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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Inhibiting Mitochondrial RNA Degradosome Complex SUV3 and PNPT1 Increases dsRNA in the Cytoplasm, Triggers Viral Mimicry Response and Sensitizes AML Cells to Immune Mediated Killing

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Eukaryotic cells have two separate genomes; nuclear DNA organized in chromosomes, and circular mitochondrial DNA located within mitochondria. Mitochondrial DNA is comprised of a double-stranded circular genome that is 16.6 kB in length, lacks introns, and encodes two rRNAs, 22 t-RNAs and 13 of the 90 proteins in the mitochondrial respiratory chain. To maintain homeostasis, mitochondria possess RNA degradation machinery to regulate mitochondrial RNA turnover. The ATP-dependent helicase, SUV3 (encoded by the gene SUPV3L1) and the exonuclease PNPase (encoded by the gene PNPT1) function in a complex to degrade mitochondrial dsRNA.

We identified PNPT1 and SUPV3L1 through an unbiased in-silico screen using bioinformatics platform to identify novel targets in AML. Our analysis revealed a strong correlation between these genes and ontologies of exogenous dsRNA, response to viruses, and RNA catabolic processes.

PNPase and SUV3 protein were increased in 7/7 AML patient samples and 10/10 of AML cell lines compared to the normal hematopoietic cells by immunoblotting. Analysis of the TARGET AML dataset revealed that AML patients with increased expression of SUPV3L1 ($p = 0.051$, $p= 0.045$) and PNPT1 ($p = 0.0013$, $p = 0.018$) had decreased overall survival and event-free survival respectively.

We knocked down or knocked out PNPT1 and SUPV3L1 with shRNA or sgRNA in AML cells, to study the importance of these genes in AML. Genetic knockdown or knockout of PNPT1 or SUPV3L1 decreased the growth and viability of OCI-AML2, TEX, K562, U937, NB4 and 8227 leukemia cells. Moreover, SUPV3L1 & PNPT1 ranked top 5.2% and 7.4% of essential genes in 26 leukemia cell lines in CRISPR screens and 2.7% and 4.9% in RNAi screens (https://depmap.org/portal). Knockdown of PNPT1 & SUPV3L1 also reduced the clonogenic growth of AML cells.

Demonstrating the functional importance of PNPT1 & SUPV3L1 on leukemia-initiating cells in vivo, genetic knockdown of PNPT1 and SUPV3L1 significantly reduced engraftment of TEX cells into the marrow of immune-deficient mice. Finally, primary AML cells with SUPV3L1 knockdown had reduced engraftment into the marrow of immune-deficient mice. Whereas, the knockdown of SUPV3L did not alter the primary engraftment of CD34+ enriched cord blood cells.

Mechanistically, knockdown of PNPT1 and SUPV3L1 in OCI-AML2 cells increased levels of cytoplasmic dsRNA 3-4 fold compared to control. Knockdown of PNPT1 and SUPV3L1 also increased cytoplasmic dsRNA in 143B cells, but not Rho(0) 143B cells that lack mitochondrial DNA, demonstrating a mitochondrial source for the increased dsRNA. Increased cytoplasmic dsRNA can mimic viral infection and trigger a type 1 Interferon response. Knockdown of PNPT1 or SUPV3L1 increased expression of genes (INFgR1, ICAM, IRF7 & JAK/STAT) associated with a type 1 interferon response compared to control. We also observed that PNPT1 and SUPV3L1 knockdown in AML cells sensitized the leukemic cells to T-cell mediated killing upon

co-culturing with double negative T cells. In addition, using an immunocompetent Balbc mouse model, we demonstrated endogenous T cells were required to observe the greatest reduction in tumor burden after SUPV3L1 knockdown.
In summary, the mitochondrial RNA degradosome complex SUPV3L1 and PNPT1 are overexpressed in AML and are essential for AML cells and stem/progenitors. These enzymes regulate the levels of mitochondrial dsRNA and their inhibition leads to a viral mimicry response and heightened sensitivity to immune-mediated killing.

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