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PB1813 REGULATORY MECHANISMS AND THERAPEUTIC POTENTIAL OF THE DLK1-DIO3 LOCUS IN PEDIATRIC ACUTE MEGAKARYOBLASTIC LEUKEMIA

Topic: 3. Acute myeloid leukemia - Biology & Translational Research

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Background:

Non-coding RNAs (ncRNAs) are emerging as potential therapeutic targets in pediatric acute megakaryoblastic leukemia (AMKL) due to their cell type and tissue-specific expression patterns. The *DLK1-DIO3* locus is highly expressed in megakaryocytes, and AMKL. Knockdown of several members of this locus has been shown to impair megakaryopoiesis. However, the functional role of the *DLK1-DIO3* locus in normal hematopoiesis and AMKL remains poorly understood.

Aims:

In this study, we aimed to investigate the regulatory mechanisms underlying the expression of the *DLK1-DIO3* locus in normal megakaryopoiesis and AMKL, and to explore its therapeutic potential in AMKL.

Methods:

We utilized various techniques to examine the regulatory layers controlling the *DLK1-DIO3* locus. CUT&RUN was performed for the key megakaryocytic transcription factor GATA1 and its truncated isoform GATA1s in CMK cells with inducible expression of these factors. We used peak calling to identify GATA1/GATA1s binding regions in the *DLK1-DIO3* locus. We also screened GATA1 binding regions and CpG islands of *MEG3* for enhancer activity using the dual luciferase assay. To investigate the therapeutic potential of this locus, we used CRISPR/Cas9 to target GATA1/GATA1s binding regions in AML cell lines and analyzed changes in cell proliferation capacity and *DLK1/MEG3* expression using FACS analysis and qPCR.

Results:

We found that GATA1 and GATA1s occupy several genomic locations upstream of *DLK1* and *MEG3* in CMK cells. We also identified enhancer regions within these GATA1/GATA1s binding regions, with three of these enhancer regions driving strong luciferase expression. In addition, a combination of several CpG islands attributed to *MEG3* was required to drive strong luciferase expression. Targeting the GATA1/GATA1s binding regions using CRISPR/Cas9 resulted in decreased proliferation capacity and downregulation of *DLK1* and *MEG3* expression in AML cell lines.

Summary/Conclusion:

Our study sheds light on the regulatory mechanisms underlying the *DLK1-DIO3* locus in normal megakaryopoiesis and AMKL. We identified GATA1/GATA1s as key regulators of this locus and demonstrated the potential therapeutic benefits of targeting this locus in AMKL. Our findings provide a foundation for further research to explore the clinical application of this locus as a novel therapeutic target in AMKL.