HiChIP Reveals Enhancer Hijacking as an Oncogenic Driver of Ambiguous Leukemia

Transcriptome and genome sequencing study demonstrates that an enhancer hijacking mechanism drives oncogenic *BCL11B* expression in lineage ambiguous leukemia.

Acute leukemia is the most common childhood cancer, arising from developing hematopoietic cells[1]. With two main lineages, myeloid and lymphoid, most leukemias are diagnosed based on their immunophenotypic similarity to normal hematopoietic cells.

A subgroup of leukemias collectively known as acute leukemias of ambiguous lineage (ALAL), thought to arise in immature stem and progenitor cells, do not follow conventional identification patterns. They may express no lineage defining markers, lymphoid markers, or both myeloid and lymphoid markers. This ambiguous factor makes ALAL challenging to diagnose and treat[2]. Researchers from the Mullighan lab in St. Jude Children's Research Hospital set out to understand the biological heterogeneity among ALAL and the molecular drivers of their lineage plasticity.

Montefiori et al. conducted a large pan-acute leukemia analysis study to define the genomic basis of ambiguous leukemias. Using gene expression profiling, this study identified a new subtype of lineage ambiguous acute leukemia, defined by structural variants (SVs) targeting the T cell transcription factor gene *BCL11B*. Data compiled from RNA-seq of over 2,500 transcriptomes identified a subset of cases exhibiting monoallellic expression of *BCL11B*. The majority of *BCL11B* structural alterations were in noncoding regions of the genome, with breakpoints up or down-stream of the gene. Most ALAL patients had rearrangements to genes on chr6 near the *ARID1B* locus. Normally, *BCL11B* expression is majorly restricted to T cells, but the expression data showed hematopoietic stem and progenitor cells (HSPCs) as the cell of origin for these leukemias. This was an interesting find as *BCL11B* expression is normally repressed in HSPCs. Based on this, the researchers hypothesized that the observed SVs served to ectopically activate *BCL11B* through enhancer hijacking.

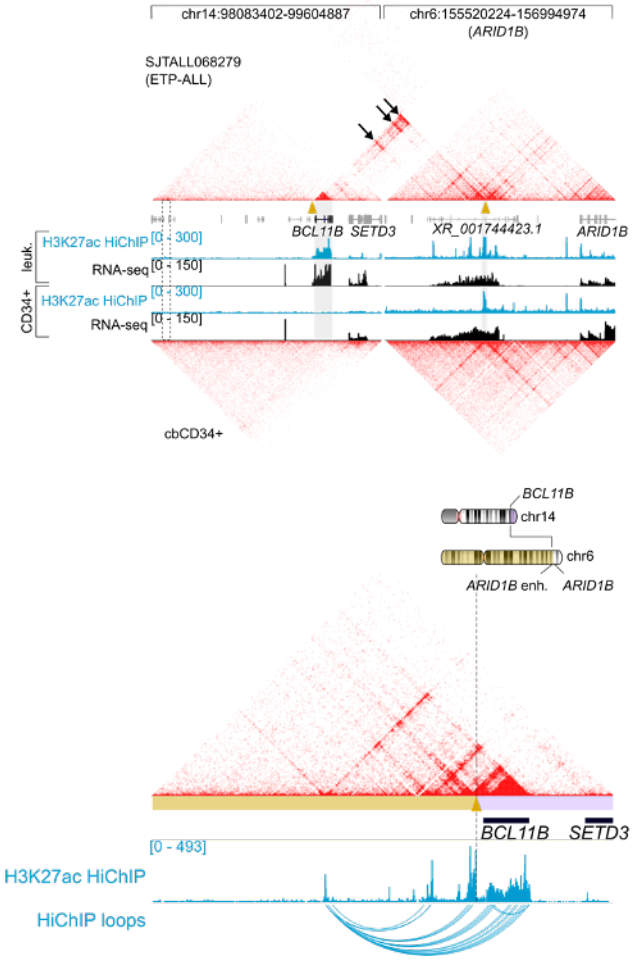


Figure 1. HiChIP was used to identify that *BCL11B* rearrangements rewire CD34+ hematopoietic stem and progenitor cell super enhancers. Black arrows indicate the ThymoD enhancer-BCL11B interaction[3].

With this working hypothesis, they performed chromatin conformation capture with Arima Genomics HiChIP to assess the presence of enhancers in HSPCs and found that *BCL11B* breakpoints occur near CD34+ HSPC super enhancers[3]. To obtain concrete evidence of enhancer hijacking, they applied the Arima HiC+ kit and a validated anti-H3K27ac antibody directly to patient samples to capture chromosome conformation around regions enriched for enhancer signal. The results demonstrate that the structural variants or rearrangements of *BCL11B* result in enhancer hijacking of super enhancers normally active in hematopoietic progenitor cells.

With WGS and RNA-seq, they also identified a new oncogenic event – a high copy tandem amplification event downstream of *BCL11B* that generates a *de novo* enhancer and presumably drives the gene's expression. This newly identified structural variant as a driver of cancer was quite common, occurring in 20% of the cases that were tested. These findings suggest continued investigations of other previously unknown classes of structural variants and gene expression deregulation mechanisms may yield further insights and discoveries. Finally, through modelling ectopic *BCL11B* expression in human and mouse progenitor cells, the study demonstrated that *BCL11B* is sufficient for driving different gene expression profiles consistent with characteristic T-lineage immunophenotype in leukemia. While assessing transformation properties in human cells is difficult, the study provides evidence from mouse data that *BCL11B* can be transforming.

"Hi-C technologies can be challenging to work with, but the Arima-HiC+ kit easily enabled us to perform H3K27ac HiChIP in both cell lines and limiting numbers of primary samples. We readily interrogated oncogenic structural variants in leukemia patient samples, including enhancer hijacking and *de novo* enhancer formations."

– Lindsey Montefiori, PhD, Postdoctoral Fellow
St. Jude Children's Research Hospital

Technology

HiChIP uses Arima-HiC+ and target-specific antibodies to analyze chromatin conformation in specific regions of the genome.

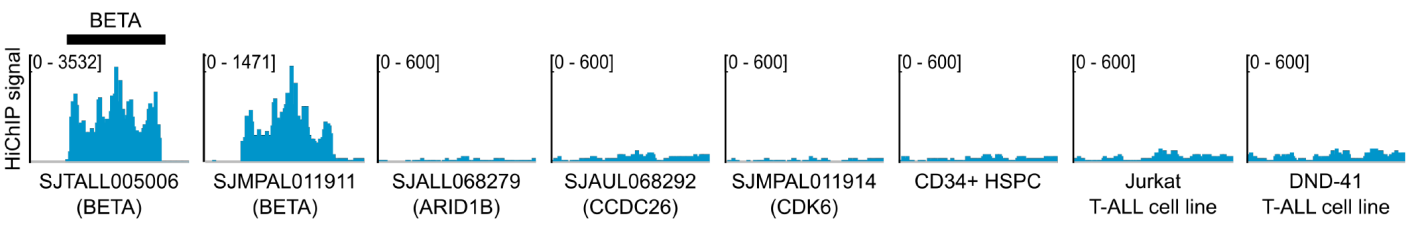
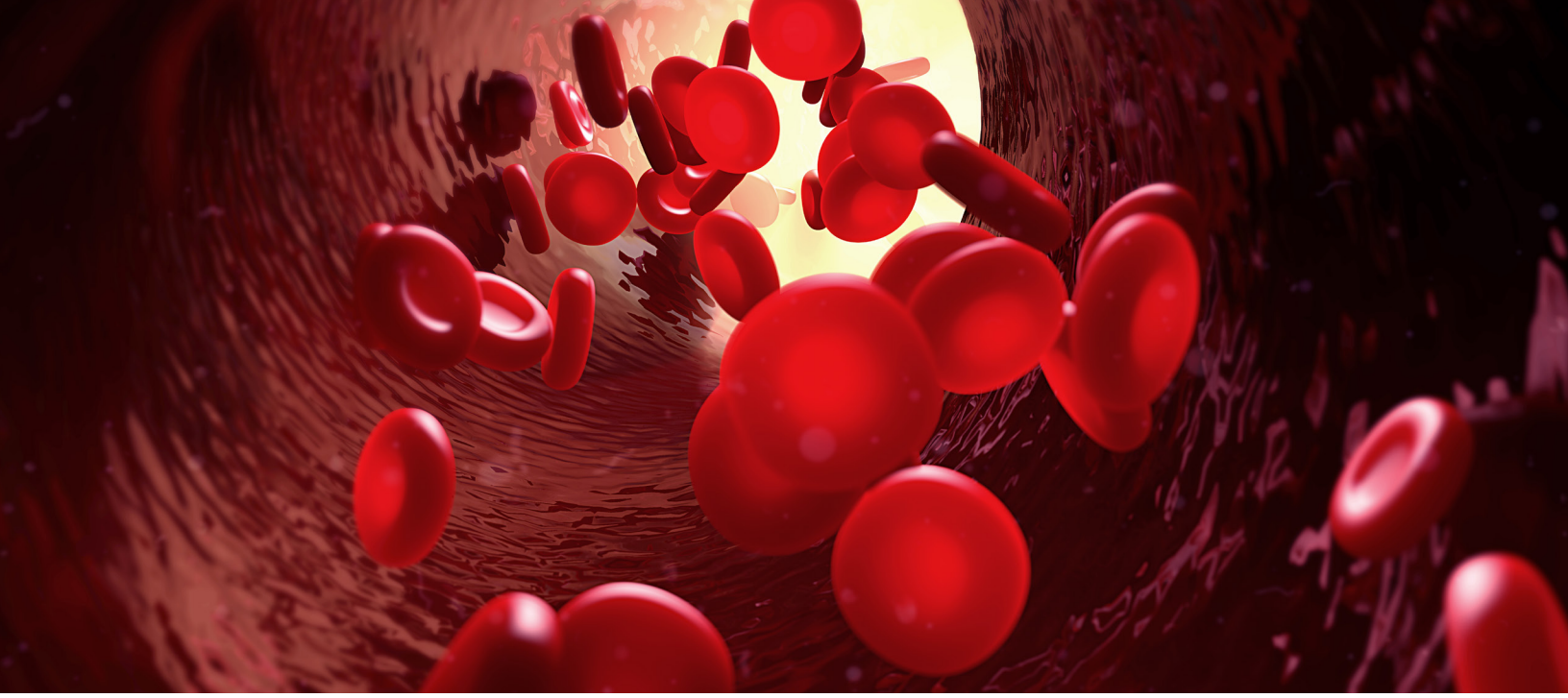


Figure 2. HiChIP H3K27 acetylation peaks demonstrated that structural rearrangements result in aberrant gene-promoter interactions that generate *de novo* super enhancers through a high copy tandem amplification event downstream of *BCL11B*[3].



Summarily, this study identified a new subtype of lineage ambiguous leukemia, defined by SVs targeting the *BCL11B* gene. Importantly, this leukemia is comprised of multiple diagnostic entities including up to 40% of T/myeloid mixed phenotype acute leukemia (MPAL) and early T-cell precursor acute lymphoblastic leukemia (ETP-ALL), and a smaller proportion of acute myeloid leukemia (AML). These alterations suggest that diagnostic distinctions based on immunophenotypes are not always sufficient to reflect the biology of leukemia.

Taking an integrated transcriptomic and genomic approach can aid in the identification of important classifications within and between diagnostic entities. This better understanding of mechanisms and structural abnormalities may influence the diagnosis, classification, and treatment of leukemias.

References

1. Linet, M. S., et al. (1999) Cancer surveillance series: Recent trends in childhood cancer incidence and mortality in the United States. *Journal of the National Cancer Institute.* 91, 1051-1058.
2. Porwit, A., and Béné, M. C. (2015) Acute leukemias of ambiguous origin. *American Journal of Clinical Pathology.* 144, 361-376.
3. Montefiori, L., et al. (2021) Enhancer hijacking drives oncogenic *BCL11B* expression in lineage ambiguous stem cell leukemia. *Cancer Discovery.*

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