Using 4-D imaging of the zebrafish model to observe pharmacodynamics of a therapeutic drug on glioblastoma

Sophie Means¹, John Gamble², Leah Wehmas³, and Julie Greenwood²

¹Department of Integrated Biology  ⁄  ²Department of Biochemistry and Biophysics  ⁄  ³Department of Environmental and Molecular Toxicology

Glioblastoma Multiform
- Type of aggressive brain cancer
- Five year survival rate of 4.70%
- Not often detected until it’s in the later stages when the symptoms appear
- Surgery is not an effective option due to invasion and proliferation of glioblastoma cells outside of the tumor mass before and after surgery

Why the Zebrafish Model?
- Transparent body
- Organism size
- Short assay period
- Little media required
- No adaptive immune system for 28 dpf
- Vertebrate anatomy and brain microenvironment

Therapeutic Inhibitor LY294002
- Phosphoinositide 3-kinase (PI3K) is involved in increased proliferation of glioblastoma cells.
- The compound LY294002 is thought to act as a potential therapeutic inhibitor, preventing PI3K from functioning.

Experimental Outline

Hypothesis: treatment with 6.25µM LY294002 and 0.5% DMSO will reduce glioblastoma cell invasion in the brain microenvironment, as well as create a morphed cell shape.

Procedure:
- Zebrafish are injected with glioblastoma cells.
- LY294002 is placed directly in the zebrafish water supply while imaging using the Zeiss 780 confocal microscope.
- The zebrafish are imaged overnight. A cell tracking software is used to measure the velocity and invasion of the glioblastoma cells in real-time.

Results

Average Minimum Velocity of Glioblastoma Cells

Average Max Velocity of Glioblastoma Cells

Average Mean Velocity of Glioblastoma Cells

Conclusion
- Glioblastoma cells treated and not treated with LY294002 move at a decreased velocity after fifteen hours.
- The compound LY294002 will be used in higher concentration or longer duration before imaging to get significant results in future experiments.
- A control with 0.5% DMSO in regular embryo media is necessary for future experiments.
- The zebrafish will be placed in the compound before imaging in future experiments to determine optimal time required for the compound to become effective.

Zebrafish Xenograft Procedure

Day 0  Pick up embryos
Day 1  PTU treat to maintain transparency
Day 3  Microinject dyed cells into cranium
Day 4  Image cranium overnight

References
- http://www.mun.ca/biology/desmid/brian/BIOL3530/DEVO_03/ch103699.jpg

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