ELSEVIER

Blood 142 (2023) 143–144

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

m6A Mediated Ribostasis of RNA Stress Granule Assembly Governs Blood Development and Regeneration

Rajesh D. Gunage, PhD[1,2], *Leonard I. Zon, MD*[3,4,2]

[1] Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital and Dana Farber Cancer Institute, Boston, MA
[2] Harvard Medical School, Boston, MA
[3] Harvard Stem Cell Institute, Cambridge, MA
[4] Howard Hughes Medical Institute, Boston, MA

Hematopoietic stem cell (HSC) form diverse cell types and requires transcription and translation regulation. Using CD34+ human hematopoietic stem and progenitor cells (hCD34+) combined with N6-methyladenosine (m6A) sequencing, we identified a novel regulatory mechanism via stress granule regulation during erythropoiesis. Our single-nucleotide resolution m6A analysis of the whole transcriptome identified ~4800 transcripts to be m6A modified and showed demethylation (>2-14 fold) during erythropoiesis. Genome-wide CHIP-seq analysis of hCD34+ cell erythropoiesis with anti-Gata1 showed an enhancer enrichment at the *ALKBH5* RNA demethylase gene. Using *ALKBH5* CRISPR Cas9 KO strategy combined with hCD34+ cell in-vitro differentiation protocol we observed a block in hCD34+ cell erythropoiesis thus validating Alkbh5 role in erythropoiesis. hCD34+ *ALKBH5* KO cells showed a failure of RNA m6A demethylation and discovered stress granule (SGs) transcripts m6A levels to be upregulated (>2-4 fold, p=0.01). Stress granules are membrane-less organelles composed of various RNA binding proteins. SGs form a hub in the cytoplasm that governs RNA metabolism and global proteome by translational regulation. Our analysis showed *ATXN2*, *G3BP1/2*, *Tia-1*,and *PABPC1* SGs transcripts are m6a modified at 3'UTR. K562 cell *ALKBH5* KO erythropoiesis model led to massive m6A upregulation on *ATXN2* 3'UTR (>14 fold, p=0.001) and other SGs transcripts. Mass spectrometry analysis of hCD34+ *ALKBH5* KO cell proteome showed downregulation of ATXN2(>2 fold, -log P value2), confirming 3' UTR m6A mediated protein synthesis regulation. *ALKBH5* KO hCD34+ cells showed aggregation of SGs protein in the cytoplasm due to altered SG protein stoichiometry(>4-10 fold, p=0.001). Zebrafish alkbh5 KO showed defective blood development and anemia. STED microscopy of the whole kidney marrow of alkbh5 KO fish showed the presence of G3BP and ATXN2 SGs with m6A labeled RNAs. *alkbh5* KO zebrafish blood and kidney marrow analysis also showed increased myeloid and lymphoid lineages indicating early lineage bias. We used the *alkbh5* KO zebrafish model and subjected it to phenyl hydrazine-induced anemia to study its role in stress-induced regeneration. Using FACS analysis of whole kidney marrow, we show red cell recovery failure in alkbh5 KO zebrafish models. Temporal analysis of alkbh5 KO marrow cells during PHZ-induced acute anemia and regeneration showed a systemic failure to resolve SGs compared to controls. We confirmed these results in the mouse *ALKBH5* KO model establishing the role of ALKBH5 in stress erythropoiesis. Furthermore, hCD34+ *ATXN2* KO combined with Methocult-colony formation assay showed defects in erythroid and myeloid lineages. Super-resolution microscopy of hCD34+ *ATXN2* KO cells phenocopied the presence of disease-associated ALS (amyotrophic lateral sclerosis) stress granules with TDP-43, G3BP1/2, PABPC1, and Tia-1 enriched with m6A labeled RNAs. Using the K562 *ALKBH5* KO erythropoiesis model and ATXN2 lentiviral overexpression, we effectively resolved stress granules and blocks in erythropoiesis. In summary, m6A marks on *ATXN2* mRNA regulate SG biology, affecting HSC differentiation and impacting blood development. SG-mediated regulation of HSC fate may highlight new therapeutic strategies for blood disorders.

Disclosures No relevant conflicts of interest to declare.

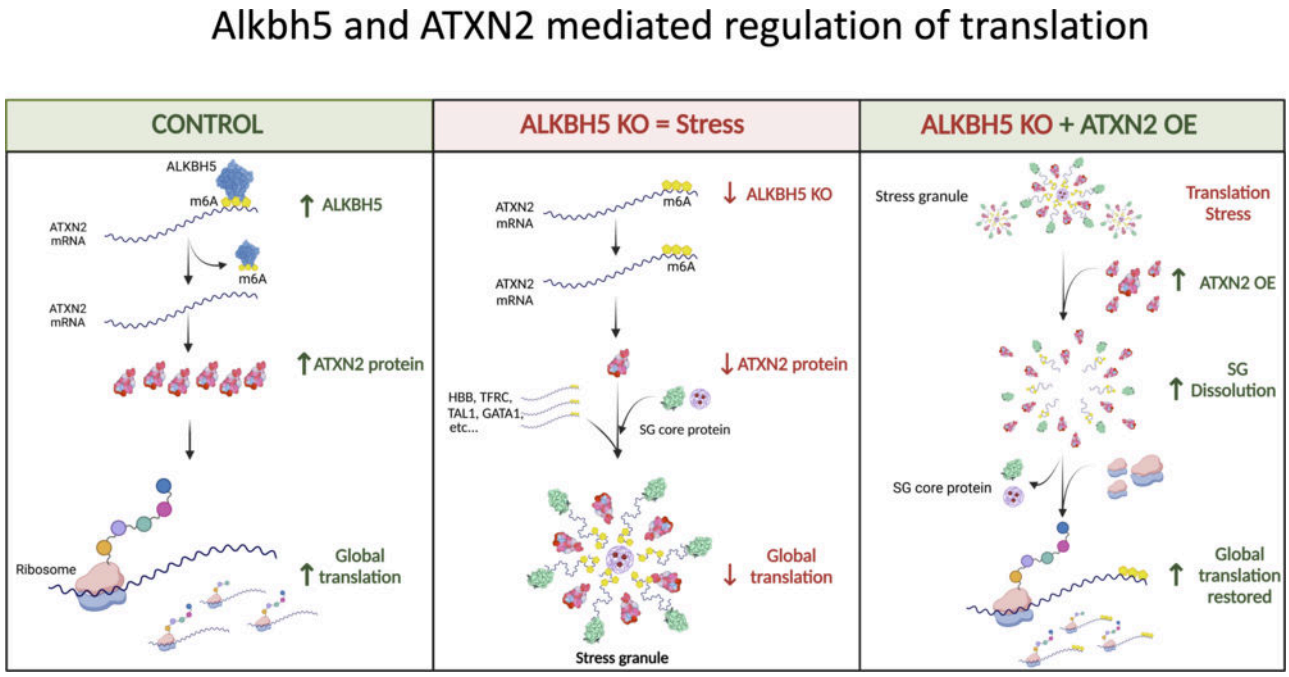

Figure 1

https://doi.org/10.1182/blood-2023-186560