ELSEVIER

Blood 142 (2023) 405–406

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Identifying Stress Granules As Determinants of Leukemia Stem Cell Maintenance and Stress Adaptation

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The development of novel leukemic stem cell (LSC)-targeted therapeutics, which spare hematopoietic stem cells (HSC), is an urgent goal towards reducing the high, LSC-driven, relapse rates in acute myeloid leukemia (AML) patients. Towards elucidating key post-transcriptional networks underlying LSC maintenance we previously performed an in vivo pooled CRISPR dropout screen targeting 128 RNA binding proteins (RBPs) highly expressed in AML LSCs while having low expression in HSCs. This screen highlighted 12 RBPs crucial for murine AML LSC-mediated leukemic growth, of which there was a significant enrichment of stress granule (SG) RBPs. Further, gene set enrichment analysis shows the SG proteome is enriched in human AML LSCs vs non-LSCs while normal HSCs have reduced SG RBP expression relative to multipotent progenitor cells. SGs are dynamic biomolecular condensates, composed of ribonucleoprotein complexes, which may form as a protective mechanism against a variety of stress conditions. SGs are gaining increasing attention as contributors to cancer metastasis and chemoresistance, but their role in LSCs is virtually unexplored.

The intriguing enrichment of SG RBPs in our screen hints at a larger role for SGs in LSC maintenance and/or stress resistance. To address this, we focused on the core SG nucleation RBP, G3BP1, a molecular switch that guides SG assembly. Fluorescence microscopy was first performed to describe SG dynamics in leukemia vs primitive normal cells where interestingly, a subset of patient AML cells contained G3BP1 SGs in the absence of added stress. In contrast, primitive healthy murine stem cells had reduced heat shock-induced SG formation in comparison to downstream normal progenitors. A series of shG3BP1 knockdown experiments performed in human leukemia cell lines and patient AML specimens demonstrated that shG3BP1-transduced cells showed increased apoptosis and differentiation and reduced colony forming capacity and competition in vitro compared to control cells. The same set of assays showed minimal impact of SG impairment to healthy umbilical cord blood cells. Importantly, in vivo xenotransplantation demonstrated that LSCs with G3BP1 knocked down were significantly compromised in their capacity to generate leukemic grafts whereas normal HSCs assessed in a similar manner display much less G3BP1 dependency.

Towards determination of the SG mechanisms essential for bulk AML and LSC regulation, we performed matched RNA-sequencing and proteomics in G3BP1 knocked down AML cells as well as BioID in AML cell lines, primary patient AML cell subsets, and healthy hematopoietic cells to allow for the uncovering of an AML specific SG proteome. These analyses highlighted numerous potential SG mechanisms of cancer cell stress resistance and/or stem cell regulatory processes including sequestration of apoptotic factors and oncogenic signalling pathways. Of note, RNA-seq and proteomics in SG impaired shG3BP1 leukemia cells showed both a negative enrichment of the SG proteome and LSC signature as well as a positive enrichment of the innate immune response. Interestingly, our BioID uncovered that the AML specific SG proteome is also

enriched for positive regulation of RIG-I signaling pathway (GO: Biological Process). Together this data suggests SGs play a protective role through the downregulation of overactive innate immune signalling in AML. Work using optimized small input approaches to map SG RNA and protein interactomes in LSCs vs HSCs to confirm similar regulatory relationships as well as identify LSC-context specific ones is ongoing.
Overall, these results elucidate SGs as key AML LSC stress adaptive regulatory networks and highlights them as novel targets for consideration in AML therapeutics.

Disclosures Dick: *Trillium Therapeutics Inc/Pfizer:* Patents & Royalties: Trillium Therapeutics; *Celgene/BMS.:* Research Funding; *Graphite Bio:* Consultancy, Membership on an entity's Board of Directors or advisory committees.

https://doi.org/10.1182/blood-2023-180919