Literature Presentation

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Research Paper: ATF4-dependent fructolysis fuels growth of glioblastoma multiforme

Hypothesis and Methodology

- Types of Omics: Proteomics and Genomics
- Hypothesis: Glucose deprivation affects fructose consumption
- Methods: treated the GBM cell lines including U87, LN229, and A172, genetically characterized primary GBM cells TJ46, as well as GBM stem cell line GSC23 with glucose-deprived media for 18 hours. The researchers then titrated the extracellular glucose concentration needed to activate fructose metabolism by treating the U87 and LN229 cells with media containing different glucose concentrations. To investigate the role of ATF4-dependent fructolysis in GBM growth, we intracranially injected luciferase-expressing U87 cells without or with *ATF4* KO or ATF4 binding-deficiency in the promoters of *SLC2A5* or *ALDOB* into athymic nude mice. To investigate the therapeutic potential of pharmacological blockage of fructose utilization in vivo, we intracranially injected the luciferase-expressing TJ46 and GSC23 cells into athymic nude mice.

Findings

- Treatment of U87 and LN229 cells with the PERK inhibitor GSK2656157 or the GCN2 inhibitor A-92 partially blocked glucose-deprivation-induced mRNA and protein expression of *SLC2A5* and *ALDOB*.
- The data strongly suggest that glucose deprivation induces expression of fructolytic genes depending on PERK-eIF2α and GCN2-eIF2α, but not AMPK signaling pathways.
- ATF4 is required for glucose deprivation-induced fructolysis
- ATF4 binds to promoters of fructolytic genes to activate fructolysis upon glucose deprivation
- ATF4-dependent fructolysis rescues proliferation and colony formation of GBM cells under glucose-deprived condition
- ATF4-dependent fructolysis is required to maintain GBM growth
- Pharmacological blockage of fructose utilization suppresses proliferation and colony formation of GBM cells under glucose-deprived condition
- Pharmacological blockage of fructose utilization shows therapeutic potential
- The expression levels of ATF4, GLUT5, and ALDOB positively correlate with each other in GBM specimens and indicate a poor prognosis in GBM patients

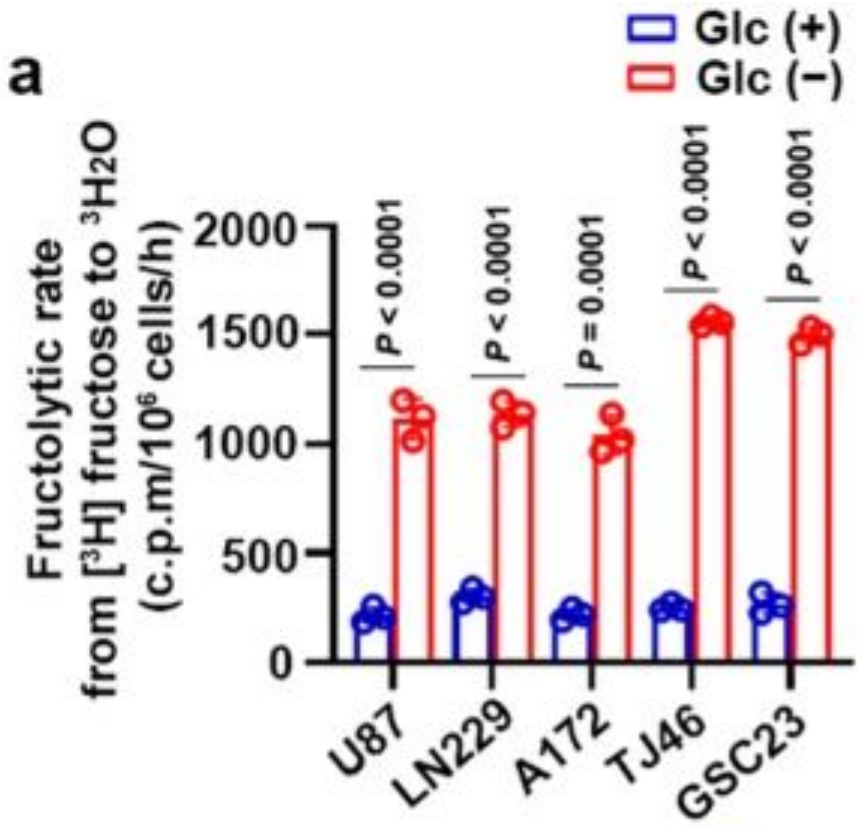
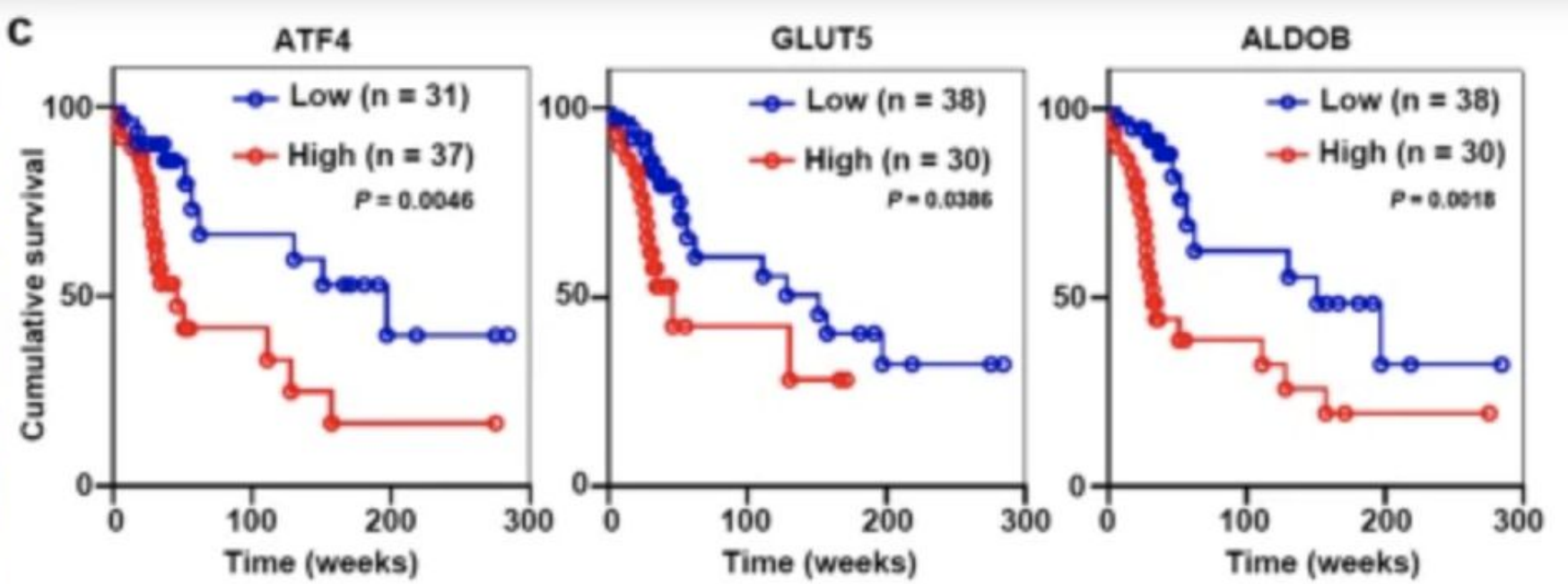


Figure 1a.

- glucose deprivation promotes fructolysis
- shows the fructolytic or fructose metabolic rate of GBM cells that were deprived of glucose for 18 hours
- fructolytic rate measured through conversion of D-[5-3H] fructose to 3H2O
- appropriate method to display data

Figure 6c.



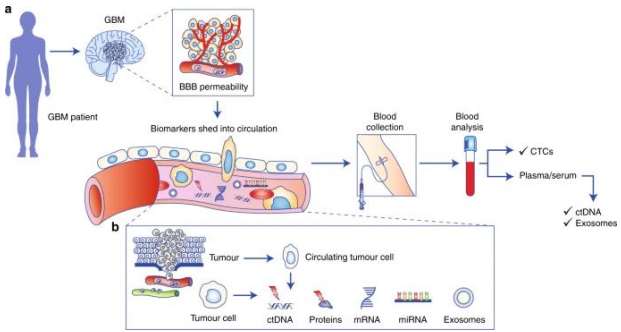
- data being used comes from 68 patients with glioblastoma multiforme w/ antibodies
- compares the survival time of patients with low levels vs. high levels of ATF4, GLUT5, and ALDOB
- appropriate method as it plots survival over time while also taking into account the expression levels of ATF4, GLUT5 and ALDOB

Review Paper: Circulating biomarkers in patients with glioblastoma

Current Approach to Glioblastoma Treatment

- Diagnosis
  - Neuroimaging followed by a biopsy (usually a tissue biopsy) to diagnose, grade, and characterize the tumor
  - Certain tumors are inaccessible or cause side effects upon removal
- Treatment
  - Usually surgery followed by chemotherapy and/or radiation
- Prognosis
  - MRI scans
    - Difficulty differentiating between tumor progression and post-radiation effects (pseudoprogression)
    - Most common technique to advise treatment options and surgery

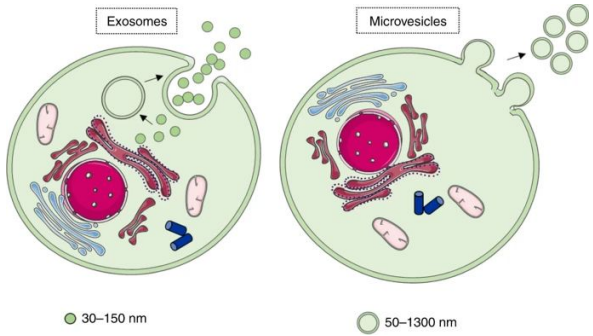
Liquid Biopsies



- Samples from the blood, urine, or saliva
  - Tumors shed contents into circulation
  - Majority of biomarkers have a short half-life
  - Assumes contents can pass through the blood brain barrier (BBB)
- BBB is a tight junction
  - Integrity determined by various proteins (claudin 3,5,12, etc.)
  - GBM induces a proangiogenic environment -> “leaky” BBB
  - Hypoxia induced by GBM also seems to promote the proliferation brain microvascular endothelial cells -> “leakly” BBB
  - “Leakiness” is clinically supported by the penetration of gadolinium through BBB under MRI scans
  - Sheds the possibility of performing liquid biopsies with systemic circulating fluids

Markers

- Circulating Tumor Cells (CTCs)
  - Very rare in glioblastoma due to the BBB and low survival rate of metastasis
  - Isolation is done by using specific protein markers to filter out the CTCs in the blood sample
  - Ex: EpCAM, an epithelial cell adhesion marker
  - Small sample size, but using CTCs as a biomarker seems to have promising applications
- Circulating Tumor Nucleic Acids or Cell-free DNA (cfDNA)
  - Suggested that main source is from apoptotic tumor cells that release RNA and DNA
  - Need to know the tumor variations in the genome
  - Ex: miR-21, associated with lower survival
  - Potentially lncRNA
- Extracellular Vesicles (EV)
  - Historically thought to be signs of apoptosis
  - Recently recognized as a tool for cell-to-cell communication
  - Detected by using cell surface markers (Ex. integrins)
  - Contents inside of EVs are usually maintained
  - Exosomes and Microvesicles



References

Chen, C., Zhang, Z., Liu, C., Wang, B., Liu, P., Fang, S., Yang, F., You, Y., & Li, X. (2022). Atf4-dependent fructolysis fuels growth of glioblastoma multiforme. *Nature Communications*, *13*(1). https://doi.org/10.1038/s41467-022-33859-9

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