DNMT AND HDAC INHIBITION INDUCE DISTINCT TRANSCRIPTIONAL PROGRAMS AND ACTIVE CHROMATIN REMODELING IN CAR19-NK CELLS

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Chimeric Antigen Receptor Natural Killer (CAR-NK) cells are a novel and highly promising approach to cancer immunotherapy because of their strong cytotoxicity, favorable safety profile and reduced risk of graft-versus-host disease [1]. However, the clinical efficacy of this treatment is still constrained. Cellular exhaustion, insufficient antitumor activity and limited persistence necessitate further research [2]. Epigenetic regulation is a central determinant of immune cell identity and functional plasticity, yet its role in shaping transcriptional states of engineered CAR-NK cells remains incompletely understood, despite its potential to improve the therapeutic efficacy and persistence.

In this study, we examined how pharmacological inhibition of DNA methylation and histone deacetylation influences global gene expression patterns and active chromatin landscapes in CAR19-NK cells. CAR19-NK cells were treated with zebularine (a DNA methyltransferase inhibitor) or BML-210 (a histone deacetylase inhibitor), followed by transcriptome profiling using RNA sequencing. Comparative analysis revealed that the two epigenetic modulators induced partially overlapping but clearly distinct transcriptional programs. Zebularine predominantly affected genes associated with extracellular matrix interactions, focal adhesion, PI3K–Akt signaling, and immune-related pathways, suggesting transcriptional reprogramming linked to cellular interaction and environmental responsiveness. In contrast, BML-210 treatment resulted in broader transcriptional activation, with enrichment of pathways related to RNA processing, ribosome biogenesis, DNA replication, cell cycle progression, and chromatin remodeling, indicative of enhanced biosynthetic and proliferative capacity.

To mechanistically link these transcriptional changes with chromatin regulation, we performed chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) analysis of the active histone mark H3K4me3 at selected gene promoters. Epigenetic modulation led to differential enrichment of H3K4me3 at promoters of genes involved in immune regulation and differentiation (FOXO1, HOXA10, STAT5), as well as components of the Polycomb repressive complex 2 (EED, SUZ12, EZH2). Notably, pronounced H3K4me3 alterations at the HOXA10 and EZH2 promoters indicate remodeling of transcriptionally permissive chromatin states that may underlie the observed RNA-seq profiles.

Together, these data demonstrate that DNMT and HDAC inhibition drive distinct yet complementary transcriptional and chromatin-level responses in CAR19-NK cells. This integrated transcriptomic and epigenetic analysis provides mechanistic insight into how targeted epigenetic modulation can shape CAR-NK cell states, offering a rational framework for future optimization strategies in engineered NK-cell-based immunotherapies.

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[2] Z. Pang et al., “Current Progress of CAR-NK Therapy in Cancer Treatment,” Cancers, vol. 14, no. 17, p. 4318, 2022.