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CHCHD2 Knockout Alters Mitochondrial Metabolism, Increases Sensitivity to Sulfasalazine, and Decreases Proliferation and Invasive Potential of Glioblastoma Cells Expressing EGFRvIII

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Glioblastoma (GBM) is the most common and malignant form of brain cancer in adults, with a poor median survival time of 14.6 months driven by hyperproliferative zones and diffusively invasive cells. Tumors are heterogeneous and characterized by hypoxic foci. Amplification, overexpression, and mutation of the receptor tyrosine kinase (RTK) EGFR is prevalent in GBM, and the constitutively active EGFRvIII mutant shows the poorest clinical outcomes. However, RTK inhibitors have failed clinically against GBM. We are investigating compensatory signaling pathways active within GBM tumor margins as a possible source of resistance to molecular subtype therapies. Previous studies have reported coiled-coil-helix-coiled-coil-helix domain-containing protein 2 (CHCHD2) as a bi-organellar protein localized predominantly in the mitochondrial inter-membrane space but also present in the nucleus, where it acts as a transcription factor. We hypothesized that this co-localization enables GBM cells to adapt to hypoxic stress in the tumor microenvironment, particularly via mitochondrial retrograde signaling. Our previous work demonstrated that U87 GBM cells harboring EGFRvIII (U87vIII) displayed nuclei enriched with CHCHD2 compared to isogenic U87 cells not expressing EGFRvIII. The objective of this study was to characterize the effect of CHCHD2 knockout (KO) on mitochondrial metabolism, therapeutic sensitivity, and proliferation and invasion of U87vIII cells. Stable U87vIII CHCHD2^{KO} cells were derived using CRISPR-Cas9 genome engineering, and knockout was verified at the gene and protein level. CHCHD2^{KO} cells displayed decreased basal and maximal oxygen consumption rate and decreased spare respiratory capacity compared to U87vIII CHCHD2^{WT}, determined using the Seahorse XFp Extracellular Flux Analyzer. U87vIII CHCHD2^{KO} cell growth was significantly decreased over 72 h in both normoxia (21% O2) and hypoxia (1% O2). Mitochondrially targeted, genetically encoded fluorescent redox biosensors revealed decreased levels of oxidized GSSG in the mitochondrial matrix of CHCHD2^{KO} cells. Further, CHCHD2^{KO} cells were more sensitive to treatment with the drug sulfasalazine, an inhibitor of the glutamate-cystine antiport system x_c^-. We next used engineered gliomas (EG) formed by microfluidic templating as a model of the GBM microenvironment. A three-dimensional invasion assay within these EGs revealed significantly increased invasion of U87vIII cells in hypoxia compared to normoxia, but this effect was abrogated in CHCHD2^{KO} cells, which displayed decreased invasion compared to U87vIII under both oxygen tensions. Collectively, these data demonstrate the multifaceted nature of CHCHD2 in the context of cell metabolism, proliferation, invasion, and therapeutic resistance, all outcomes which promote the malignancy and recurrence of GBM. Future work will further interrogate the function of CHCHD2 in the nucleus and the gene expression signature it may regulate in GBM.

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