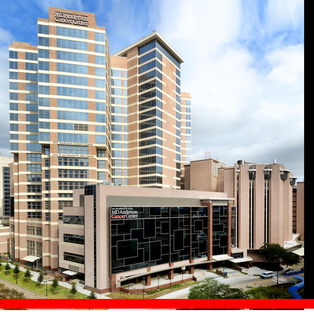

Characterization of Glioblastoma Stem Cells

Joelly Cortes[1] , DiAngelo Gonzalez[2] , Sam McLeod[2] , Aadhya Tiwari[2], Sadhan Majumder[2]

[1] Neuroscience, Yale College

[2] Department of Genetics, The University of Texas MD Anderson Cancer Center

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Background

Glioblastomas are exceedingly aggressive brain tumors with a maximum survival duration as low as 15 to 18 months. In addition to the tumor cells, glioblastomas are also comprised of a small population of cancer stem cells known as Glioma stem cells (GSCs). The GSCs are known to potentiate tumor progression and metastasis and play a crucial role in therapy resistance. Stem cells are characterized by their self-renewal and differentiation capabilities. While noncancerous stem cells are essential for the growth and regeneration of heathy tissues, cancer cells with stem-like qualities are not desirable. The exact origin of these cancer stem cells remains unknown, but their presence has posed a considerable challenge in tumor eradication.

In this project, we learned to characterize glioblastoma stem cells (GSCs) by verifying the presence of stem cell markers that indicate the presence of stemness in these cells. Based on literature, we hypothesize that the GSCs we received have stem cell marker expression but no neuronal marker expression. To test this hypothesis, the expression levels of synapsin, SOX2, and β-III tubulin were analyzed in glioblastoma stem cells and primary neurosphere cultures.

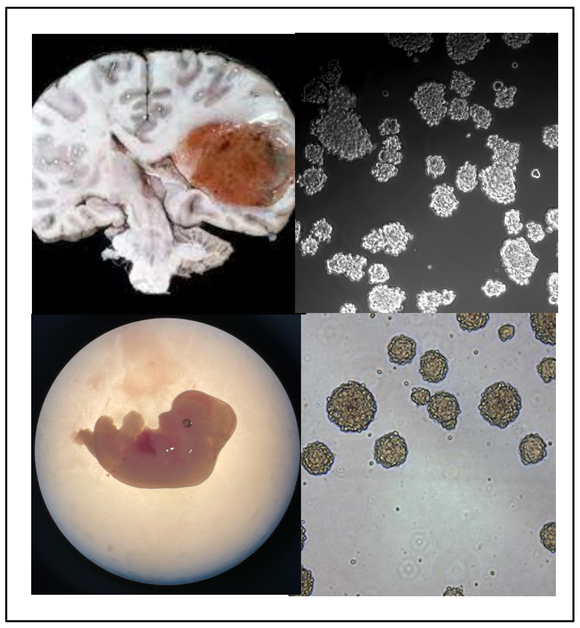

Figure 1. On the top left, a glioblastoma tumor can be appreciated, and, on the right, the glioblastoma spheres they can form when cultured. Underneath, there's the mice embryo from which neural stem cells were derived, and, on the right, the neurospheres they form.

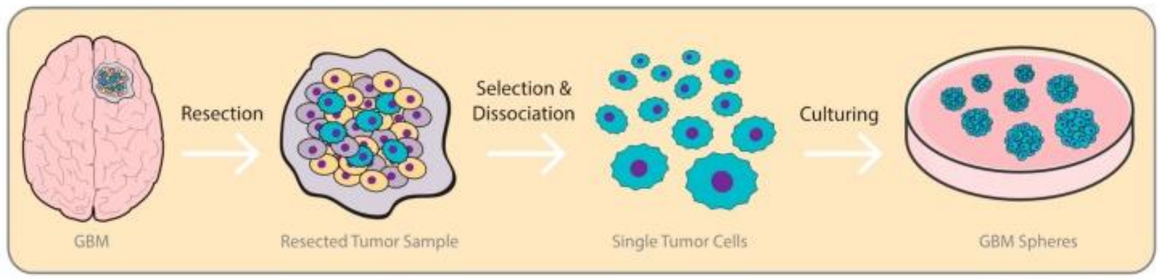

Figure 2. Schematic representation of the establishment of glioblastoma cell cultures derived from tissue removed from cancer patients through surgery.

Introduction

SOX2 is a transcription factor capable of recruiting other growth factors and activating pluripotency. SOX2 is an established neural stem cell marker, which is known to be expressed in Glioblastoma stem cells (GSCs). Contrastingly, β-III tubulin and synapsin are specific neuronal markers that are expressed when neural stem cells differentiate into neurons as β-III tubulin is involved in axonal guidance and synapsin is involved in synapse maturation. As neural stem cells differentiate into neurons, the expression of stem cell markers like SOX2 declines, and the expression of neuron-specific differentiation markers such as β-III tubulin and synapsin augment.

Nuerospheres are heterozygous bodies that contain neural stem cells, neurons, and glial cells. As such, they are known to be positive for stem cell markers and for neuronal differentiation markers. Glioblastomas are known to originate from astrocytes, a type of glial cell. While glial stem cells can differentiate into neurons in response to certain stimuli, glial stem cells typically specialize into glial cells like astrocytes and oligodendrocytes. In order to examine the expression of stem cell and differentiation markers in the GSCs, we used mice embryonic E12.5 primary neurospheres (NSCs) as a control.

Methods

Glioblastoma stem cells (GSCs)_ were obtained by dissociating a tumor sample removed through a patient receptive surgery, selecting single tumor cells, and then culturing the sample. Neural stem cells (NSCs) were derived by removing part of the frontal cortex of mice embryos on the 12th day of embryonic development and then culturing until neurospheres were observed. Western blot was conducted on the GSC cell line and neurospheres in order to analyze the protein expression levels in each cell type for synapsin, SOX2, and β-III Tubulin. The results were quantified using actin as a control.

Results

The western blot shows that SOX2 is present in both the GSCs and the neurospheres which confirms the presence of stem cells in both samples. On the other hand, the expression level of synapsin was almost negligible in the GSCs but high in the neurospheres. For β-III Tubulin, GSCs had a low expression while the neurospheres had a relatively high expression.

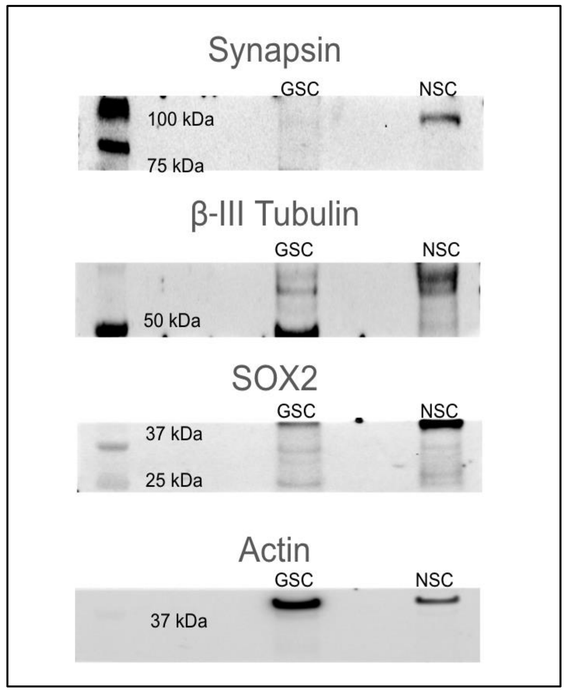

Figure 4. Protein expression bands developed through a western blot procedure.

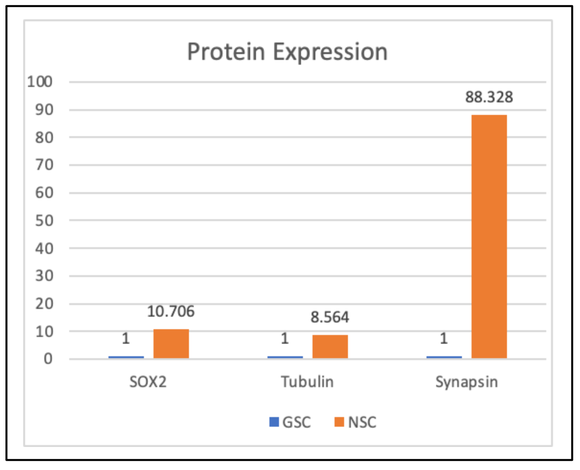

Figure 5. Quantification of protein expression levels in glioblastoma cancer stem cells and noncancerous neural stem cells.

Conclusion

In accordance with our hypothesis, our findings show that GSCs have stem cell marker expression, and almost negligible level of neuronal markers, which confirms that we are working on pure Glioblastoma stem cell population.

Given that SOX2 is a stem cell marker and the GSCs are isolated cancer stem cells, it was expected that the GSCs will have SOX2 expression, and this is confirmed by our results. Considering that β-III tubulin is a marker of early neuronal differentiation and that synapsin is a marker of late differentiation, the GSCs should not have expressed these markers because glial stem cells do not typically differentiate into neurons. While synapsin expression was almost negligible in the GSCs, β-III tubulin was expressed in a very low amount as compared to the neurospheres, and this suggests that the GSC line is mostly the pure glioblastoma stem cells. The mild level of β-III tubulin expression could perhaps be a result of some of the glioblastoma stem cells differentiating because the culture conditions were not maintained stringently.

Since the expression of differentiation markers was not anticipated, a possible future direction for this study could be investigating why the GSCs expressed mild levels of β-III tubulin, the early differentiation marker.

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