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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Ubiquitin Ligase SCF-FBXO11 Controls a Network of RNA-Binding Proteins and Splicing in MDS

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The F-Box protein FBXO11 is a substrate receptor of Skp-Cullin-F-Box (SCF) ubiquitin ligase complexes. Loss-of-function mutations in *FBXO11* are associated with diffuse large B-cell lymphoma, and FBXO11 has been shown to regulate epigenetic drivers of erythroid cell maturation. In our prior study, we found that deletion of *FBXO11* improved cell survival under cytokine deprivation in an MDS cell line. Furthermore, we had observed a striking decrease in FBXO11 protein levels in AML patient samples. However, how FBXO11 contributes to myeloid malignancies is unclear. Here, using RNA-sequencing, proteomics, and CRISPR-Cas9 screening, we reveal broad ubiquitylation of RNA-binding proteins (RBPs) and alternative splicing as a mechanism by which deregulation of FBXO11 enhances malignant cell function. In addition, we report the presence of mutations in the uncharacterized N-terminal extension of FBXO11 in both lymphoid and myeloid malignancies.

We first observed that heterozygous deletion of *FBXO11* increases colony-forming ability of MDS-AML cell lines *in vitro* whilea2-fold increase in expression results in decreased cell growth and apoptosis. In a transplant model with a retrovirally-driven *Runx1* mutation, heterozygous deletion of *Fbxo11* exacerbated neutropenia and shortened overall survival. However, depletion of FBXO11 in healthy human and murine HSPCs significantly impaired myeloid progenitor colony-formation, indicating a unique requirement for FBXO11 in myelopoiesis. Based on its tumor suppressive activity, we evaluated whole-exome sequencing data from patient samples of both lymphoid and myeloid malignancies for *FBXO11* mutations. This analysis uncovered rare mutations in *FBXO11* in myeloid malignancies and revealed a substantial cluster of previously unidentified mutations in the Q-rich, N-terminal extension of FBXO11. We found that this uncharacterized, extended protein isoform is predominantly expressed in both normal and malignant hematopoietic cells. In colony-forming assays, expression of the long isoform and several of the N-terminal mutations inhibited CD34+ progenitor function. On a cellular level, lack of this N-terminal extension in the short isoform resulted in aberrant subcellular localization of FBXO11 within the nucleus.

To elucidate the mechanism of FBXO11 in myeloid malignancy, we performed immunoprecipitation of FBXO11 followed by proteomics to identify endogenous FBXO11 complexes in MDS-AML cells. We integrated these data with ubiquitin proteomics of WT and *FBXO11*-KO MDS cells to identify candidate substrates of SCF-FBXO11-mediated ubiquitylation. FBXO11 complexes are significantly enriched for RNA-binding proteins affecting mRNA splicing, a process commonly deregulated in MDS. *FBXO11*-KO MDS cells displayed broad depletion of ubiquitylation in a network of RBPs. As expected, targeting FBXO11 did not affect the level of transcripts of interacting proteins; however, there were variable changes in total protein levels indicative of post-transcriptional regulation. We then applied a focused CRISPR-Cas9-KO screen of candidate SCF-FBXO11 substrates in MDS-AML cells depleted of FBXO11. We observed significant bi-directional effects in colony-forming ability mediated by several of the FBXO11-regulated RBPs including TRIM28, HNRNPU, NPM1, and SYNCRIP. Though *FBXO11* is significantly decreased in MDS compared to healthy controls, we saw no difference in *FBXO11* expression between splicing factor WT and mutant samples, suggesting that the function of FBXO11 in regulating mRNA splicing in MDS is independent of these mutations. Compared to healthy controls, *FBXO11*-low MDS samples exhibited a greater number of alternative splicing events, predominantly in skipped or mutually-exclusive exons. To experimentally test whether FBXO11 indeed affects RNA splicing, we utilized a bichromatic splicing reporter assay in MDS-AML cells which showed that depletion of FBXO11 is sufficient to alter splicing of the reading frame in the cassette exon, read out as a shift from GFP to RFP. Finally, STRING

protein network analysis revealed that the alternative splicing events occurring in FBXO11-low MDS patient samples affect key nodes in protein translation and metabolism.
Collectively, our data support a model whereby the change in FBXO11-mediated ubiquitylation of RBPs drives alternative exon usage that exacerbates malignant cell function in MDS.

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