Investigating the Contribution of Cis-regulatory Elements Involved in Glioblastoma Response to Temozolomide with Single-cell CRISPR Base-editing

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Glioblastoma (GBM) is the most aggressive form of brain cancer with an average survival time of 12-18 months and only 5% of patients surviving past 5 years.[1] Temozolomide (TMZ) is the standard-of-care chemotherapeutic for GBM, which extends survival by approximately two months. TMZ induces many O^6-methylguanine base lesions in the DNA ultimately causing cytotoxicity. Specifically, the base lesions undergo cyclical processing by the DNA mismatch repair (MMR) pathway, eventually resulting in a double strand break in the progeny DNA and subsequent cell cycle arrest leading to apoptosis. Previous studies have shown that changes in gene expression within the MMR pathway can yield GBM cells exhibiting a strong TMZ resistance.[2] Although the role of these MMR genes has been widely studied, the cis-regulatory elements (CREs) of the genes associated with TMZ mechanism of action are not well understood.

To investigate the involvement of CREs in MMR genes to TMZ resistance, we will apply clustered regularly interspaced short palindromic repeats (CRISPR) base-editing to multiple GBM cell lines. We will use the BE4max[3] cytidine deaminase to model base pair transitions generated by TMZ. As an initial proof-of-concept, we will deliver single guide RNAs (sgRNAs) targeting coding regions of MMR genes that eliminate MMR activity. First, we will transduce BE4max CRISPR into multiple GBM cell lines using lentivirus. Then for each of these BE4max CRISPR GBM cell lines, we will transduce unique single guide RNAs (sgRNAs) that target genes in the MMR pathway and non-targeting controls using the CROP-seq system for pooled CRISPR screens at single-cell resolution.[4,5] We will expose perturbed and control cells to TMZ and subject them to droplet based single-cell RNA-sequencing. This framework and the multiplexing ability to single-cell genetic screens will be used in future studies to understand the role of CREs that regulate genes in the MMR pathway as a response to TMZ enabling the dissection of the molecular mechanisms promoting GBM reoccurrence.

References

1. Glioblastoma multiforme - Brain Tumour Research. Accessed June 7, 2022. https://www.braintumourresearch.org/info-support/types-of-brain-tumour/glioblastoma-multiforme

2. McFaline-Figueroa JL, Braun CJ, Stanciu M, et al. Minor Changes in Expression of the Mismatch Repair Protein MSH2 Exert a Major Impact on Glioblastoma Response to Temozolomide. *Cancer Res*. 2015;75(15):3127-3138. doi:10.1158/0008-5472.CAN-14-3616

3. Gearing M. CRISPR 101: Cytosine and Adenine Base Editors. Accessed June 6, 2022. https://blog.addgene.org/single-base-editing-with-crispr

4. Hill AJ, McFaline-Figueroa JL, Starita LM, et al. On the design of CRISPR-based single-cell molecular screens. *Nat Methods*. 2018;15(4):271-274. doi:10.1038/nmeth.4604

5. Datlinger P, Rendeiro AF, Schmidl C, et al. Pooled CRISPR screening with single-cell transcriptome readout. *Nat Methods*. 2017;14(3):297-301. doi:10.1038/nmeth.4177