Novel CRISPR Knockout of Hif-1a in U251 Glioblastoma Cells

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Hypoxia (reduced oxygen) is common in many brain tumors, including gliomas. Hypoxia Inducible Factor 1 Alpha (Hif-1a) is upregulated and stabilized under hypoxic conditions, leading to tumor growth. This experiment targets Hif-1a because normal cells generally don't require Hif-1a. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) can be used to knock out genes. A lab at Huntsman Cancer Institute has previously treated the glioblastoma cell line U251 with CRISPR-Cas9 targeting Hif-1a. However, they do not know if this treatment worked. The U251 cell clones were screened by ELISA (Enzyme-Linked Immunosorbent Assay), DNA sequencing, and a T7 endonuclease assay. Using these, this experiment tested if CRISPR-Cas9 successfully knocked out Hif-1a. Hypoxic cell lysate (prepared previously) was used for the ELISA. The T7 assay showed that the negative and positive controls worked, and three of the four clones have possible mutations. Sequencing results and HIF-1a protein levels determined by ELISA corroborated this, along with c-Met, a gene activated by Hif-1a. Interestingly, this also showed that one of the clones was still producing low levels of Hif-1a, despite the T7 and sequencing results. This may be because it is heterozygous, but more testing would be needed to verify this. A cost analysis was done and showed that the T7 assay had the best cost to time ratio. This experiment shows that CRISPR can knock out Hif-1a and potentially be used in cancer treatment. These CRISPR knockout cells will be used to test the interaction of chemotherapy, radiation, and hypoxia in glioblastoma animal models.

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