Abstract

Uveal Melanoma is a rare ocular cancer predominantly associated with mutations at the 209 amino acid site in GNAQ and GNA11 genes. Despite GNAQ and GNA11 genes sharing 90% of their amino acid sequence, emerging evidence suggests potential differences in their impact on Uveal Melanoma prognosis. To address these discrepancies, the creation of isogenic cell lines is imperative to allow for comparative analysis and evaluate the effects of the 209 site mutations in GNAQ and GNA11. Three Uveal Melanoma cell lines were selected for use: Mel 285, which has no mutations at the GNAQ or GNA11 209 amino acid site, and 92.1 and Mel 202, which have identified mutations at the GNAQ 209 amino acid site. To test the effects of GNAQ gene silencing, CRISPR Cas9 induced gene knockouts were designed for the 92.1 and Mel 202 cell lines to create Uveal Melanoma isogenic cell lines with a silenced 209 mutation site. To test the differences in GNAQ and GNA11, CRISPR Cas9 induced gene knockins were designed for the Mel 285 cell line to create isogenic Uveal Melanoma cell lines with the mutant 209 amino acid sites in GNAQ and GNA11 genes.

I started the experiment by performing knockouts in the Mel 285 cell lines as a preliminary step to test the CRISPR-Cas9 system and PCR primer designs, given the ease of testing with knockouts and the opportunity to gain experience before attempting the procedure in the anticipated 92.1 and Mel 202 cells. However, this experiment encountered a standstill post-transfection, with lack of successful PCR amplification. Consequently, although not reflected on the poster, transfection of the 92.1 and Mel 202 cells occurred this week, employing the CRISPR Cas9 system components, but in a plasmid this time, targeting the 209 amino acid site directly. This is expected to enhance PCR efficiency, because this site has been targeted in lab previously with primers and conditions that are known to work. I anticipate obtaining knockout cell lines within the next two weeks. Additionally, knockins in the Mel 285 cells are the next step in the experimental process.