The Effect of Radiation on Cellular Metabolism in Glioblastoma and GBM Stem-like Cell Lines

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Overview: Glioblastoma

- Glioblastoma Multiforme is the most aggressive brain tumor with a median survival of 14 months

- Standard treatment of care:
  - Maximum surgical resection
  - Chemotherapy with Temozolomide (TMZ)
  - Radiation therapy

- Even with all of the treatment, GBM usually comes back. Recurrence is largely due to GBM stem-like cells that are radio-resistant
  - Tumor stem-like cells can proliferate into any type of GBM tumor cell

- Targeting these cells and making them radio-sensitive is a huge focus of research in order to decrease the chance of recurrence
All cancer cells employ the Warburg Effect:
- A phenomenon in which tumor cells prefer glycolysis over oxidative phosphorylation in order to produce energy

Due to cancer’s high rate of glycolytic activity, we must look at proteins that assist in this process

Observing the effect of radiation on GLUT1 and GLUT3 proteins
- **Glucose Transporter 1**: mammalian glucose transporter; high levels in GBM
- **Glucose Transporter 3**: neuronal GLUT; up regulated in GSCs
Cell lines

- We use different cell lines because each one has its own characteristics and mutations; it will give us a bigger picture

- Normal Astrocytes
  - Adherent
  - Normal human cells derived from brain tissue
  - Star-shaped cells in the brain and spinal cord

- U251
  - Adherent GBM tumor cells

- GBM1
  - Stem-like cells
  - Live in neurospheres

- NSC11
  - Stem-like cells
  - Live in neurospheres
Methods

- **Flow Cytometry:** measures glucose uptake
  - Cells had a PI stain to measure viability
  - Used a florescent glucose analogue (2-NBDG)
- **RT-qPCR:** observe gene expression for GLUT1 and GLUT3
  - Extract the cell’s RNA
  - qPCR to quantitate expression levels
- **(Attempted) Western Blotting:** look at protein levels of GLUT1 and GLUT3
  - Membrane proteins are very easy to degrade
  - The antibodies used to detect the proteins were very difficult to work with, and I didn’t really get a signal with the GLUT1 or GLUT3 antibody
All three cell lines (tumor cells and stem-like cells) take in more glucose after they are irradiated.

- Indicates a higher metabolic rate
- The tumor cells use more glucose than the stem-like cells
- This glucose uptake increases the most after irradiation

Elizabeth Spehalski Ph.D. (in prep)
Results: RT-qPCR

- After irradiation, the GLUT1 expression decreased in tumor cells.
- In GBMJ1s (stem-like cell line), GLUT1 expression actually increased after irradiation.
- In the NSC11s, the gene expression stayed relatively stable.

- After irradiation, GLUT3 expression exhibits an upward trend in all three cell lines.
  - In tumor cells and GBMJ1s, GLUT3 expression definitely increased.
  - In the NSC11s, the gene expression didn’t really change.
Both the tumor cells and stem-like cells take in and utilize more glucose AFTER irradiation. This indicates an elevated rate of glycolysis in all three cell lines.

The gene expression levels for both proteins are sensitive to radiation in the tumor cells and the GBMJ1 stem-like cell line.

- The expression levels in the NSC11 stem-like cell line don’t seem to be affected.

For the future...

- Look at different time points following irradiation
- Get my Western Blots to work!!!
What I learned...

- Research takes FOREVER!!
  - It takes almost 3 days to do one experiment
  - It is really hard to start projects on Fridays because the cells usually sit for 24 hours, and I didn’t want to go in on the weekends
- Sometimes experiments just don’t work.
- Since the fiscal year ends in September, Christmas at NIH is in August
- Pipets are really easy to break
- Everything looks like water, so you really have to label all of your test tubes
- Everyone is actually really normal and friendly!!!
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