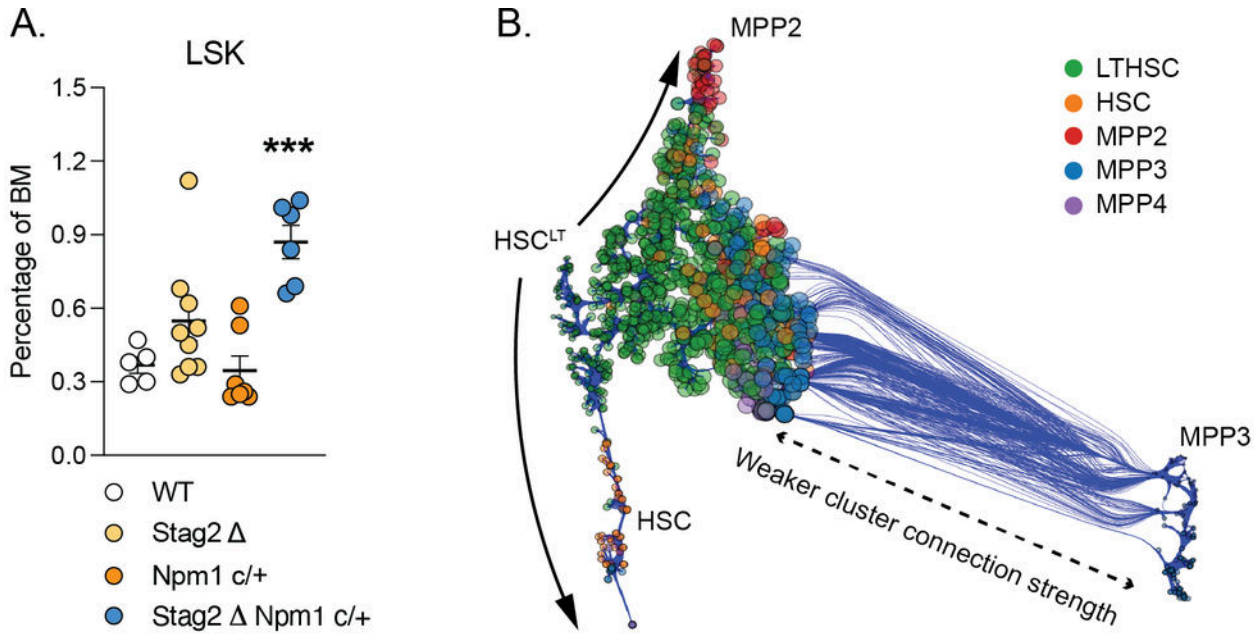

Figure 1 Stag2$^{\Delta}$Npm1$^{c/+}$ leads to expansion of LSK cells and altered MPP differentiation kinetics. (A) Immunophenotyping of LSK population at 4 weeks post mutation activation. (B) CellDancer analysis of LSK scRNAseq shows trajectory and pseudotime transition of Stag2$^{\Delta}$Npm1$^{c/+}$ HSPC.

Figure 1

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