

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. In the Strahl lab, we have 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.