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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

UBE2N Regulates Oncoprotein Networks in Myeloid Malignancies

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Acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) originate from clonal mutant hematopoietic stem and progenitor cells, referred to as leukemic stem and progenitor cells (LSPCs). Ubiquitination is an essential process for various cellular maintenance functions, including protein degradation and scaffolding, which is often perturbed in a variety of cancers. Ubiquitination of protein substrates involves three related enzymes: the E1 activating enzyme, the E2 conjugating enzyme, and the E3 ligase. In humans, there are up to 40 E2 conjugating enzymes, among which Ubiquitin conjugating enzyme E2 N (UBE2N) stands out as one of the most crucial and overexpressed E2 enzymes in AML cells. Our recent study reported that UBE2N is a druggable target in MDS and AML (Barreyro et al., Science Translational Medicine, 2022), and we found that its catalytic active site cysteine 87 is required for various AML mouse models, including MLL-AF9, FLT3-ITD, AML1-ETO9a, and MN1 (unpublished). These observations indicate that the catalytic function of UBE2N is critical for AML cells but dispensable for normal hematopoiesis.

To examine the active state of UBE2N, we investigated the "charged" version of UBE2N, which contains a ubiquitin bound to cysteine 87, in normal hematopoietic cells and in a AML cell line panels. Our findings confirmed that the activated form of UBE2N is highly expressed in human AML cells compared to normal hematopoietic cells. UBE2N exclusively synthesizes Lysine-63 (K63)-linked polyubiquitin chains on target proteins in conjunction with selected E3 ligases, leading to the stabilization and/or activation of protein substrates. To identify the relevant targets of UBE2N in AML, we conducted a ubiquitin-enrichment followed by a proteomic analysis in isogenic UBE2N-deficient AML cells compared to WT AML cells. We identified ~300 proteins that are ubiquitinated by UBE2N. Importantly, UBE2N regulates a network of leukemia-associated oncoproteins, including SYNCRIP, HUWE1, NPM1, TYK2, IKBKG, VAV1, BAX, IGF2BP2, IRF4, IRAK4, RBMX, STAT3, and BTK.

UBE2N can interact with various E3 ligases in a cell- and context-dependent manner. To gain insight into the major E3 ligase that cooperates with activated UBE2N in AML, we performed a proximity-based labeling assay followed by mass spectrometry. This interactome screen identified Tripartite motif containing 21 (TRIM21) as the top E3 ligase that partners with UBE2N in AML cells. TRIM21 is overexpressed in AML patients compared to healthy controls. Moreover, deletion of TRIM21 in AML cells impaired cell viability and proliferation in vitro and prolonged survival in a xenograft model in vivo, which phenocopies the loss of UBE2N. Importantly, restoration of TRIM21 rescued the functional defect of UBE2N-deficient AML cells, indicating that the UBE2N-TRIM21 axis is operational in AML.

To prioritize the UBE2N-dependent oncoprotein network, we focused on UBE2N-TRIM21 regulated signaling in AML. For this, a gene expression analysis was performed in UBE2N-deficient AML cells that were restored with TRIM21. The top UBE2N-TRIM21 oncoprotein network that emerged was related to interferon-STAT signaling. Additionally, both UBE2N and TRIM21 were found to regulate STAT3 ubiquitination. Deletion or inhibition of UBE2N catalytic activity significantly reduced total and activated STAT3 in AML cells. Finally, the expression of constitutively active STAT3 partially restored the functional defect of AML cells treated with a UBE2N inhibitor, indicating that STAT3 function, a known driver of LSPCs, is regulated via the UBE2N-TRIM21 axis in AML.

In summary, our data reveal that UBE2N regulates oncoprotein networks in AML and that inhibiting UBE2N catalytic function, such as with small molecule inhibitors, effectively suppresses LSPCs.

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