

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

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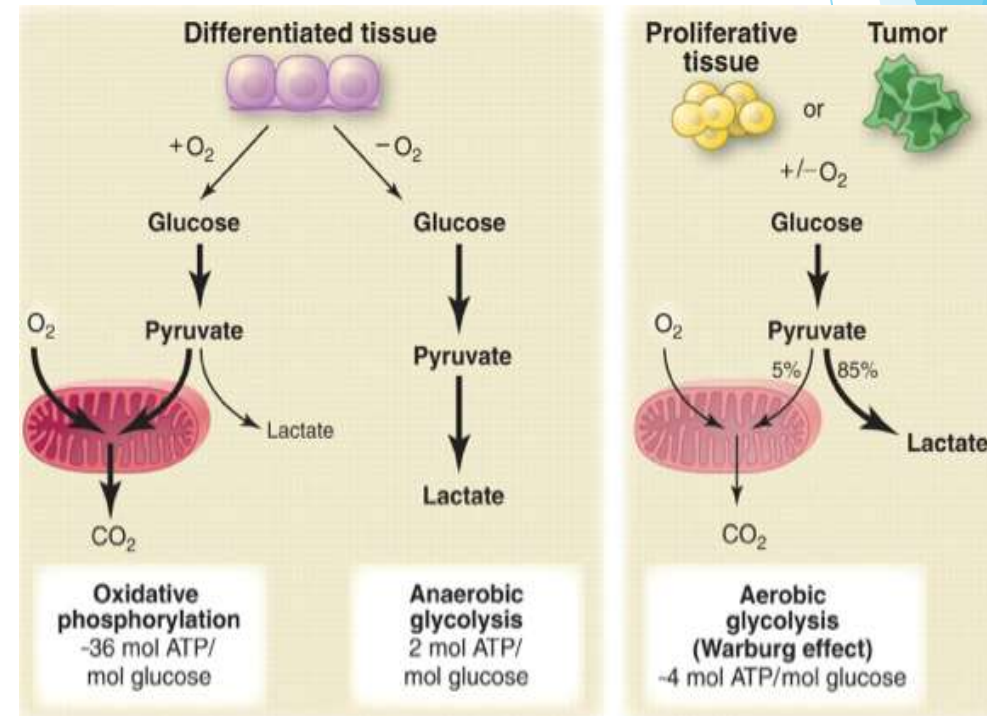
National Cancer Institute, National Institutes of Health

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

My project

- ▶ 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



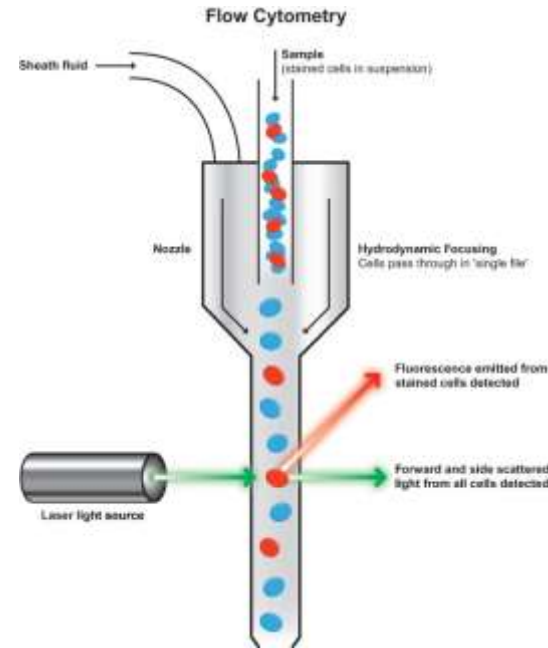
Cell lines

- ▶ 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
- ▶ GBMJ1
 - ▶ 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 fluorescent 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

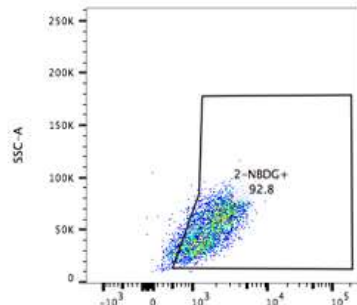
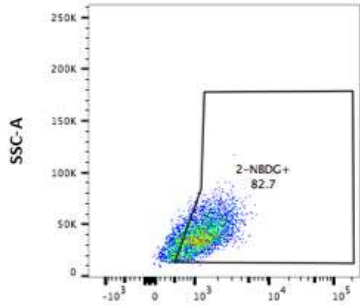


Results: Flow Cytometry

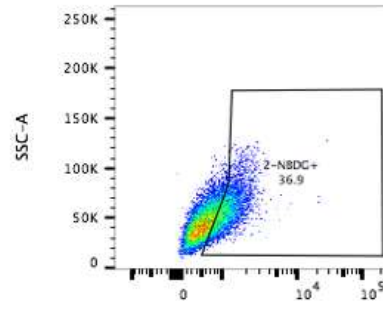
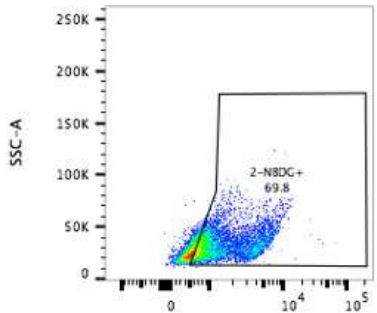
No IR

6 GY IR

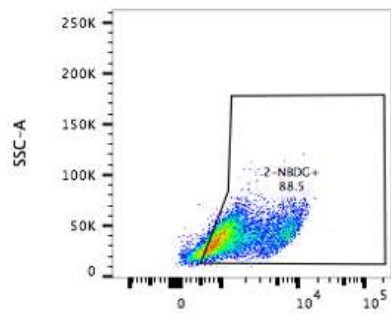
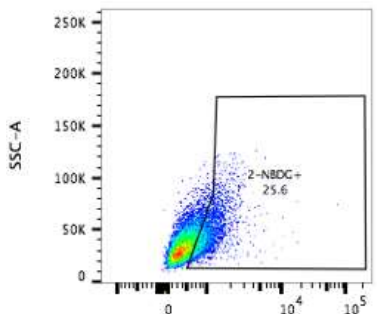
U251



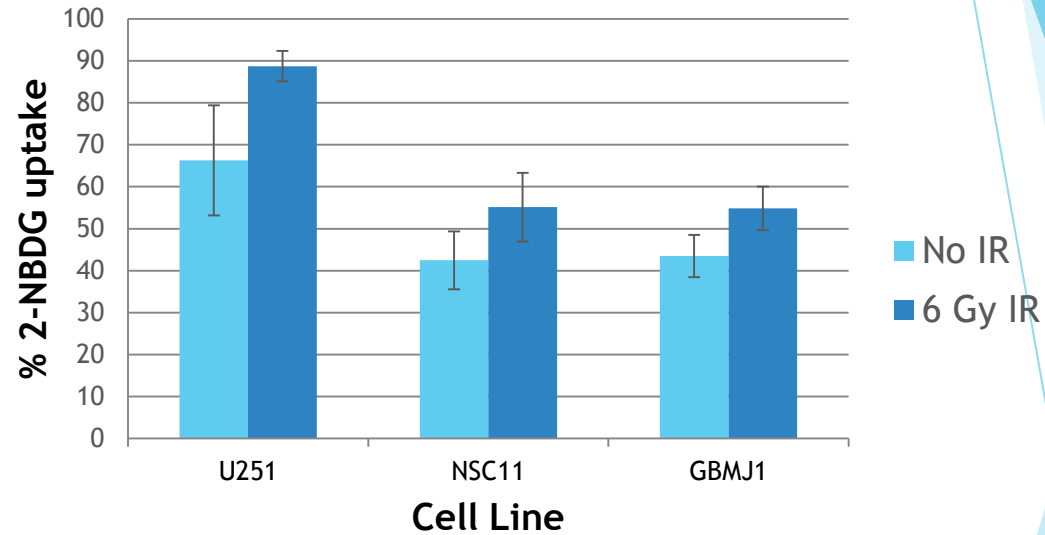
GBMJ1



NSC11



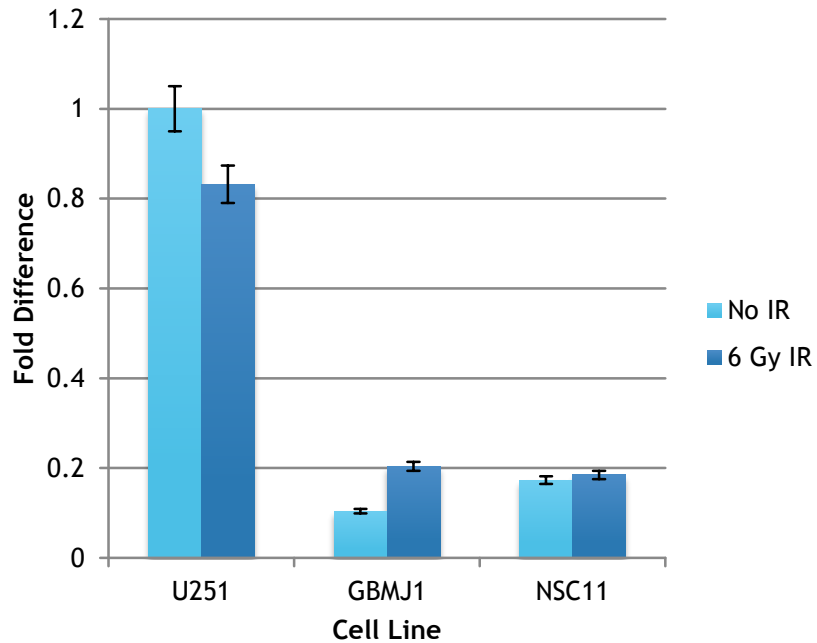
Glucose uptake



- ▶ 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

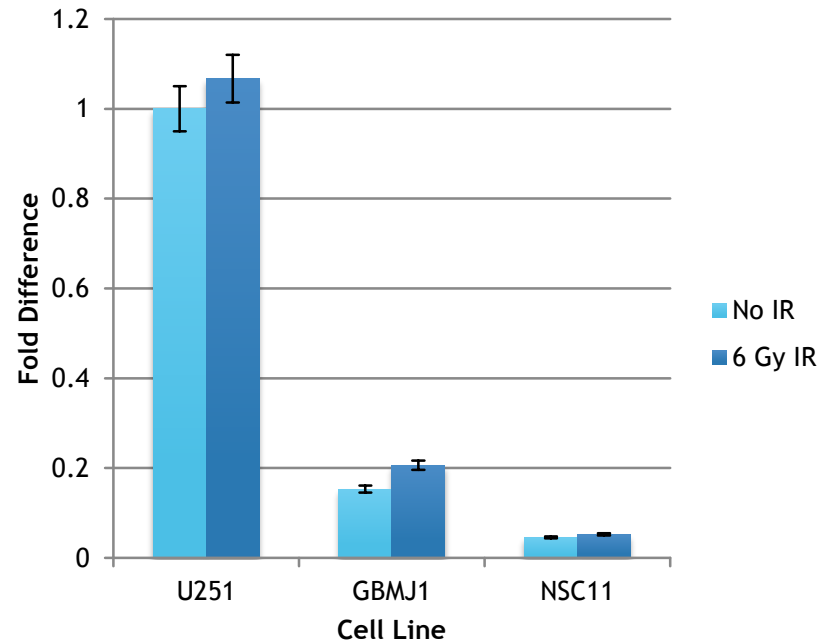
Results: RT-qPCR

SLC2A1 Expression



- ▶ 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

SLC2A3 Expression



- ▶ 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

Summary

- ▶ 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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