Determining the Histone Post-translational Modification Binding Specificities of ASH1L

NC-LSAMP North Carolina Louis Stokes Alliance for Minority Participation STEM Pathways and Research Alliance

Tiffanie Lee, Burkholder, N., Krajewski, K., Strahl, B.

Department of Biochemistry and Biophysics at UNC Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Abstract

ASH1L (absent, small, or homeotic discs 1 -like) is a **histone reader** and **methyl transferase** that has been implicated in MLL (**mixed lineage leukemia**) cancers, which commonly affect children and may be acute and treatment-resistant. The Strahl Lab found ASH1L to bind to H3K4me3 and H3K27ac which we suspect facilitates ASH1L's recruitment of the LEDGF histone reader, which is part of the MLL protein complex, to the transcription start site of leukemia target genes. Past studies have shown that MLL cell lines with an ASH1L knockdown had reduced viability and that mice transduced with ASH1L shRNAs and transplanted with MLL leukemia cells survived for longer, strongly suggesting the potential of ASH1L as a therapeutic drug target. The objective of my project is to determine the histone post-translational modification binding specificities of the ASH1L histone reader domains. ***We hypothesized that the BRD domain binds to H3K27ac and the BAH and PHD domains bind to H3K4me3 based on the binding specificities of BRD/PHD/BAH domains from other histone reader proteins.*** I used site directed mutagenesis and affinity purification to create, clone, and purify ASH1L constructs with mutated, putative-nonfunctional histone reader domains. Then, I used pulldown assays to test binding of wild-type and mutant proteins to differentially histone peptides and nucleosomes. **The purified WT ASH1L protein showed binding on K4me3 and increased binding on doubly modified histone.** In the future, we would like to test the mutants with defects in binding in HEK (human embryonic kidney) and MLL cancer cells to determine if disrupting binding at these domains is effective in treating MLL cancers in humans.

Introduction

- **ASH1L and MLL cooperate to maintain hematopoiesis and transcription in immune cells in the blood.** Normally, the two cooperate to maintain hematopoiesis and transcription of immune cells in the blood, but when MLL gets rearranged with the gene coding for a "super elongation complex", the expression of MLL translocations can lead to increased transcription and cancer [1].
- **Acute cases frequently result from MLL translocations, and some MLL-rearranged leukemias are resistant to chemotherapy, resulting in poor outcomes [2].**

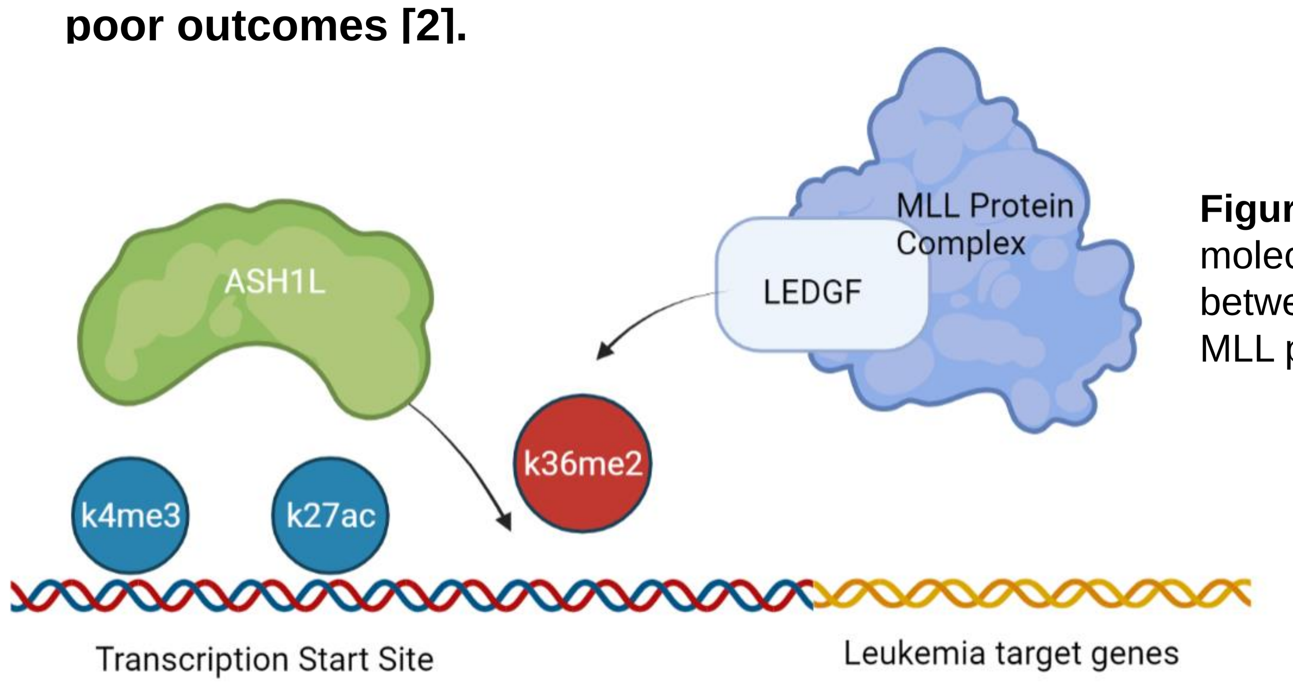

Figure 1. Predicted molecular interaction between ASH1L and MLL protein complex.

Inhibiting ASH1L binding can potentially treat MLL-rearranged leukemias.

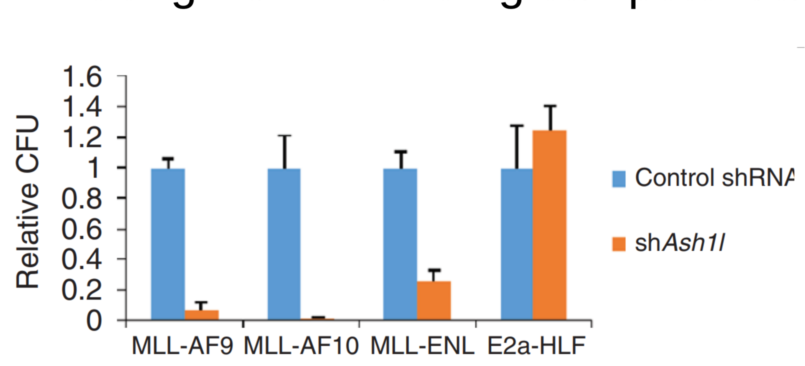

Figure 2. MLL cell lines with an ASH1L knockdown had reduced viability [1].

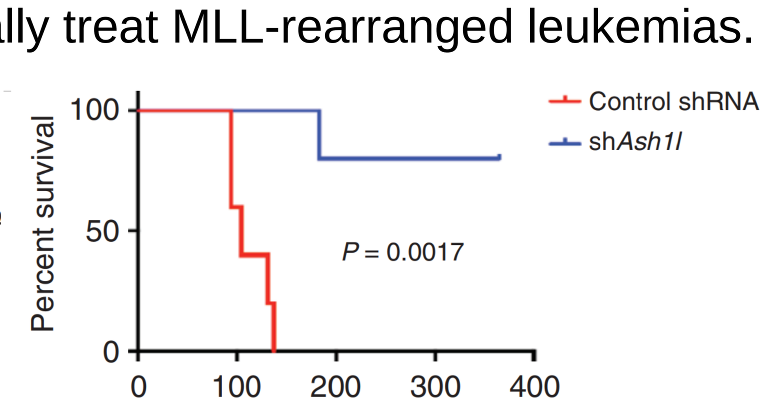

Figure 3. Mice transduced with ASH1L shRNAs and transplanted with MLL leukemia cells survived for longer [1].

Research Question and Hypothesis

How does ASH1L read chromatin? What histone reader domains are involved in binding?
- BRD – K27ac
- BAH and PHD – K4me3

Testing with in-vitro histone binding of WT mutants

Using Site Directed Mutagenesis to Create ASH1L Histone Reader Mutant Constructs

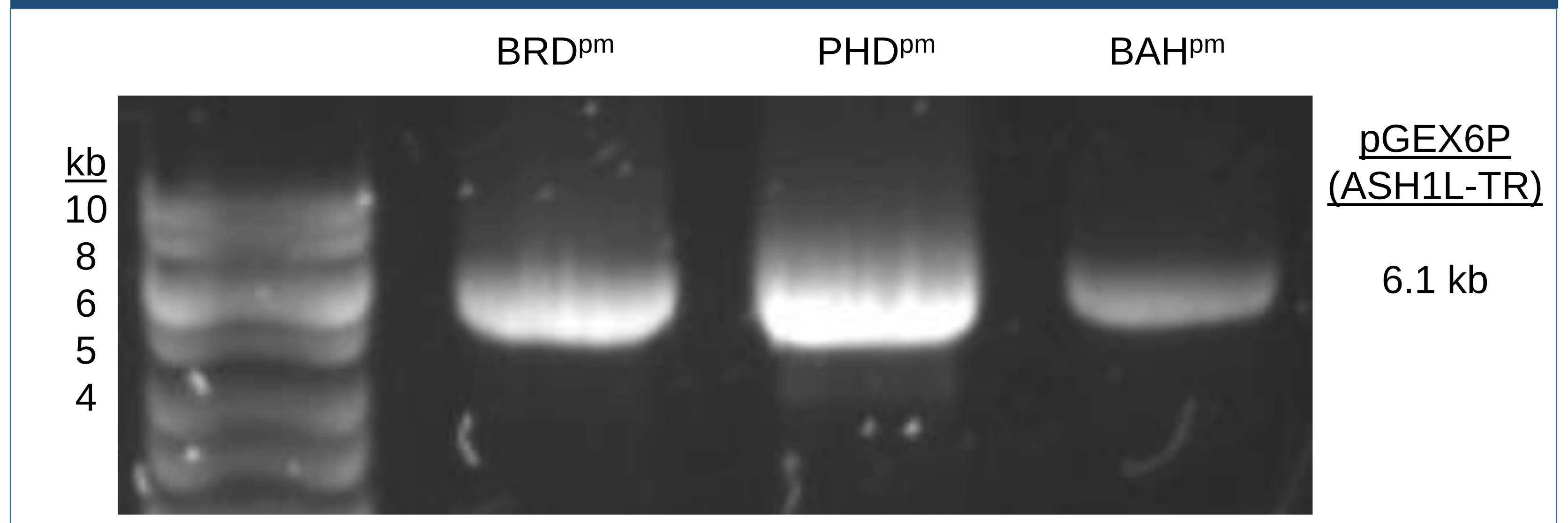

Figure 4. DNA gel shows successful creation and cloning of mutant constructs. Site directed mutagenesis was used to create three single point mutants with mutations in the BRD (Y2525F), PHD (D2595A), and BAH (D2700A) domains using pGEX6P vectors with ASH1L triple readers. **Positive signal at the right size (6.1 kb) means that PCR amplification for single point mutants is working despite primers showing mismatches for adding in mutations.**

Affinity Purification of GST-ASH1L-TR

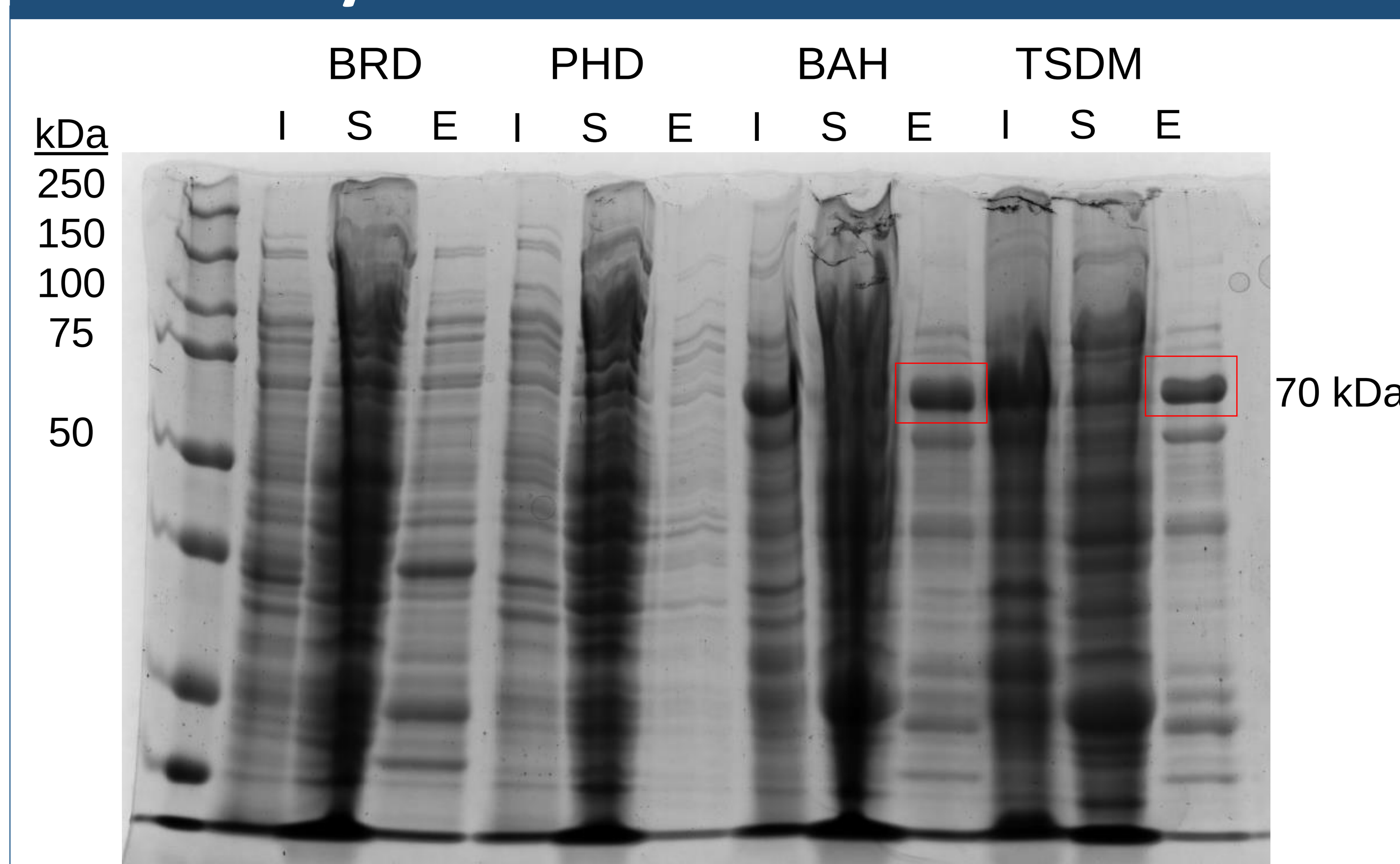

Figure 5. Western blot of protein purification products (I, insoluble; S, soluble; and E, elution) of three single-point mutants and a triple mutant with a single point mutation in each reader domain (TSDM). The elution products show that the BRD construct is not the right size and that the PHD construct was not expressed. **Both the BAH and TSDM mutants expressed and purified successfully based on the elution of correct sized protein (~70 kDa).**

Peptide Pulldown Assays to Determine Binding

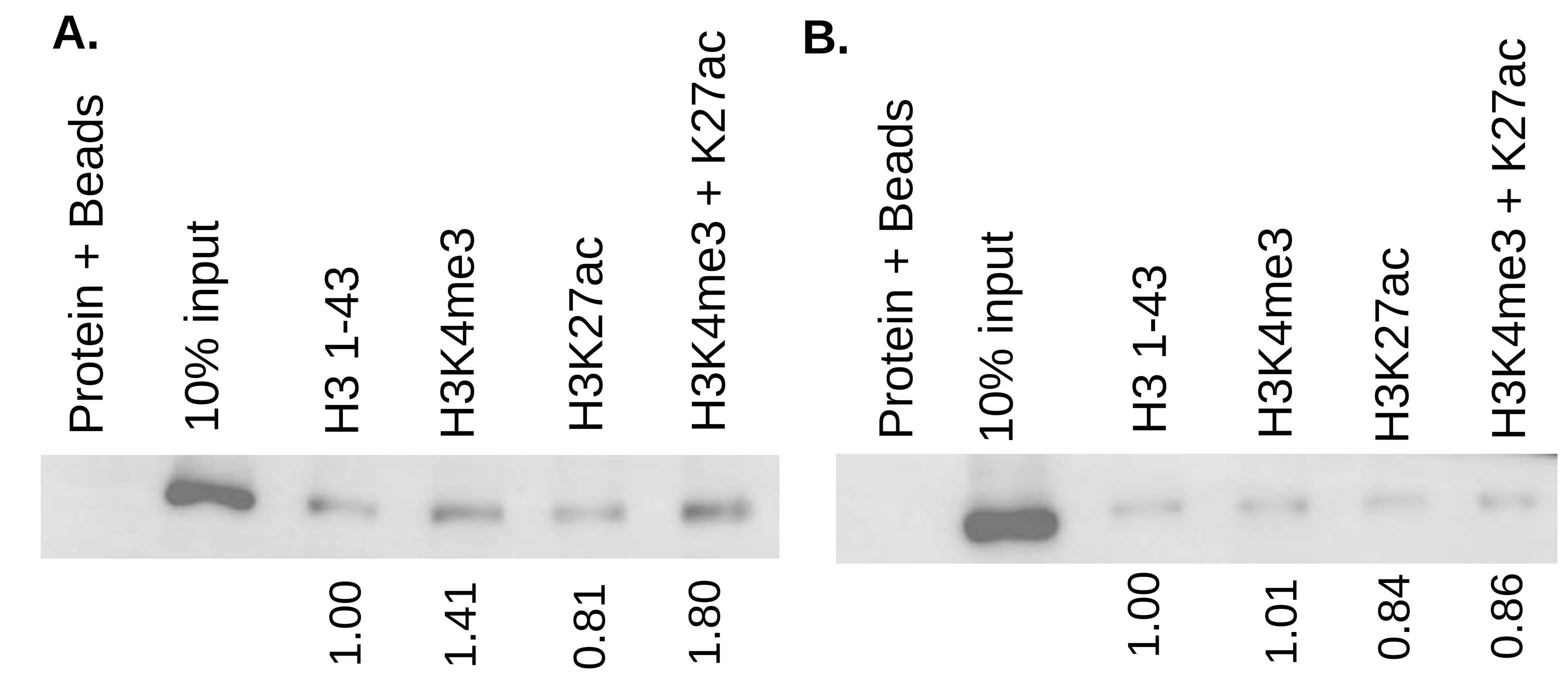

Figure 6. A.) Peptide pulldown assays with purified WT ASH1L protein show binding on K4me3 and increased binding on doubly modified histone. B.) TSDM triple mutants show lower signal and lost specificity but are not enough to eliminate histone binding. The BRD mutant band is not as clear, as K27ac binding is not as strong as K4me3, suggesting the BAH and PHD domains may be stronger binding targets.

Conclusion

- Successfully made mutant constructs of GST-ASH1L mutants in histone reader domains
- Affinity purified single mutants and triple mutant
- Confirmed binding of ASH1L TR on H3 peptides

Future Directions

- Reconfirm sequencing and determine if issue with BRD and PHD single point mutants is due to transcription or stability of proteins themselves. If mutation disrupts protein folding, identify and develop alternative mutations to test.
- Determine binding specificities of single and double point mutants
- Test on nucleosomes, which should show higher levels of binding specificities because they're more native to readers.

References

1. **Zhu, L., Li, Qin., Wong, S. H. K., Huang, M., Klein, B. J., Shen, J., Ikenouye, L., Onishi, M., Schneidawind, D., Buechele, C., Hansen, L., Duque-Afonso, J., Zhu, F., Martin, G. S., Gozani, O., Majeti, R., Kutateladze, T. G., and Cleary, M. L.** *Cancer Discovery*. 2022. ASH1L Links Histone H3 Lysine 36 Dimethylation to MLL Leukemia. **12**(7):771-783.
2. **Winters, A. C. and Bernt, K. M.** *Frontiers in Pediatrics.* 2017. MLL-Rearranged Leukemias—An Update on Science and Clinical Approaches. **5**(4):1-21.

Acknowledgements

- Special thanks to all the members of the Strahl Lab, the SMART program, and the Chancellor's Science Scholars program for their support.
- This research was supported by the NC LSAMP SPRA program, which was funded through NSF Award #2010124, as well as by the UNC Office for Undergraduate Research.
- We gratefully acknowledge the NIH (R35 GM126900-01; R01 CA198482-01; R61 DA047023), NIH/NCI (T32 CA009156), Qura Therapeutics.