CRISPR Cas9 Knockout and Knock-in of GNAQ Mutant Alleles for the Creation of Isogenic Uveal Melanoma Cell Lines

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Background

• Uveal Melanoma (UM) is a rare type of cancer affecting the uveal tract of the eye.

• Approximately 85% of Uveal Melanoma cases exhibit a known point mutation in GNAQ or GNA11 genes at codon 209, resulting in a glutamine to leucine mutation in GNAQ and GNA11 or a glutamine to proline mutation in GNAQ.[1]

• GNAQ and GNA11 share about 90% of their amino acid sequence, leading to the general assumption that these genes are functionally identical.

• Mel 285 cell lines lack GNAQ and GNA11 mutations, while 92.1 and Mel 202 cell lines possess GNAQ mutations at the 209 amino acid site.[2]

Objectives

• To generate an isogenic cell line containing the GNAQ Q209L mutation in the chromosome of the Mel 285 UM cell line.

• To generate isogenic cell lines without the Q209L mutation in the chromosome of 92.1 and Mel 202 UM cell lines.

• Use these cell lines in future experiments for comparative analysis of GNAQ and GNA11 genes and the 209 amino acid site mutations.

Introduction

• The CRISPR (clustered regularly interspaced short palindromic repeats) Cas9 system will be utilized to create the mutations in the Mel 202, 92.1, and Mel 285 cell lines.

• Knockout and knock-in techniques will use the CRISPR system to knockout the 209 mutation in Mel 202 and 92.1, and create the 209 site mutation in Mel 285 cells.

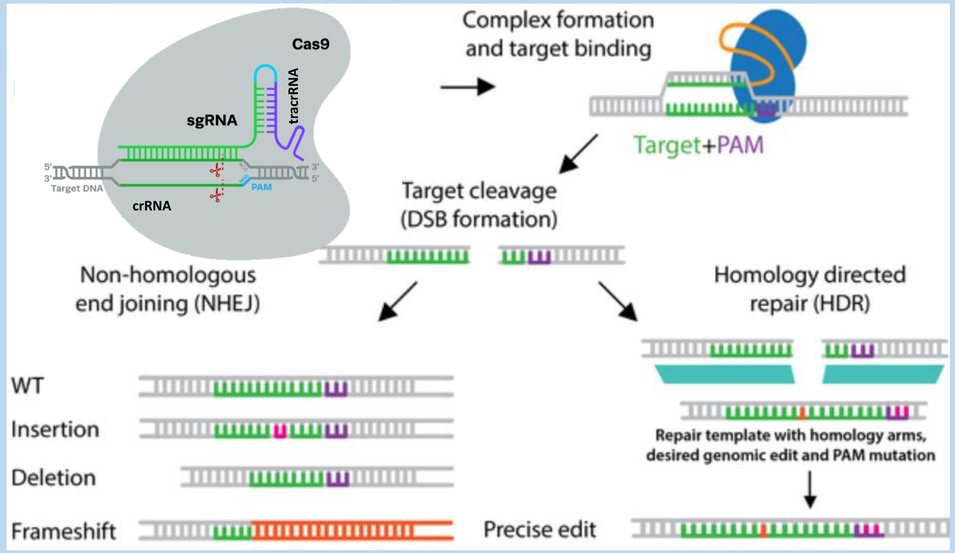

Figure 1. sgRNA binding to gDNA for Cas9-induced DSB, and DSB repair options.[3]

• CRISPR Cas9 system uses single-guide RNA (sgRNA), composed of CRISPR RNA (crRNA) and trans-activating RNA (tracrRNA), with the Cas9 enzyme to create a DNA double-stranded break (DSB) at the site of interest.
• The DNA will repair itself through nonhomologous end joining with the knockout cells, or with a homology directed repair (HDR) template with the knock-in cells.

Methods

1. crRNA design for sgRNA of knock-ins and knockouts
2. Creation of sgRNA through ordered crRNA and tracrRNA.
3. CRISPRMAX lipofectamine transfection of cell lines.
4. Harvest half of transfected cells, purify genomic DNA (gDNA) of harvested cells, and continue passaging the other half.
5. Design primers for PCR of transfected gDNA and perform PCR reactions.
6. Purify PCR product and send for Sanger sequencing to ensure proper mutation. Use ICE analysis to analyze the sequences.
7. Utilize flow cytometry to sort single cells for clonal outgrowth.
8. Maintain isogenic cell lines.

Results

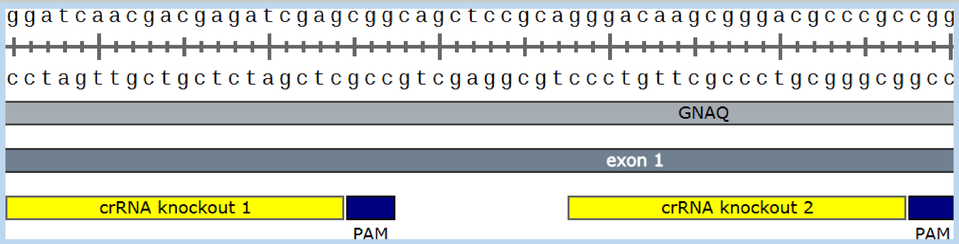

Figure 2. crRNA designs for CRISPR knockout transfections.

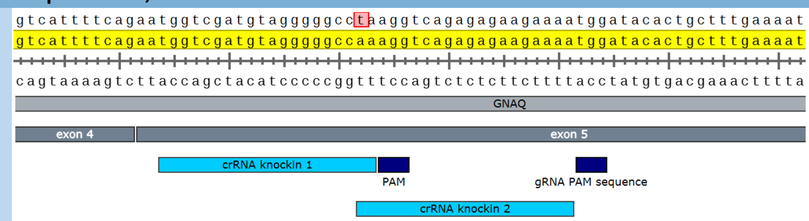

Figure 3. crRNA designs for CRISPR knock-in transfections. HDR template sequence with single point mutation seen at the top.

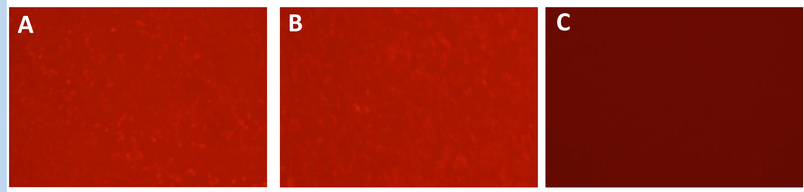

Figure 4. 24 hours post-transfection of Mel 285 cells. Knockout 1 in image A, knockout 2 in image B, and positive control in image C.

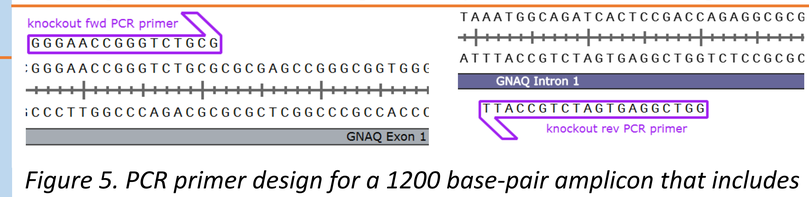

Figure 5. PCR primer design for a 1200 base-pair amplicon that includes the knockout mutation.

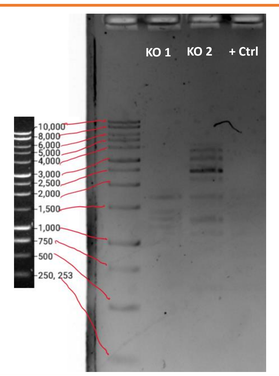

Figure 6. Gel with 1kb ladder, knockout 1, knockout 2, and + control PCR reactions.

Conclusion and Future Direction

• After several attempts at PCR, a properly sequenced amplicon was not obtained (see Figure 6 as an example).
• Therefore, the knockouts performed in the Mel 202 and 92.1 cell lines will use a Cas9 and sgRNA plasmid that targets the 209L site in exon 5 directly.
• Knock-ins of the Mel 285 cell lines still need to be performed and sequenced.

References

1. Chromosomal Alterations than Gα-Protein GNAQ Mutations." European Journal of Cancer, vol. 170, July 2022, pp. 27–41. ScienceDirect, https://doi.org/10.1016/j.ejca.2022.04.013.
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2. Jager, Martine J., et al. "Uveal Melanoma Cell Lines: Where Do They Come from? (An American Ophthalmological Society Thesis)." Transactions of the American Ophthalmological Society, vol. 114, Aug. 2016, p. T5. PubMed Central, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5161001/.
3. *CRISPR plasmids: Cut*. Addgene. https://www.addgene.org/crispr/cut/