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Chronic lymphocytic leukemia and Chronic Lymphoproliferative Disorders

EPIGENETIC REGULATION OF CD49D EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA: INSIGHTS FROM BIMODAL CD49D CASES

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Introduction: CD49d (integrin α4) is a key adhesion molecule in chronic lymphocytic leukemia (CLL) with strong prognostic relevance. While most CLLs display homogeneous CD49d expression, about 20% show a bimodal pattern with CD49d$^+$ and CD49d$^-$ subpopulations originating from the same leukemic clone. In bimodal cases, CD49d$^+$ cells expand over time, especially after therapy, showing higher proliferation, bone marrow enrichment, and accumulation in the CXCR4^{dim}/CD5^{bright} proliferative compartment. Clinically, these cases resemble CD49d$^+$ CLL and correlate with adverse outcomes. We investigated whether CD49d modulation in bimodal CLL results from genetic divergence or epigenetic regulation.

Methods: FACS-purified CD49d$^+$ and CD49d$^-$ fractions from eight bimodal CLLs collected at 2–3 longitudinal timepoints (mean follow-up 3.3 years) underwent whole-genome sequencing (WGS). Single-cell DNA genotyping (Mission Bio Tapestri) validated clonal relationships. Epigenetic analyses included ITGA4 promoter methylation, ATAC-seq for chromatin accessibility, and CUT&RUN for histone modifications (H3K4me3, H3K27me3, H3K27Ac, H3K4me1). ChIP in the CD49d$^+$ HG-3 cell line assessed transcription factor (TF) and coactivator binding. Functional assays tested inhibition of BRD4 (PLX51107, 1 µM) and histone acetyltransferases (A-485, 2 µM and Inobrodib, 500 nM).

Results: WGS revealed a median of 4,815 heterozygous SNVs and 61 driver mutations in 22 genes. Phylogenetic analysis showed shared branching evolution between CD49d$^+$ and CD49d$^-$ subsets with no clone-specific segregation. Longitudinal tracking indicated transitions from CD49d$^-$ to CD49d$^+$ cells, supporting phenotypic plasticity within genetically stable clones. Single-cell genotyping confirmed identical clonal architecture. Epigenetically, CD49d expression correlated with chromatin permissiveness at the ITGA4 locus. Promoter methylation was lower in CD49d$^+$ versus CD49d$^-$ cells. ATAC-seq revealed increased accessibility across 14 upstream regions (~170 kb) enriched for TF motifs (CTCF, EBF1, PU.1, RUNX3). ChIP confirmed TF, BRD4, and RPB1 binding, supporting enhancer–promoter interactions. CUT&RUN showed enrichment of active histone marks (H3K4me3, H3K27Ac, H3K4me1) in CD49d$^+$ and repressive marks (H3K27me3) in CD49d$^-$ subsets. Homogeneous CD49d$^+$ cases exhibited stronger enhancer activation than bimodal CD49d$^+$ fractions, suggesting reversible enhancer states. BRD4 or HAT inhibition markedly reduced CD49d transcript and surface protein expression.

Conclusions: CD49d modulation in bimodal CLL arises from epigenetically driven phenotypic plasticity sustained by a dynamic chromatin configuration at the ITGA4 locus. Reversible enhancer activation, rather than genetic divergence, explains the coexistence of CD49d$^+$ and CD49d$^-$ subclones. Targeting the BRD4–histone acetylation axis may thus represent a therapeutic strategy to modulate CD49d expression and limit CLL progression.