Cancer and CRISPR: A Story of Two Assays

Bawden, Neve (School: Millcreek Junior High School)

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 do not generally require Hif-1a. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) in correlation with Cas-9, has previously been used to treat the glioblastoma cell line U251 in a lab at the Huntsman Cancer Institute. DNA sequencing was done on U251 samples and genome editing did occur in three of the U251 cell samples CR2, CR5, and CR6. Hypoxic cell lysate was prepared and used in the Enzyme-Linked Immunosorbent Assay (ELISA), Luciferase Functional Assay (LFA), and 660 protein assay. The ELISA, in conjunction with the protein assay, showed that two samples are not producing Hif-1a and that three samples are. This is interesting because U251CR5 sequencing showed that the genes were edited, but the cells were still producing Hif-1a. However, the LFA showed that U251CR5 had no active Hif-1a. This shows that ELISA and protein assay by itself is not the best way to screen if a protein is still active or not. Additionally, CRISPR can be used to knock out Hif-1a effectively and possibly be used as a cancer treatment.

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