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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

The Exportin, CSE1L, Regulates Ribosome Biogenesis and Is a Selective Dependency in Childhood and Young Adult Acute Myeloid Leukemia

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Nuclear transport receptors, termed importins and exportins, regulate protein transport through the nuclear pore. Although genetic alterations in the nuclear pore complex are more common in young AML patients (e.g., NUP98 rearrangements), nucleocytoplasmic transport has not been studied extensively in childhood/young adult AML. We compared the expression of genes associated with nucleocytoplasmic transport in young (<40 years; mean age 27 in BeatAML & 29 in TGCA) and older (>40 years, mean age 64 in BeatAML & 61 in TCGA) AML patients and identified enrichment of nucleocytoplasmic transport pathways in childhood/young adult vs older AML (BeatAML P=2.43e-3, FDR=0.01, TCGA P=2.39e-4, FDR=3.46e-3). Young patients with nucleoporin (NUP) rearrangements had high expression of nucleocytoplasmic transport genes, but this pathway was also upregulated in young patients without NUP rearrangements, compared to older patients.

Of the nucleocytoplasmic genes, *CSE1L* was the top differentially expressed exportin in young vs older AML patients. *CSE1L* is an exportin with a role in recycling importin alpha proteins. Recently, *CSE1L* was found to export circular RNA. Its function in childhood/young adult AML is unknown. By immunoblotting, we confirmed increased expression of CSE1L protein in childhood/young (n=10, mean age 23) vs older (n=10, mean age 77) AML. *CSE1L* mRNA expression predicted outcome exclusively in young patients (<40 years), where increased *CSE1L* predicted for decreased remission rates (P=0.027), event free survival (P <0.0001) and overall survival (P<0.0001), but was not predictive in older patients (TARGET, Beat AML) .

To further understand the dependency of childhood/young adult AML on *CSE1L*, we knocked down the gene with shRNA in AML cell lines derived from young patients (NB4, MV4-11, THP-1) and TEX cells, derived from transducing cord blood with a high-risk pediatric fusion, *FUS-ERG*. *CSE1L* knockdown reduced AML growth, viability and clonogenicity. We confirmed loss of AML viability and clonogenicity using *CSE1L* CRISPR knockout. Gene dependency scores indicated a greater reliance on *CSE1L* than *XPO1* in young AML cell lines (P<0.0001). Knockdown of *CSE1L* in primary AML cells from a young chemo-refractory patient with high *CSE1L* expression, reduced leukemic engraftment into mouse marrow. *CSE1L* depletion also reduced AML engraftment in secondary transplants, demonstrating its essentially for leukemic stem cells. In contrast, knockdown of *CSE1L* did not impair engraftment of normal human hematopoietic cells into mouse marrow.

To understand the function of *CSE1L*, we interrogated a BioID database (PXD007976) and identified proteins that interact with *CSE1L*. Among the *CSE1L* interactors, ribosome biogenesis was the top biologic process identified. We validated the interaction with ribosome biogenesis proteins and BioID hits, RRS1 and RPL29 using Co-IP and proximity ligation assay. Knockdown of *CSE1L* in TEX cells led to nuclear accumulation of RPL29 and a reduction in cytoplasmic RPL29 (3.3 fold). Using a GFP reporter assay that incorporates into the large ribosomal subunit precursor (pre-60S), we induced depletion of *CSE1L* and confirmed decreased cytoplasmic RPL29-GFP. Pre-ribosome export is closely coordinated with rRNA maturation and pre-ribosome assembly. *CSE1L* knockdown decreased the expression of pre-60S and ribosome biogenesis pathways and quantitively reduced rRNA transcription rates by PCR (3 fold), consistent with a role for CSE1L in large ribosome subunit export and ribosome biogenesis.

Given increased *CSE1L* expression in young patients and its role in ribosome biogenesis, next we investigated ribosome biogenesis in AML patients. Ribosome biogenesis pathways were the top differentially expressed gene sets in young vs older

AML patients (BeatAML P=1e-10, FDR=7.9e-8, TCGA P=2.34e-4, FDR=3.4e-3). Expression of ribosome biogenesis pathways positively correlated with expression of the nucleocytoplasmic transport pathway.
In summary, nucleocytoplasmic transport and ribosome biogenesis are upregulated in young AML patients. The nuclear exportin, *CSE1L*, is a regulator of ribosome biogenesis and its depletion leads to impaired ribosome biogenesis and leukemic cell death in childhood/young adult AML. Thus, our findings reinforce growing evidence that childhood and older adult AML are fundamentally different diseases, driven by different biologic processes.

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