Dissecting Glioblastoma: CRISPR Knockout of Hif-1ALPHA Disrupts Downstream Targets

Bawden, Neve (School: Bountiful High School)

Hypoxia Inducible Factor 1 Alpha (Hif1a) contributes to cancer growth in most solid tumors. Because normal cells do not generally require HIF1, it is a potential target. How cancer will adapt to inactivation is unknown. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) in conjunction with Cas-9, has previously been used to inactivate HIF1a in the glioblastoma cell line U251. DNA sequencing showed genome editing did occur in three of the U251 cell clones: CR2, CR5, and CR6. Hypoxic whole cell lysate was prepared and used in the enzyme linked immunosorbent assays (ELISA) to measure HIF1a and its target proteins GLUT1, VEGF and PDK1. Nuclear extract was used to measure HIF1/DNA binding. The ELISA and DNA binding showed that CR2 and CR6 are not producing HIF1a but CR5 is, and it binds DNA. CR5 sequencing showed that the gene was edited. However, previous research showed that CR5 had no active HIF1a. Surprisingly, the GLUT1 assay showed that CR2 and CR6 make the same amount of GLUT1 as the control. CR5 is producing less GLUT1, but is still showing to be active. VEGF results mirror the results of HIF1a, as expected, while PDK1 shows no difference in any clones. Others have shown GLUT1 and PDK1 are regulated by HIF1a. This shows that cells can adapt to HIF1a inactivation and regulate them through alternate pathways. This indicates CRISPR can be used to knock out Hif1a effectively to create cell lines for detailed study of HIF1a in glioblastoma.

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