Title: Bayesian networks modeling identifies a reliance of TP53 mutant AML on NF kappa B signaling

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In AML, TP53 mutations (TP53Mut) confer the worst prognosis. Leukemia stem cells (LSCs) are AML cells endowed with self-renewal capacity, allowing them to recapitulate leukemia after therapy and cause relapse. In human AML, the pathways that mediate self-renewal in human TP53Mut AML are not well-known.

To define the signaling pathways activated in TP53Mut human LSCs, we used CyTOF, a form of flow cytometry that can measure 40+ proteins sat single-cell resolution. We compared the levels of activated signaling molecules in the LSC-enriched (CD34+CD38-) subset of TP53Mut (n=7) and TP53WT (n=7) AML samples. Phosphorylated NF kappa B (pNFkB), pSTAT1, and pP38 are significantly higher in TP53Mut AMLs. In contrast, Ki67 and pMAPKAPKII are higher in TP53WT AML. These data demonstrate a unique signaling activation state in TP53Mut AML.

The influence of signaling molecules on downstream targets can vary by cellular context. Therefore, we asked whether the interaction of the activated signaling molecules vary between TP53Mut and TP53WT LSCs. We performed Bayesian networks, a machine learning algorithm that can be used to discover statistical correlations and dependencies between signaling molecules. We found that TP53Mut model showed pNFkB have strong influences on pSTAT1, pERK, and beta-catenin levels. Furthermore, Bayesian networks has modeled pNFkB as a central signaling hub. Next, we compared the transcriptomic profiles of TP53Mut and TP53WT samples in the BEAT AML dataset and found significant enrichment of all the NFkB gene sets (n=19) in the TP53Mut samples, providing more evidence for a unique reliance of TP53Mut human AML on NFkB signaling.

Therefore, we used shRNA constructs to determine the influence of TP53Mut on signaling in human AML. We found that knockdown of TP53Mut reduced NFkB protein levels in Kasumi AML cells (TP53Mut) but not in MOLM13 AML cells (TP53WT). RNA sequencing of transduced Kasumi cells showed that TP53Mut knockdown led to loss of NFkB and LSC self-renewal signatures. Knockdown of TP53Mut in primary human TP53Mut AMLs led to a similar loss of NFkB and LSC signatures in these primary samples as well. Colony formation in Kasumi cells and TP53Mut primary human AML were also abrogated after TP53Mut knockdown. These data show TP53Mut promotes NFkB activation in human AML and suggests that human TP53Mut LSCs may be dependent on NFkB for self-renewal. These data implicate NFkB as a possible therapeutic strategy to target TP53Mut human LSCs.