Stony Brook University
The Graduate School

Doctoral Defense Announcement

Abstract

Antisense-Targeted Exon Skipping of *H3-3A* for

H3.3K27M-Altered Diffuse Midline Glioma Therapy

By

Lucia Yang

Diffuse midline gliomas (DMGs) are a deadly class of pediatric brain cancers with a 5-year survival rate after diagnosis of < 1%. Many DMGs feature a dominant, somatic, heterozygous point mutation in the non-canonical histone H3.3 coding gene *H3-3A*. This gain-of-function mutation replaces lysine 27 with methionine (K27M), not only preventing trimethylation of K27 (H3K27me3) but also disrupting global di- and tri-methylation of wild-type histone H3 proteins. This mutant H3.3K27M onco-histone is considered a major driver of tumorigenesis.

To prevent the translation of H3.3K27M, we aimed to target exon 2 of *H3-3A* to promote exon skipping, as this constitutive exon comprises not only the K27M mutation but also the only in-frame start codon of the gene. First, we designed splice-switching ASOs that targeted pre-mRNA regions that interact with the spliceosome at the 5' splice site. The lead ASO specifically induced *H3-3A* exon 2 skipping and restored global H3K27me3 marks. In a patient-derived xenograft tumor mouse model, our lead ASO extended survival and reduced proliferative marks compared to vehicle and scramble-sequence controls. Second, we identified a H3.3K27M mutation-specific interaction with two members of the RNA binding protein family RBFOX that induce mutant-allele specific exon skipping. Third, we have continued work to characterize the efficacy of thiomorpholino oligonucleotides (TMOs) to increase allele-specific knockdown of *H3-3A* in the context of H3.3K27M-altered DMG. Our results show the potential of a splice-switching ASO targeting *H3-3A* exon 2 for exon skipping as a therapeutic for H3.3K27M DMG. More generally, our work represents a new strategy of using ASOs to induce skipping of a constitutive exon to effectively achieve gene downregulation.

Date: March 14, 2025 **Program**: Graduate Program in Genetics
Time: 2:00 PM **Dissertation Advisor**: Adrian R. Krainer
Place: Hawkins Conference Room, Wendt Building, Cold Spring Harbor Laboratory
To attend virtually, contact the Program Director at martha.furie@stonybrook.edu.