Northern Illinois University

STUDENT ENGAGEMENT FUND
SEF Final Report Cover Sheet

Please Note: If more than one individual worked on this project, **each SEF student will need to submit a separate cover sheet.**

Student Information:

Project Title:	**Culturing Glioblastoma Cancer Stem Cells**
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Student Signature: Rebecca Maas | **Date: 5/5/2017**

Faculty Mentor: **Linda Yasui** | **Date: 5/5/17**

Glioblastoma multiforme is a malignant tumor that grows in the human brain. Patients diagnosed with Glioblastomas (GBM) require very extensive and invasive treatment that includes surgery, chemotherapy, and radiation. Although these treatments are effective for other types of cancer, the rate of recurrence for Glioblastomas are extremely high. After being diagnosed, patients survive an average of 12-15 months afterwards (Ogura, et. al.,). The believed reason for this rate are the Glioblastoma stem cells present in the tumor prior to treatment. These stem cells are essentially identical to Glioblastoma cells that make up the bulk of the tumor, however, they have certain characteristics that allow them to have resistance to cancer treatments. It is difficult to identify stem cells because they are relatively rare and there is an absence of markers to identify them, increasing the difficulty to study them. Stem cells also show continual cell genesis in the central nervous system resulting in the production of functional progeny, allowing for the return of the malignant Glioblastoma tumor (Identification and Characterization of Neural Stem Cells: Why is this so darned difficult?, 2010). Current research in this subject has become especially pressing since there is a promise of a cure for Glioblastomas through STEM cells. Research with STEM cells in order to decrease its resistance to radiation is the main focus. Therefore, through this project we hoped to contribute some understanding and insight for better treatment options for GBMs.

The first step in this experiment was to culture the GBM cells in a DMEM:F12 medium that was supplemented with 10% fetal bovine serum and penicillin/streptomycin. Next, these cells were then formed into cell spheres by being placed in a neural stem cell medium, containing recombinant human epidermal growth factor, basic fibroblast growth factor, and B27 supplement, which was changed every two days. The U87 cell line spheres began growing within 3-5 days and the U251 cell line spheres took up to two weeks. Next, the expression of the

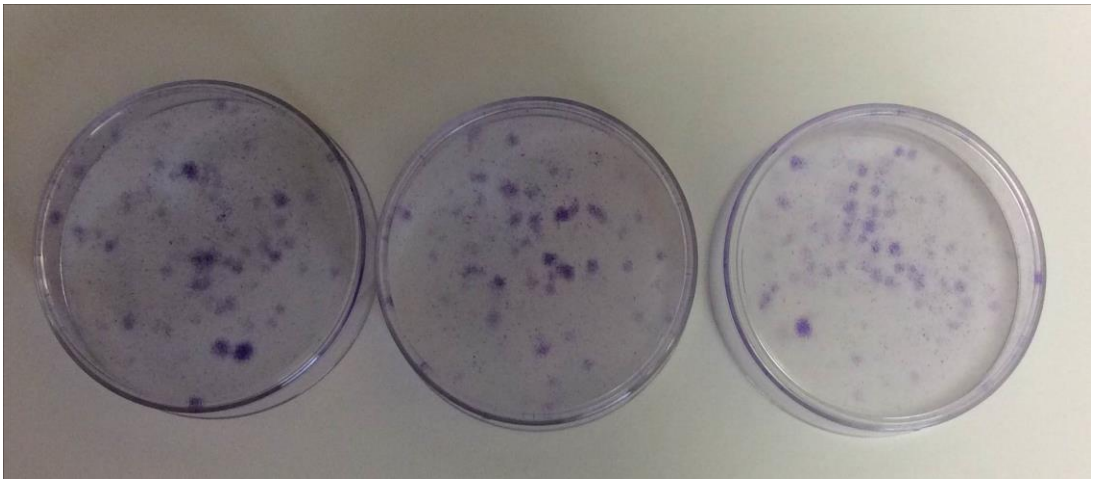
cancer stem cell markers were tested. This was done by qPCR by RNA extraction. There are multiple possible markers to observe however, the most common found in the literature review was CD133, Musashi, and Nestin. Unfortunately, no single marker had been found to be sufficient to confer stem-like properties. Therefore, a combination of different markers must be used in future tests to determine these characteristics. A clonogenicity assay was performed on the cells to prove their ability to self-renew. In this step, one cell was plated per plate and allowed to replicate. The cells were passaged every 1-2 weeks for up to 4 passages, and U87 spheres cells formed clones within the plates, proving that characteristic. In order to further prove the characteristics of self-renewal and proliferation, TEM pictures were taken of the cells on different days to see their growth rate. The U251 cells, that were irradiated, actually continued to grow, which is what was expected. The reason that GBM tumors reoccur so frequently is because the stem cells are essentially resistant to radiation, which is was what shown in this process. Another key characteristic of stem cells is their lack of organelles. So, it was determined that our cells also lacked organelles after careful observation of the ultrastructure through imaging. Lastly, in order to prove that the stem cells can differentiate into different cell types, a multipotency assay was performed. In this test, we stained the bulk cells to show the GFAP marker, which we found to be successful.

Dr. Yasui and I were very happy with our results over the length of the experiment. Although we cannot say we definitively grew stem cells, the data and results we have at this point is very promising. From here, we will continue to perform the necessary tests until we prove the cells we grew are indeed stem cells. This summer we plan to carry out a tumorgenicity assay in which we will inject the stem cells into immunocompromised mice. If a tumor grows in that place then we can finally say we grew stem cells. After that, we will then incorporate the

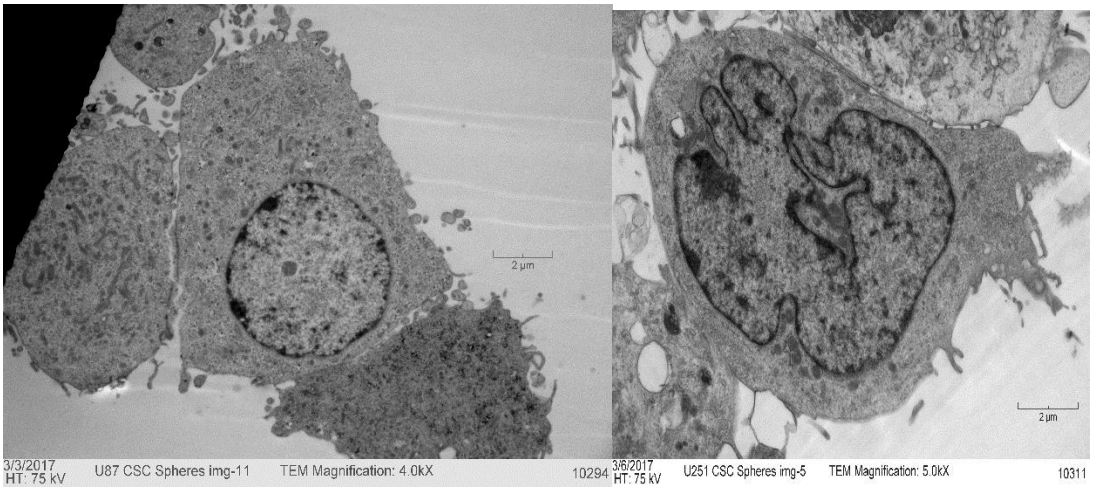
stem cells into further novel therapeutic approaches in hopes of developing a better method of treatment for patients with GBMs.

Participation in the SEF program has allowed me to explore my academic interests further in an environment that is not a controlled classroom. This is extremely significant since experiences like this one will prepare me for my professional future and allow me to develop skills and characteristics I would lack otherwise. Working closely with a successful professor has given me insight into academia and has allowed me to develop close relationships with not only Dr. Yasui but other students as well, which has already proven to be very beneficial for my academic career.

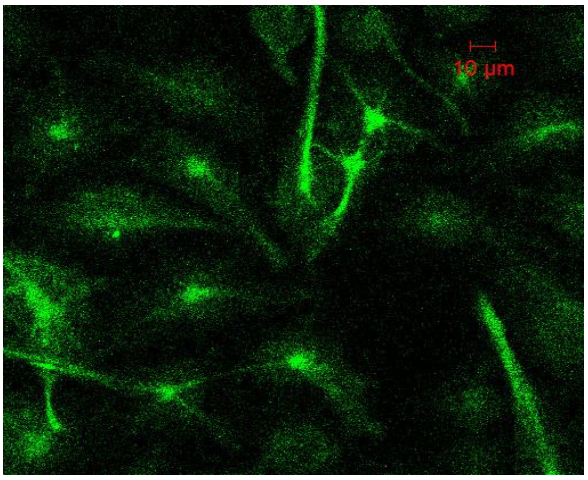
Other Materials



This is the clonogenicity assay in which we proved the cells' ability to self-renew.



Above are the ultrastructure pictures of the U87 cell line (left) and the U251 cell line (right).



Above is an image of the U251 bulk tumor cells which was taken during the multipotency assay to prove the cells' differentiation potential.

Literature Cited

Ogura, Kengo, Takashi Mizowaki, Yoshiki Ogura, Katsuyuki Sakanaka, Susumu Miyamoto, and Masahiro Hiraoka. "Initial and Cumulative Recurrence Patterns of Glioblastoma after Temozolomide-based Chemoradiotherapy and Salvage Treatment: A Retrospective Cohort Study in a Single Institution." *Radiation Oncology*. N.p., n.d. Web. 01 Oct. 2016.

"Identification and Characterization of Neural Stem Cells: Why Is This so Darned Difficult?" *Identification and Characterization of Neural Stem Cells: Why Is This so Darned Difficult?* N.p., 8 Mar. 2010. Web. 01 Oct. 2016.

Budget

Culture plates	596.77
Packets of powered growth medium	81.79
Stem cell growth supplements and growth factors	1,197.92
CSC markers	638.00
TOTAL	2,514.48

All listed prices reflect NIU's negotiated contract prices from Fisher Scientific. All commodities listed are necessary to grow and maintain CSCs (Cancer Stem Cells). Two kind of culture plates are included in the culture plate category. These culture vessels are specifically treated to permit CSC growth into sphere cultures or for a limiting dilution assay used to determine the proportion of CSCs in the population.

The powered growth medium packets are conveniently packaged to make 1 liter of growth medium. We save commodity dollars by filter sterilizing our own medium.

The stem cell growth supplements and growth factors are essential elements for CSC growth. These include B27 supplements, epidermal growth factor and fibroblast growth factor.

CSC markers will be used to confirm that we have cultured CSCs.

The small overage above $2500 will be covered by commodity funds provided by Dr. Yasui's Biological Sciences Departmental Commodities allocation.