SMARCA5 Controls Gene Expression in AML1-ETO-Expressing Cells

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Abstract:

The (t;8;21) is the most frequent chromosomal translocation associated with acute myeloid leukemia (AML). It fuses the N-terminal portion of AML1 to most of ETO forming the AML1-ETO (AE) oncogenic transcription factor. Using Kasumi-1 hybrid cells expressing the fusion protein AML1-ETO, we found that SMARCA5 directly regulates a subset of the AML1-ETO gene repression signature, including the canonical target, CD82. Though SMARCA5 regulates only a small subset of the AML1-ETO gene repression signature, its main function is regulating CTCF sites.

Preliminary Data: SMARCA5 associates with AML1-ETO

A. Venn diagram of the significant SMARCA5 Cut&Run peaks overlapped with either the significant up (bottom panel) or down (top panel) regulated peaks at 6hr +/- 1.5kbp. B. Venn Diagrams of the SMARCA5 Cut&Run peaks overlapped with either the significant up (bottom panel) or down (top panel) regulated peaks at 24hr +/- 1.5kbp.

Background: Who is SMARCA5?

“Degron tags” that are engaged by proteolysis targeting chimeras (PROTACs) to trigger ubiquitination of a specific protein with the potential to transform our understanding of transcriptional regulators for which small molecule inhibitors are not available. We used CRISPR-Cas9 D10A “Nickase” to confirm SMARCA5 localization at these targets, including the canonical target, CD82. Though SMARCA5 regulates only a small subset of the AML1-ETO gene repression signature, its main function is regulating CTCF sites.

Methods: Endogenous PROTAC tag

We used CRISPR-Cas9 D10A “Nickase” to induce two ssDNAs breaks at the end of the coding region of SMARCA5 and homology directed repair to insert an FBX212 peptide tag and a FLAG tag epitope tag into the endogenous locus of SMARCA5 in the t(8;21) containing cell line Kasumi-1. This approach yielded an available targeting site for SMARCA5.

Results: SMARCA5 loss impairs AML1-ETO cell growth

A. Parental or SMARCA5-FKBP12 (30) cells after 24hr degradation with dTAG-47 treatment over a 3 day period. B. Annexin V staining for apoptosis of Kasumi-1 cells after 24hr degradation with dTAG-47 treatment. C. CD82 staining for apoptosis of Kasumi-1 cells after 24hr degradation with dTAG-47 treatment. D. Flow cytometry analysis of cell cycle analysis by BrdU and 90% staining of Kasumi-1 cells after 24hr degradation with dTAG-47 treatment.

Results: The role of SMARCA5 in transcription

A. Venn diagram of the overlapping significant SMARCA5 Cut&Run annotated peaks with either the significant up (bottom panel) or down (top panel) regulated peaks at 6hr +/- 1.5kbp. B. Venn Diagrams of the SMARCA5 Cut&Run peaks overlapped with either the significant up (bottom panel) or down (top panel) regulated peaks at 24hr +/- 1.5kbp.

SMARCA5 regulates AML1-ETO mediated transcription.

A. Venn diagram of the significant SMARCA5 Cut&Run annotated peaks overlapped with either the significant up (bottom panel) or down (top panel) regulated peaks at 6hr +/- 1.5kbp. B. Venn Diagrams of the SMARCA5 Cut&Run peaks overlapped with either the significant up (bottom panel) or down (top panel) regulated peaks at 24hr +/- 1.5kbp.

Results: SMARCA5 regulates Nucleosome Free Regions

A. Heatmap of the significant SMARCA5 Cut&Run peaks called by diffbind, and DESeq2 that have a p value of less than 0.05 and a fold change greater than 1.5 after 2hr, 6hr, and 24hr dTAG-47 treatment B. Heatmap of the significant SMARCA5 Cut&Run peaks called by diffbind, and DESeq2 that have a p value of less than 0.05 and a fold change greater than 1.5 after 2hr, 6hr, and 24hr dTAG-47 treatment. C. Heatmap of the ATAC-seq signal around the significantly called SMARCA5 peaks that are 40% of the RNA-seq targets

Conclusion and Future directions:

• SMARCA5 regulates only a small subset of AML1-ETO targets. 10 out of 59.
• The main function of SMARCA5 is regulating CTCF binding.

Future direction: How is SMARCA5 regulating CTCF binding?

Measure nucleosome repeat length within ATAC-seq dataset. Perform Hi-C or 3C around specific gene targets

References:


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