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Blood 142 (2023) 4119–4120

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

A Potential Link between hnRNP K and Cebpα: Implications for Ribosomal Dysfunction in Acute Myeloid Leukemia (AML)

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Deletion of the long arm of chromosome 9, del(9q), is a recurrent genetic abnormality involving the haploinsufficient loss of the RNA binding protein hnRNPK and is often associated with poor treatment responses in AML. In addition to reduced hnRNP K expression, other critical proteins, not localized to this locus, are also aberrantly expressed in del9q AML. One protein of critical interest is CEBPα, a well-established myeloid differentiation transcription factor. Recently, CEBPα was discovered to have a novel role in ribosomal RNA (rRNA) synthesis. However, as opposed to CEBPα, hnRNP K's role in RNA stability, rRNA processing, and ribosomal functions is already firmly rooted. Collectively, these results suggest that del(9q) AML dysregulations in hnRNP K and CEBPα expression may have a multi-faceted influence on ribosomal biogenesis and protein translation. Previously, we used animal models and patient samples to demonstrate that *Hnrnpk* haploinsufficiency alters CEBPa expression, suggesting that reduced hnRNP K expression contributes to decreased CEBPα levels and activity. These findings led us to hypothesize that these distinct, yet interconnected proteins, play a cooperative role in regulating rRNA synthesis in two major ways. Firstly, hnRNP K directly governs rRNA processing and ribosomal loading. And secondly, via hnRNP K's mediated regulation of the nucleolar-localized isoform of CEBPα (p42 isoform), which was recently shown to enhance recruitment of RNA polymerase I to rRNA genes.

To explore these relationships, we used AML patient samples, *Hnrnpk* haploinsufficient animal models, si- and sh- *HNRNPK* AML cell lines, and biochemical analyses. Initially, RPPA, CyTOF, and western blot analyses were used to investigate how reduced hnRNP K levels affect proteins involved in ribogenesis and protein translation. Here, we observed a direct correlation between hnRNP K expression and defects in proteins and pathways involved in protein translation, such as mTOR, phos-S6, and eEF- and eIF-family members, and CEBPa ($\log_{10}$pAdj $< .0001$). Subsequently, we performed polysome assays that revealed 40S and 60S subunit formation was impaired in AML cell lines with reduced hnRNP K levels. Across multiple cell lines, reduced hnRNP K expression directly led to a significant increase in monosomes (unbound/inactive ribosomes; $p < 0.01$) and a significant decrease in polysome-bound RNA ($p < 0.05$) . Critically, *HNRNPK* haploinsufficiency and si- and sh- *HNRNPK* knock down experiments disrupted the abundance and processing of the 47S transcript ($p < 0.05$).

To understand the impact that hnRNP K has on CEBPα in the context of rRNA biology, we performed co-immunoprecipitation of hnRNP K followed by RNA-seq (RIP-Seq) in human AML cell lines KG-1 and KG-1a. 1076 interacting RNAs were identified, which were strongly enriched in transcription factors implicated in myeloid biology, including *CEBPA* ($\log_2$pAdj $< .0001$). Biochemical analyses of sequence-specific motifs conferred direct and functional interactions between hnRNP K and the 5' and 3' UTRs of the human *CEBPA*. These interactions support our in vivo findings that del9q AML and *Hnrnpk* haploinsufficiency lead to reduced *CEBPA* expression, specifically in the p42 isoform, potentially impacting its role in nucleolar localization and rRNA transcription regulation.

Currently, we are evaluating the significance of these interactions at both the biological and translational levels. Using biochemical and cell-based assays, we are evaluating the direct effect that *Hnrnpk* haploinsufficiency has on rRNA abundance

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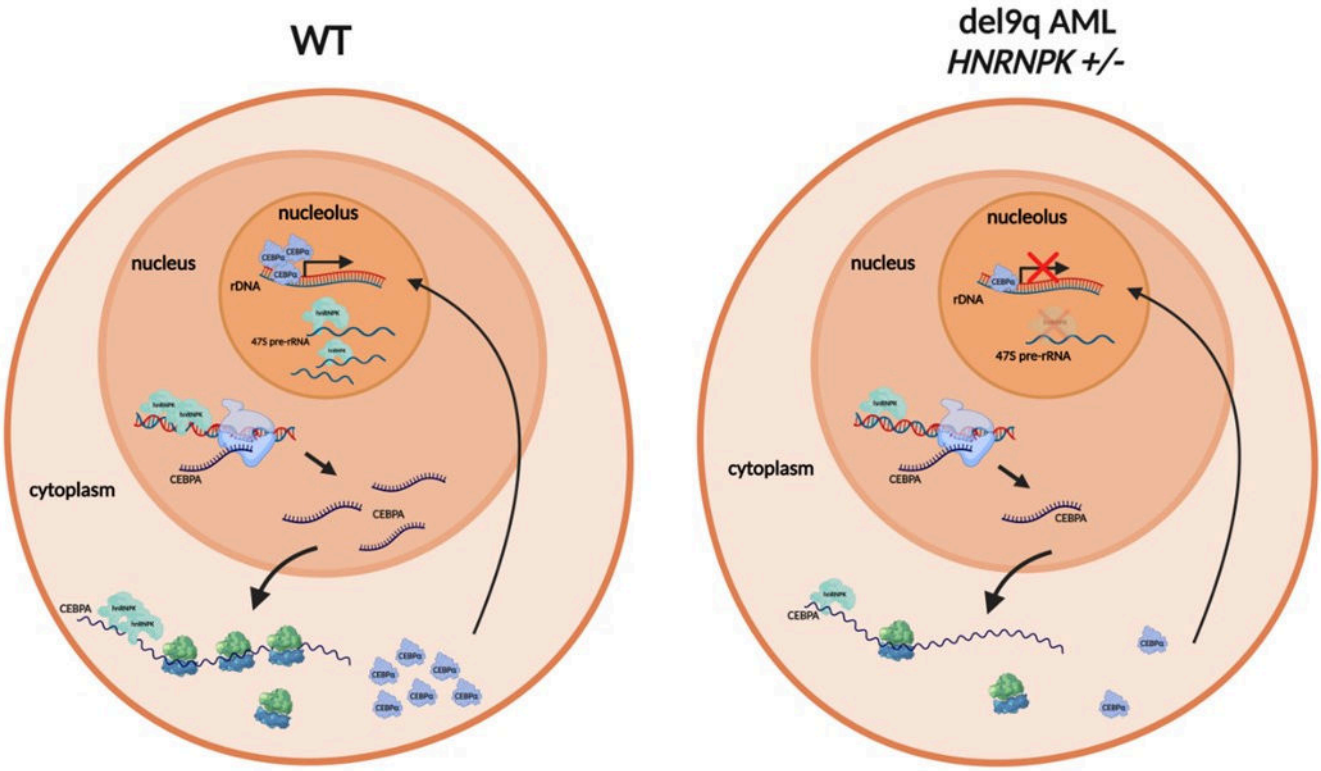

Figure 1: Schema depicting the dysregulated role of hnRNP K in del(9q) AML, impacting *CEBPA's* control of rRNA synthesis. (Left) In the wild-type setting, hnRNP K interacts with the *CEBPA* transcript, increasing overall CEBPα levels, resulting in enhanced 47S pre-rRNA expression. (Right) In del(9q) AML, hnRNP K levels are diminished, leading to decreased CEBPα levels and a subsequent reduction in 47S pre-rRNA.

Figure 1

and on the processing of ITS products in the context of *CEBPA*. Through the use of viability screens, we are examining the efficacy of inhibitors of protein translation, translation initiation, and RNA Pol I on leukemic cells with 9q deletions of reduced hnRNP K expression.

Altogether, these findings identify an uncharacterized relationship between del9qAML (*HNRNPK* haploinsufficiency), CEBPα expression, and ribogenesis, providing valuable insight towards mechanisms driving this subtype of leukemia. Collectively, these data also suggest that del9q AML represents a novel and currently uncharacterized ribosomopathy.

Disclosures Post: *LinBioscience:* Research Funding. **Crysandt:** *Pfizer:* Other: Travel grant.

https://doi.org/10.1182/blood-2023-190737

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