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THE EFFECTS OF SELECTED TRANSCRIPTION FACTORS AND SMALL MOLECULES ON CONVERTING GLIOBLASTOMA CELLS TO GABAERGIC NEURONS

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ABSTRACT

Previous studies have demonstrated that the overexpression of the transcription factor Ascl1 can reprogram human glioma cells to functional neurons both *in vitro* and *in vivo*. (Pedder et al., 2012; Suvà et al., 2014). In this study, a combination of six small molecules were added with the single transcription factors Ascl1 or NGN2 to convert cultured human glioblastoma cells to GABAergic neurons within 12 days with a conversion efficiency of 72% and 52%, respectively. Quantification of neuron markers DCX and MAP2 also reveals that the small molecules play a role in the overall increase of neuronal reprogramming efficiency. Interestingly, transcription factors alone can reduce the cell proliferation significantly, and addition of small molecules cannot further reduce the proliferation of glioma cells. The results indicate that both Ascl1 and NGN2 combined with the six small molecules, successfully converted glioma cells to neurons. However, NGN2 converted glioma cells into both glutamatergic and GABAergic neurons, whereas Ascl1 converted glioma cells significantly to GABAergic neurons. This study opens a new avenue to treating glioblastoma through less invasive approach when compared to conventional chemotherapy, and may have broad implications in cancer treatment in future trials.

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Chapter 1

Setting the Stage for Gliomblastoma Cell Conversion

Facts and Trends of High Grade Glioma

Gliomas are the most common primary tumor of the central nervous system and are derived from glial cells such as astrocytes or supportive cells in the brain. They account for almost 30% of all primary brain tumors. There are different types of gliomas including astrocytomas, ependymomas and oligedengrogliomas that originate from astrocytes, ependymal cells lining the ventricles, and oligodendrocytes respectively. Gliomas can be classified into four grades (I, II, III, IV) based on their malignancy, with Grade I gliomas being benign and Grade IV being high-grade malignant gliomas. Glioblastoma multiforme (GBM) is the most common and aggressive high-grade glioma (astrocytoma WHO grade IV) (Zhao, et al., 2012). They are relatively incurable with a survival time of 15 months as they are resistant to treatments and are responsible for tumor recurrence (Deleyrolle et al., 2011; Pedder et al., 2012). Despite the therapeutic improvements through combined neurosurgery, chemotherapy, and radiotherapy available today, the patients' survival has barely been prolonged. Therefore, treatment of glioma remains as a major challenge for neuro-oncologists today.

The primary high-grade glioma tumors contain a heterogeneous population of cells ranging from highly tumorigenic stem cells to more differentiated cancer cells. The actual origin of these tumors, however, are still not well defined but previous studies have noted evidence that suggests that these tumors may have originated from stem or progenitor cells found in the adult human brain (Guichet et al. 2013). Despite the definite cause of the primary brain tumors remaining unknown, some potential risk factors that may increase the possibility of a brain tumor

include: old age, although certain gliomas such as astrocytomas, ependymomas and pilocytic may occur in young adults or children; exposure to radiation from atomic bombs, or repeated ionization radiation from radiation therapy; and family history and genetics (Llaguno et al., 2009).

The deadly nature of such gliomas originates from their exponential growth and invasive behavior, as they tend to contain a fraction of multipotent stem-like cells that are highly tumorigenic. These cells are undifferentiated and have high proliferation rates (Guichet et al. 2013). Therefore, one potential way of blocking tumor growth and proliferation would be to convert them to terminally differentiated cells, particularly neurons. Previous studies have discussed gene transfer as a treatment of malignant glioma. Studies have demonstrated using specific transcription factors to convert human glioma cells into functioning neurons (Su et al., 2014; Zhao et al., 2012). However, there have not been many previous studies on reprogramming glioma cells specifically to GABAergic neurons using a combination of a single transcription factor and small molecule combinations to increase the efficiency of conversion. This study, therefore, focuses on combining a single transcription factor Ascl1 or NGN2 with a combination of small molecules to increase the efficiency of glioma cell conversion to GABAergic neurons.

Benefits of Neuronal Reprogramming of Glioma

This research project focuses on converting glioma cells to GABAergic neurons using a combination of transcription factors. Malignant gliomas are highly aggressive and very difficult to treat. Current clinical treatments mostly aim to improve the neurological deficits that are seen in patients and prolong the survival time rather than finding a cure. They are considered incurable and all the treatment methods available are only able to prolong survival of most

patients for a few months. Therefore, finding alternative methods to treat these tumors are necessary and one possible solution being neuronal reprogramming. Being able to convert glioma cells into neurons would inhibit the glioma cells from their proliferative state, especially if they are converted to GABAergic neurons (Yang et al., 2017). GABA is the main inhibitory neurotransmitter and GABA receptor presence have been linked to the attenuation of glioma proliferation.

Gliomas are also often accompanied by seizures. Previous studies have revealed the role of tumor derived excitatory glutamate (Glu) accumulation on the occurrence of epileptogenesis. The high Glu levels may also cause excitotoxicity and potentially aid in the glioma growth by vacating space for tumor expansion (Takano et al, 2001; Ye et al., 1999). However, the peritumoral glutamate contribution is not only responsible for tumor growth and epilepsy. GABAergic interneurons are essential to counteract the excitatory activity in maintaining the balance of excitation-inhibition in normal functioning brain. Studies have indicated GABAergic inhibition of peritumoral neurons as well as reduction of GABAergic immunoreactive neurons in the epileptic cortex of low-grade glioma patients, may also contribute to the development of epilepsy (Cambell et al. 2015; Blanchart et al. 2017; Park et al., 2017). Thus, the generation of GABAergic neurons could potentially not only reduce tumor growth by converting them to a terminally differentiated state such as a neuron, but it can also reduce the epileptic symptoms and restore the excitation-inhibition balance in patients suffering from high-grade glioblastoma.

Selecting Transcription Factors for Glioma Conversion

Previous studies have discussed gene transfer as a treatment of malignant glioma. Researchers have demonstrated the overexpression of transcription factors Pax6, P53, and Pten

affect glioma growth, however, despite the reduction of glioma growth, the glioma cells still held their proliferative states (Klatzmann et al., 2008; Li et al., 1998; Gomez-Manzano et al., 1996). Other studies have demonstrated using transcription factors such as Ascl1, Brn2, SOX-11, and NGN2 to convert fibroblasts into functioning neurons (Su, et al., 2014; Zhao et al., 2012). One of the transcription factors we decided on is Acheate scute homolog-1 (Ascl1). Ascl1 is a major bHLH proneural transcription factor necessary and sufficient for neuronal generation. Overexpression of Ascl1 has previously induced full neuronal differentiation from neural precursor cells or somatic cells (Park et al., 2017). Increasing Ascl1 expression has also been associated with glioblastoma stem cells restoring their neuronal lineage potential and reducing tumor-like proliferative properties by opening new sites in the chromatin for neurogenic gene expression program activation. Ascl1 binds to chromatin and activates gene expression. It is able to function as a pioneer factor by opening closed chromatin leading to the expression of neuronal fate genes and differentiation. Studies previously demonstrated that in human glioblastoma (GBM) cells two-thirds of the binding regions are contained in closed regions and Ascl1 promotes chromatin accessibility at enhancer regions to transcriptionally activate the neuronal target genes promoting differentiation (Park et al., 2017; Guichet et al., 2013). Ascl1 also suppresses precursor and glial fate in favor of neuronal fate as it initiates fate determination program (Park et al., 2017). Researchers have also found Ascl1 to rapidly generate GABAergic induced neuronal cells from human pluripotent stem cells (Yang et al., 2017). Therefore, increasing Ascl1 expression to induce GABAergic neurons could potentially reduce glioma proliferation, therefore tumorigenesis, and reduce certain symptoms related to brain cancer such as epileptic seizures (Haglund et al., 1992).

Another transcription factor we decided to use is Neurogenin2 (NGN2). NGN2 belongs to the bHLH transcription factor family involved in neuronal differentiation and specification. Previous studies have demonstrated NGN2 involved in converting astrocytes and fibroblasts to generate neurons directly (Zhao et al., 2015). Neurogenin2 has been involved in converting human fetal lung fibroblasts to cholinergic neurons with high efficiency as well as successfully reprogrammed astroglia to excitatory neurons (Heinrich et al., 2010; Liu et al, 2013). However, researchers had demonstrated that NGN2 have been seen to generate excitatory neurons over inhibitory neurons (Nehme et al., 2018; Heinrich et al., 2010). Therefore, NGN2 is being used to compare the GABAergic neuron generation by Ascl1, a transcription factor previously linked to GABAergic neuron, versus by NGN2 that have been linked to generation of other subtypes of neurons. It also allows to analyze the effects of the small molecule cocktail on improving the generation of GABAergic neurons when combined with each of the single transcription factors.

Selecting Small Molecules Combination for Glioma Conversion

Several previous studies have established that certain drug cocktails or small molecules can help with cell reprogramming by regulating transcription factors and modifying epigenetic mechanisms (Li et al., 2014; Hou et al., 2013). Small molecules are low molecular weight compounds (less than 9000 Daltons) that regulate biological processes. Most drugs are considered small molecules. They can modulate cellular pathways involved in proliferation, differentiation, or cell death and, therefore, could potentially be used to reprogram glioma cells to neurons and increase the efficiency of GABAergic neuronal conversion when combined with transcription factors.

It has been previously demonstrated that such small molecules cocktails can accelerate differentiation of human pluripotent stem cells into functional nociceptors, which are sensory receptors for pain. LDN193189 and SB431542 were used to inhibit SMAD signaling followed by CHIR99021, a GSK kinase inhibitor and DAPT, a γ-secretase inhibitor that blocks Notch signaling, to initiate neural induction (Chambers et al., 2012). In other cases, embryonic fibroblasts have been successfully converted successfully to pluripotent stem cell, and neurons using cocktails of small molecules (Hou et al, 2013; Cheng et al., 2014; Mak et al., 2012). In other cases, researchers have demonstrated how the sonic hedgehog (Shh) could be substituted with hedgehog signaling activators such as the small molecules purmorphamine (Pur) and smoothened agonist (SAG) during dopaminergic maturation. Previous studies from my lab have also used several combinations of small molecules to convert human fetal astrocytes to neurons through modulation of multiple signaling pathways, recently narrowing down from nine small molecules to four (Zhang et al., 2015; Yin et al., 2019). For this thesis project, I have narrowed down the small molecules to CHIR99021, DAPT, LDN193189, and SB431542, which will be referred as Core, along with SAG and PMM.

My honors thesis reveals a novel method in which human glioblastoma can be reprogrammed into GABAergic neurons by using a single transcription factor combined with six small molecules to assist the conversion. Reprogramming of human glioblastoma cells to GABAergic neurons can potentially open a new approach to treating glioma by reducing the proliferation of brain tumors as well as addressing epilepsy, which is often seen to co-occur with tumorigenesis in the brain.

Chapter 2

Materials and Methods

Plasmid Construction and Retrovirus Production

The mouse NGN2 and Ascl1 plasmids were constructed from our PCR product and inserted into a pCAG-GFP-IRES-GFP retroviral vector (Zhao et al., 2006) (gift of Dr. Fred Gage) to generate pCAG-NGN2-IRES-GFP or pCAG-Ascl1-IRES-GFP. Viral particles were packaged in gpg helper free human embryonic kidney (HEK) cells to generate vesicular stomatitis virus glycoprotein (VSV-G)-pseudo typed retroviruses encoding neurogenic factors in CellMax hollow fiber cell culture system (Spectrum Laboratories). The titer of GFP, NGN2, and Ascl1 viral particles was about 2 x 10^5 pfu/ml, 8 x 10^7 pfu/ml, and 2 x 10^6 pfu/ml respectively, which were determined after transduction of the HEK cells. Similar titer was achieved by adding around 200ul GFP, 5ul NGN2, 60ul Ascl1 virus containing medium to each well (24-well plates).

Human GBM Cell Culture

Human GBM cell line, U251, was purchased from Sigma. The U251 cells were cultured in glioma culture medium (GCM), which included MEM (GIBCO), 0.2% penicillin/streptomycin (GIBCO), 10% FBS (GIBCO), 1mM Sodium Pyruvate (GIBCO), 1% Non-Essential Amino Acids (NEAA, GIBCO), and 1 x GlutMAX (GIBCO). The cells were kept at 37°C in humidified air with 5% CO2.

Reprogramming Human GBM cells to Neurons

For chemical reprogramming, U251 cells were seeded in poly-D-lysine-coated (PDL) coverslips in 24-well plates at least twelve hours before the retrovirus infection reached a density of 10,000 cells per coverslip. The retrovirus was used to increase the transcription factor NGN2 or Ascl1 expression. GFP, NGN2 or Ascl1 retrovirus was added in GBM cells with 8 μg/ml Polybrene (Santa Cruz Biotechnology). Culture medium was replaced by neuronal differentiation medium (NDM) on the next day to help with neuronal differentiation and maturation. NDM included DMEM/F12 (GIBCO), 0.4% B27 supplement (GIBCO), 0.8% N2 supplement (GIBCO), 0.2% penicillin/streptomycin, 0.5% FBS, Vitamin C (5 μg/ml, Selleck Chemicals), Y27632 (1 μM, Tocris), GDNF (10 ng/ml, Invitrogen), BDNF (10 ng/ml, Invitrogen) and NT3 (10 ng/ml, Invitrogen). Cells were maintained at 37°C in humidified air with 5% CO2.

The day after adding the retrovirus, used to overexpress the transcription factors, the NDM medium was replaced with Core+SAG+PMM small molecule combination which was prepared in Induction medium. The small molecule combination was composed of SB431542 (5 uM, Tocris #1614) LDN193189 (0.25 uM, Sigma #SML0559), CHIR99021 (1.5 uM, Tocris #4423) DAPT (5 uM, Sigma #D5942) which made up the Core, and SMO agonist (SAG) (0.1 uM, Cayman #11914) and Purmorphamine (PMM) (0.1 uM, Cayman #10009634). The drug was refreshed every four days. On the 12th day of post infection, the cells were fixed for staining or the medium was changed to regular NPC medium again, which was then refreshed as needed.

In vitro Immunofluorescent Staining to Analyze Protein Expression

After infection and small molecule treatment of U251 Glioma cells, on targeted days (12 days' post infection, 24DPI), the coverslips were picked from the wells and fixed with room

temperature 4% PFA in 1X PBS and incubated for 15 minutes at room temperature. Then, the cells were washed with 1X PBS for three times for 5 minutes each. Afterwards, blocking buffer (0.3% Triton, .5% normal donkey serum, in 1X PBS) was administered for at least 1 hour. Depending on the transcription factors and cancer biomarkers being tested, a combination of primary antibodies was made (see *Table 1: Antibody List*) with the blocking buffer. After blocking, the cells were incubated with primary antibody for at least 2 hours at room temperature covered or 24 hours/ overnight in 4ºC in the dark wrapped in shrink wrap and tin foil.

Following primary antibody incubation, the cells were washed with 0.05% or 0.3% Triton in 1X PBS three times, 5 minutes' interval each. Secondary antibodies conjugated with Alexa Fluor 488, Alexa Fluor 594, and Alexa Fluor 647 were prepared using the same blocking buffer in order to counterstain the primary antibody (see *Table 1: Antibody List*). The secondary antibodies were incubated for at least 2 hours at room temperature covered. When finished, another wash using 0.3% Triton in 1X PBS was administered for 2 times for 10 minutes each. After the two washes, the final wash was administered using a DAPI solution diluted 1:5000 with the 0.3% Triton in 1X PBS washing buffer for 10 minutes.

After the final wash, the coverslips were mounted on micro-slides covered with anti-fading mounting solution containing DAPI (Invitrogen) and sealed with nail polish (EMS) and let to dry at room temperature in the dark before storing them in 4ºC.

Microscope Images

Olympus Confocal Laser Scanning Microscope. The Confocal Laser Scanning microscope (Olympus) was used to take the fluorescent images. *In vitro* coverslip images were taken using the normal single-layer snap function as well as Z-stacks and tile images to cover large areas.

Most images focused in at 20x magnification. Some images zoomed into a magnification of 40x using an oil lens.

REVOLVE4 Upright, Inverted, Brightfield, Fluorescent Microscope (Echo). The Revolve Fluorescent microscope (Echo) was used to take multiple single layer snap images of the coverslips for quantification.

Quantification Converted GABAergic Neurons

The statistical analysis on the conversion of infected glioma cells with transcription factors and small molecule treatment was done through cell counting using the ImageJ software. The most representative fields in the three batches of each of the two tested transcriptional factor group (Neurogenin2 (NGN2), and Acheate-scute (Ascl1) plus GFP control) were used for counting. The statistical significance and p-value was determined using one-way ANOVA test followed with Dunnett's test.

Chapter 3 Results

Screening for Small Molecule Combinations

Based on the literature sources various transcription factors and small molecules were identified to convert human glioblastoma to GABAergic neurons. We found previous publications discussing the use of Ascl1 directly in order to convert certain types of glioma cells to neurons (Yang et al., 2017; Haglund et al., 1992). Ascl1 had particularly been used for the generation of GABAergic neurons (Park, et al., 2017). Later, another transcription factor, Neurogenin2 (NGN2), which also had been involved in neuronal reprogramming, was used to compare the conversion of GABAergic neuron generated by using Ascl1 versus NGN2 when combined with small molecules. For this experiment, the U251 human glioblastoma was infected either with Ascl1, NGN2 or GFP control. Then the next day, either a combination of small molecules or DMSO was added. For the experiment, there were 6 candidates that met our selection criteria based on previous findings as well as the research being done in the small molecule project in Dr. Gong Chen's lab. The previous studies had demonstrated the use of nine small molecules: LDN193189, SB431542, TTNPB, thiazovivin, CHIR99021, VPA, DAPT, SMO agonist (SAG), and purmorphamine (PMM), successfully converted astroglial cells to functional neurons, which were recently narrowed down by a follow-up study to DAPT, CHIR99021, SB431542, and LDN193189 (Zhang et al., 2015; Yin et al., 2019). The major selection criteria were induction of neurogenesis, particularly GABAergic neurons and minimal cell death or apoptosis. The small molecules selected were SB431542, LDN193189, CHIR99021, DAPT, which made up the Core, and SMO agonist (SAG) and Purmorphamine (PMM). Initially, after introducing the retrovirus infections to the U251 human glioblastoma to

overexpress Ascl1 or NGN2, the cells were treated with either DMSO (control), Core (SB431542, LDN193189, CHIR99021, and DAPT), or Core+SAG+PMM. The drugs were refreshed every four days and checked for apoptosis and morphology change. At 12DPI the cells treated with just Core and Core+SAG+PMM showed similar amount of apoptosis. However, the Core+SAG+PMM showed more morphological changes. At 24DPI, the Core+SAG+PMM again showed more morphological changes than Core alone, while having similar amount of cell death or apoptosis. Therefore, we finalized the small molecule combination of Core, SAG and PMM to be added to the single transcription factors and GFP control to see the effect on neuronal reprogramming and specially glioma cell conversion to GABAergic neurons.

Effects of Transcription Factors and Small Molecules on Human GBM Cells *In vitro*

In this study, we initially tested the overall neuronal conversion of the U251human glioblastoma cells by staining them with immature neuronal marker Doublecortin (DCX), at the 12 days' post infection (DPI) time point. By 12 DPI, there was already change in cellular morphology seen when compared between GFP controls and the single transcription factor treated glioma cells (see Figure 1A). They changed from flat thin slightly elongated cells to longer neuron-like cells with neurites.

Immunostaining with early neuronal marker DCX (Doublecortin) at 12 DPI revealed that just using the transcription factors had significantly increased the expression of the neuronal markers compared to the GFP, DMSO control. The cells were much more elongated and neuronal-like. Introducing small molecules Core, SAG and PMM have also increased the expression of DCX in GFP and NGN2 treated U251 glioblastoma cells at 12 DPI, however, the

overall morphology of the converted cells with just the NGN2 versus with the small molecules was very similar (see Figure 1A-B).

The reprogramming efficiency of the small molecule and single transcription factor treatment was quantified based on the ratio of DCX to GFP (green fluorescent protein) expression during immunostaining results for the cell cultures after 12 DPI. With the introduction of the small molecules there was significant difference between the expression of DCX in GFP, DMSO control group (GFP, 0%) and the small molecule treated GFP glioma cells (GFP+D, 16.3%± 3.3% SEM; n = 3 batches) as can be seen in Figure 1C. There was also increase in DCX expression when small molecules were introduced to NGN2 treated glioma cells (NGN2, 71.6% ± 3.3% Standard Error Mean (SEM); NGN2+D, 85.4% ± 3.3% SEM; n = 3 batches each). When comparing the small molecule treated glioma cells with DMSO, there was not much significant difference in DCX expression between the Ascl1 in DMSO (Ascl1) and Ascl1 in small molecules Core, SAG and PMM (Ascl1+D). However, there was a significant difference in the morphology. Even though the cells were elongated and neuron-like in both the Ascl1, DMSO control group and the Ascl1+D small molecule treated group, the cells tend to cluster in the Ascl1+D group but not in the Ascl1, DMSO group.

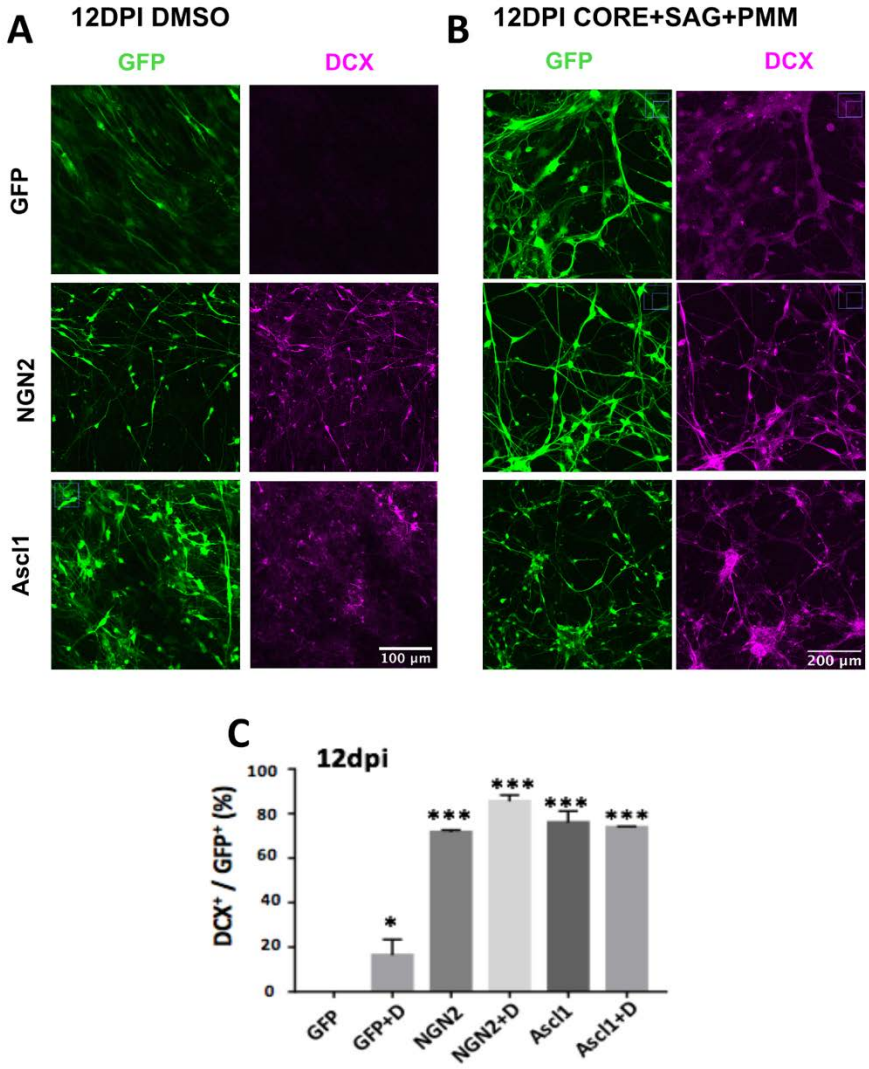

Figure 1: Single neuronal transcription factors NGN2 or Ascl1 along with Core+SAG+PMM combination converts U251 human GBM cells to neuron-like cells. **A**) NGN2-GFP or Ascl1-GFP retrovirus infection in U251 human GBM cells led to a massive number of neuron-like cells compared with GFP control retrovirus at 12 DPI. NGN2+DMSO, Ascl1+DMSO converted cells were immunopositive for immature neuronal markers Doublecortin (DCX, magenta). **B**) NGN2+D or Ascl1+D retrovirus infection in U251 human GBM cells led to a massive number of neuron-like cells compared with GFP control retrovirus at 12 DPI. **C**) Quantitative analyses of conversion efficiency at 12 days' post infection revealed by the percentage of DCX^+ cells over the total infected cells labeled by GFP (GFP, 0; GFP+D, 16.3% ± 3.3%; NGN2, 71.6% ± 3.3%; NGN2+D, 85.4% ± 3.3%; Ascl1, 86.7% ± 4.0%, Ascl1+D, 70.9 ± 3.3%). Data are represented as mean ± SEM, and were analyzed by one-way ANOVA followed with Dunnett's test. **, $p < 0.01$; ***, $p < 0.001$; $n > 250$ cells quantified from triplicate samples.

Similar pattern in morphology was seen when the converted U251 human glioblastoma cells were stained with another immature neuronal marker, β3-tubulin (Tuj1) at the same time point (see Figure 2). There was significant increase in expression of Tuj1 when compared between DMSO control group and the small molecule treated group. The expression of Tuj1 in Ascl1+D and NGN2+D was much higher as well as the more neuron-like elongated morphology. They elongated neuron-like cells were also more clustered in the small molecule treatment alongside the overall higher expression of Tuj1. Similar clustering of the neuron-like cells were seen in the small molecule treated Ascl1cells compared to the just Ascl1 treated cells in DMSO.

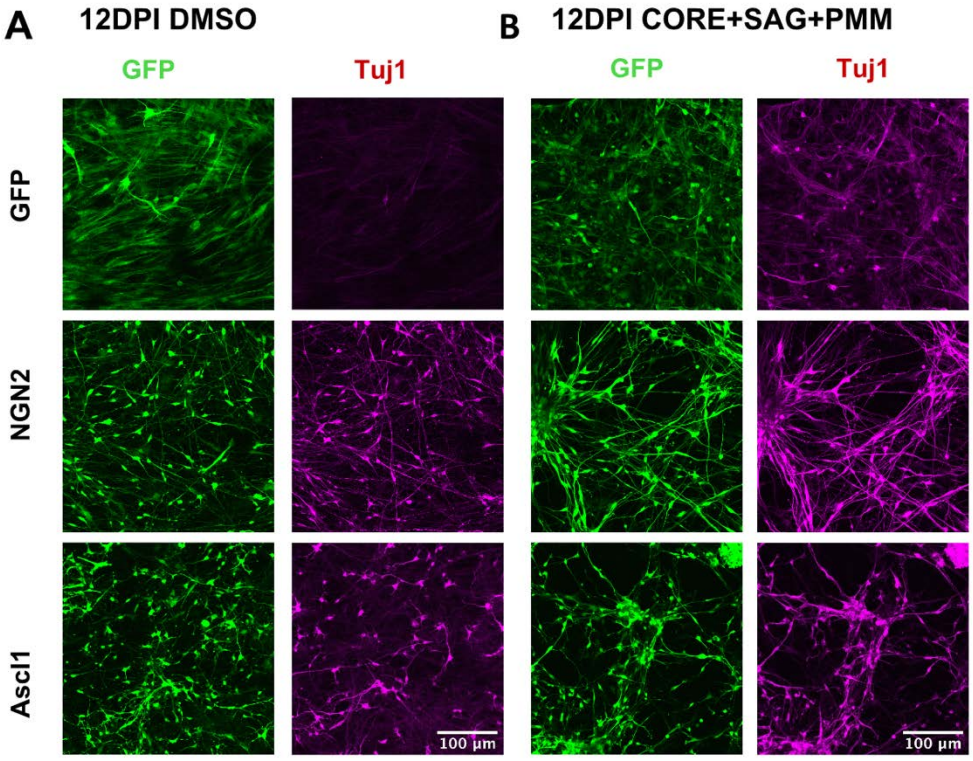

Figure 2: Human U251 GBM cells treated with NGN2 or Ascl1 in combination with small molecules were immunopositive for immature neuron marker Tuj1.
A) NGN2-GFP or Ascl1-GFP retrovirus infection in U251 human GBM cells led to a massive number of neuron-like cells compared with GFP control retrovirus at 12 DPI. NGN2+DMSO, Ascl1+DMSO converted cells were immunopositive for immature neuronal markers β3-tubulin (Tuj1, magenta). **B**) NGN2+D or Ascl1+D retrovirus infection in U251 human GBM cells led to a massive number of neuron-like cells compared with GFP control and NGN2 or Ascl1 alone.

The second time point that was analyzed and tested was at 24 DPI. At 24DPI the cells were stained with a mature neuronal marker microtubule associated protein 2 (MAP2). There was not any major change in overall morphology at 24DPI compared to the 12DPI (See Figure 3). There was similar elongated neuron-like cells seen in the single transcription factor treated U251 glioblastoma cells in DMSO and in the single transcription factor and small molecule treated cell groups at 24DPI as was seen at 12DPI. However, the elongated neuron-like cells were more clustered in the transcription factor and small molecule treated NGN2 and Ascl1 groups compared to the single transcription factor DMSO groups.

The reprogramming efficiency of small molecule and single transcription factor treatment was quantified based on the ratio of MAP2 to GFP of immunostaining results for the cell cultures after 24 DPI. When the efficiency was compared between the small molecule treated versus DMSO control group, there was significant increase in the expression of MAP2 in the single transcription factors and small molecule treated groups compared to when the cells were just treated with NGN2 or Ascl1 (NGN2, 51.2% ± 5.9% SEM versus NGN2+D, 81.7% ± 4.2% SEM; Ascl1, 36.9 ± 5.9% SEM versus Ascl1+D, 54.9 ± 4.2% SEM; n = 3 batches each).

The results displayed that by day 12 post infection, there was significant conversion of glioblastoma cells to neurons as indicated by the immunopositive expression of immature neuronal markers such as DCX and Tuj1 and by 24 days' post infection significant amount of the neurons were immunopositive for mature neuron marker, MAP2. Despite the significant increase in MAP2 expression at 24DPI when treated with Core, SAG, and PMM, there was noticeable amount of apoptosis.

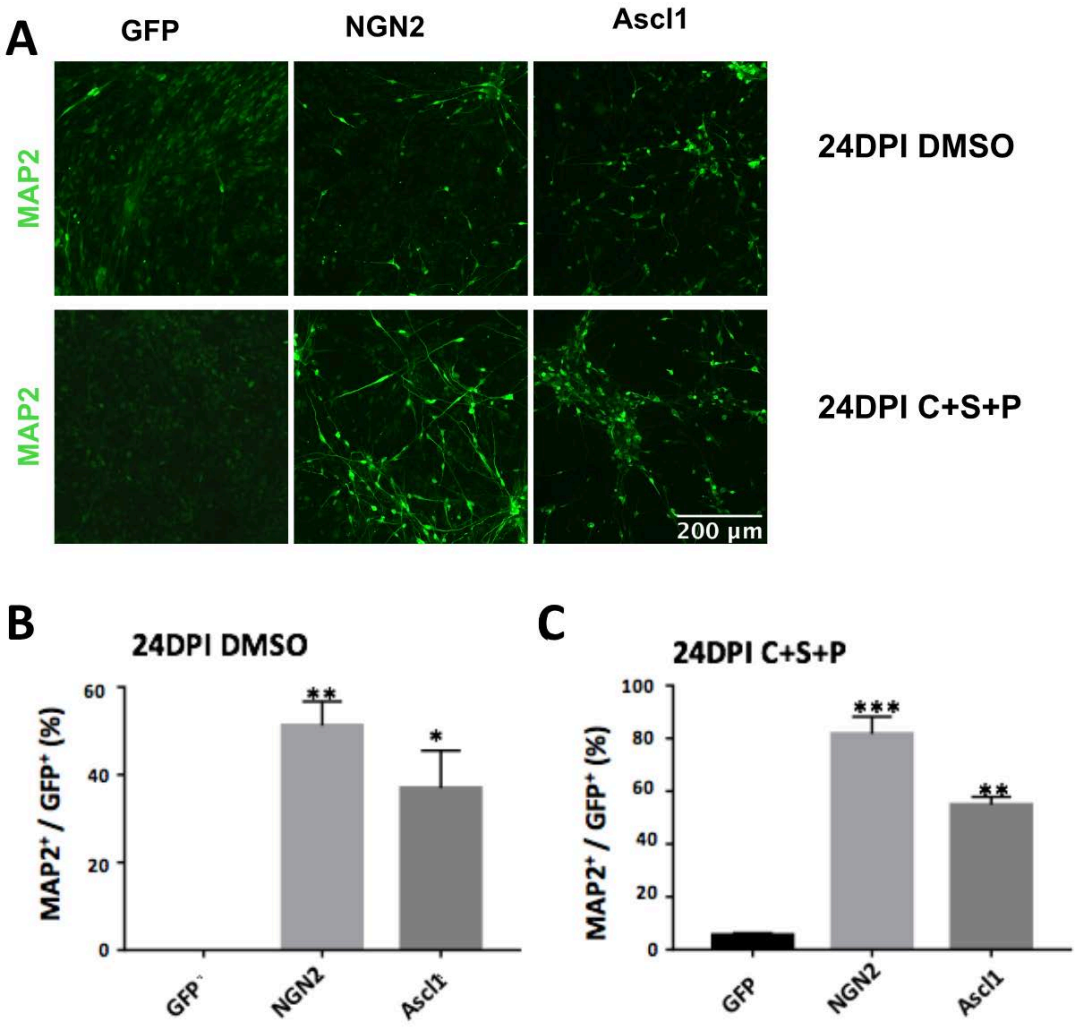

Figure 3: NGN2 or Ascl1 in combination with small molecules converts U251 human GBM cells to neurons with mature neuronal marker MAP2.
A) NGN2-GFP or Ascl1-GFP retrovirus infection in U251 human GBM cells led to a massive number of neuron-like cells compared with GFP control retrovirus at 24 DPI. NGN2, NGN2+D, Ascl1, or Ascl1+D converted cells were immunopositive mature neuronal marker Microtubule associated protein 2 (MAP2, green). B) Quantitative analyses of conversion efficiency at 24 days' post infection revealed by the percentage of MAP2+ cells over the total infected cells labeled by GFP when in DMSO (GFP, 0; NGN2, 51.2% ± 5.9%, Ascl1 37.0 ± 5.87%). C) Quantitative analyses of conversion efficiency at 24 days' post infection revealed by the percentage of MAP2+ cells over the total infected cells labeled by GFP when treated with small molecules (GFP+D, 5.6% ± 4.2%; NGN2+D, 81.7% ± 4.2%, Ascl1+D, 54.9 ± 4.2%). Data are represented as mean ± SEM, and were analyzed by one-way ANOVA followed with Dunnett's test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; $n > 250$ cells quantified from triplicate samples.

Reprogramming Human GBM Cells into GABAergic Neurons *In vitro*

After determining the efficiency of overall neuronal conversion, human glioblastoma converted neurons induced by small molecules and single transcription factors, Ascl1 or NGN2, were immunostained with neuronal subtypes markers GABA, which is an inhibitory GABAergic neurons and VGluT1, an excitatory glutamatergic neuron marker. This was done to determine the subtypes of neurons generated by the single transcription factor and the small molecules treatment. Similar to determining the reprogramming efficiency of overall neuronal conversion using DCX, the reprogramming efficiency of small molecules and single transcription factor treatment was quantified based on the ratio of GABA to GFP of immunostaining results for the cell cultures after 12 DPI.

At 12DPI, the GFP (GFP, 0; n = 3 batches) and NGN2 (NGN2, 12.9% ± 4.3% SEM; n = 3 batches) treated cells in DMSO were immunonegative for GABA while Ascl1 treated cells in DMSO were immunopositive for GABA (see Figure 4). There was no significant difference in the expression of GABA in NGN2, DMSO induced neurons when compared to GFP treated cells in DMSO, whereas Ascl1 treated cells in DMSO significantly increased the efficiency of GABA expression in comparison to the GFP, DMSO control even though the conversion had a low efficiency (Ascl1, 33.7% ± 4.3% SEM; n = 3 batches).

The addition of small molecules, Core, SAG and PMM, along with the single transcription factors increased the expression of GABA significantly (GFP, 0 vs. GFP+D, 20.2% ± 4.3% SEM; NGN2, 12.9% ± 4.3% SEM vs. NGN2+D, 51.8% ± 4.3% SEM; Ascl1, 33.7% ± 4.3% SEM vs. Ascl1+D, 71.8 ± 4.3% SEM; n = 3 batches each) (See Figure 4C). The small molecules Core, SAG and PMM were able to even convert some GFP treated human

glioblastoma cells to express the inhibitory neuronal marker, GABA, when compared to the GFP treated cells in DMSO (GFP, 0; GFP+D, 20.2 ± 4.3% SEM; n = 3 batches each).

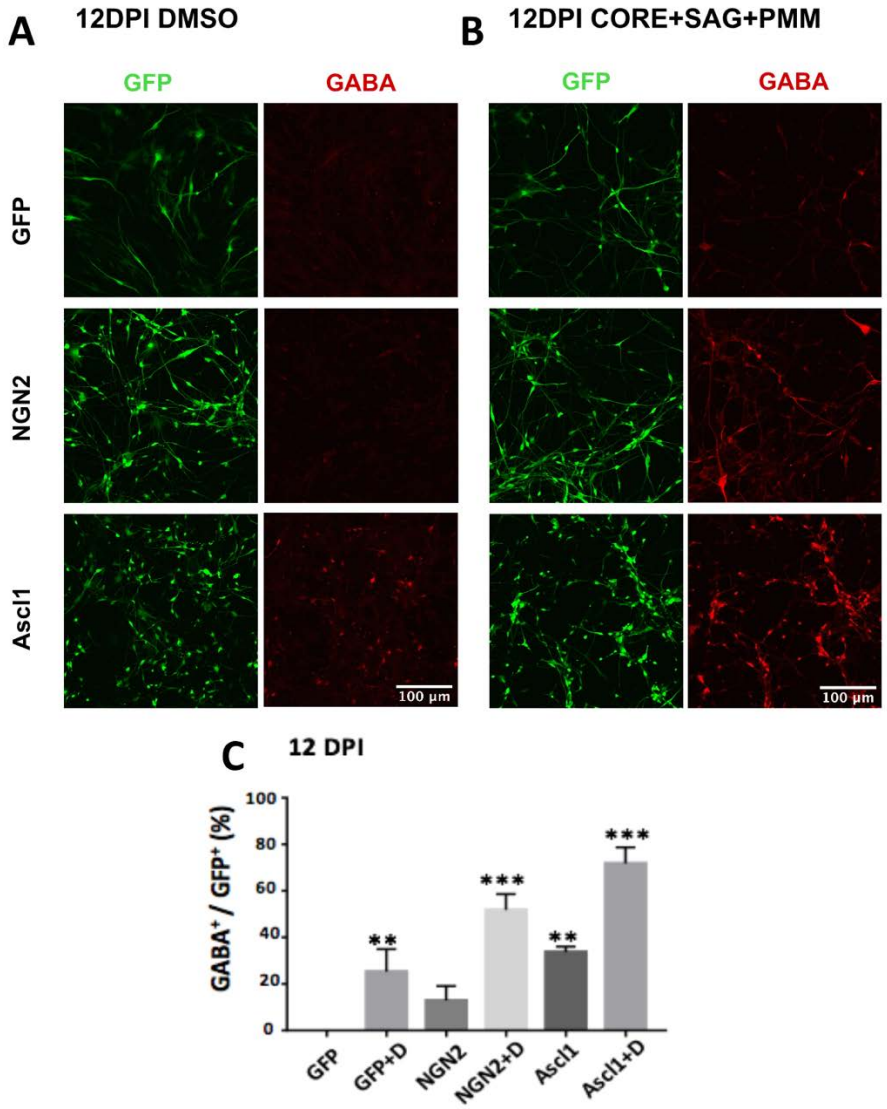

Figure 4: Combining small molecules with neuronal transcription factors Ascl1 or NGN2 increased conversion of human U251 GBM cells to GABAergic neurons.
A) NGN2-GFP or Ascl1-GFP retrovirus infection in U251 human GBM cells led to a massive number of neuron-like cells compared with GFP control retrovirus at 12 DPI. NGN2, Ascl1converted cells were immunopositive for GABAergic neuron markers GABA (red). **B**) NGN2+D or Ascl1+D retrovirus infection in U251 human GBM cells led to a massive increase of neuron-like cells compared with GFP control retrovirus at 12 DPI and were immunopositive for GABA as well. **C**) Quantitative analyses of conversion efficiency at 12 days' post infection revealed by the percentage of $GABA^+$ cells over the total infected cells labeled by GFP (GFP, 0; GFP+D, 20.2% ± 4.3%; NGN2, 12.9% ± 4.3%; NGN2+D, 51.8% ± 4.3% Ascl1, 33.7% ± 4.3%, Ascl1+D, 71.8 ± 4.3%). Data are represented as mean ± SEM, and were analyzed by one-way ANOVA followed with Dunnett's test. **, $p < 0.01$; ***, $p < 0.001$; $n > 250$ cells quantified from triplicate samples.

To further compare the effects of the single transcription factors and small molecules combinations in reprogramming human glioma cells to different types of neurons, the treated U251 human glioblastoma cells were also immunostained with excitatory glutamatergic neuron marker, VGluT1, besides just GABAergic neuron marker.

At 12DPI, unlike the expression of GABA, the NGN2 (NGN2, 78.9% ± 4.0% SEM; n =3 batches) treated cells in DMSO and Ascl1 (Ascl1, 31.4% ± 4.0% SEM; n = 3 batches) treated cells in DMSO were both immunopositive for VGluT1 (see Figure 5). The expression of VGluT1 was significantly higher in Ascl1 and NGN2 treated cells in DMSO compared to DMSO, GFP control cells. However, the conversion of glioblastoma cells to neurons using Ascl1, DMSO had a much lower efficiency, when compared to the conversion efficiency seen in the NGN2, DMSO group.

The addition of small molecules, Core, SAG and PMM, along with the single transcription factors increased the expression of VGluT1 when added with GFP or Ascl1 significantly (GFP, 0 vs. GFP+D, 28.9% ± 4.0% SEM; Ascl1, 31.4% ± 4.0% SEM vs. Ascl1+D, 82.9 ± 4.0% SEM; n = 3 batches each) (See Figure 5C). The expression of VGluT1 in cells treated with NGN2 alone already showed very high conversion efficiency and the addition of small molecules Core, SAG and PMM to NGN2 did not increase the VGluT1 expression significantly when compared to just being treated with NGN2 (NGN2, 79.0% ± 4.0% SEM; NGN2+D, 80.1% ± 4.0% SEM; n = 3 batches each). This may be because NGN2 alone demonstrated maximum conversion possible.

The addition of small molecules when combined with Ascl1 demonstrated significant increase in GABAergic neuron and glutamatergic neuron conversion, while small molecules combined with NGN2 increased GABAergic neuron conversion.

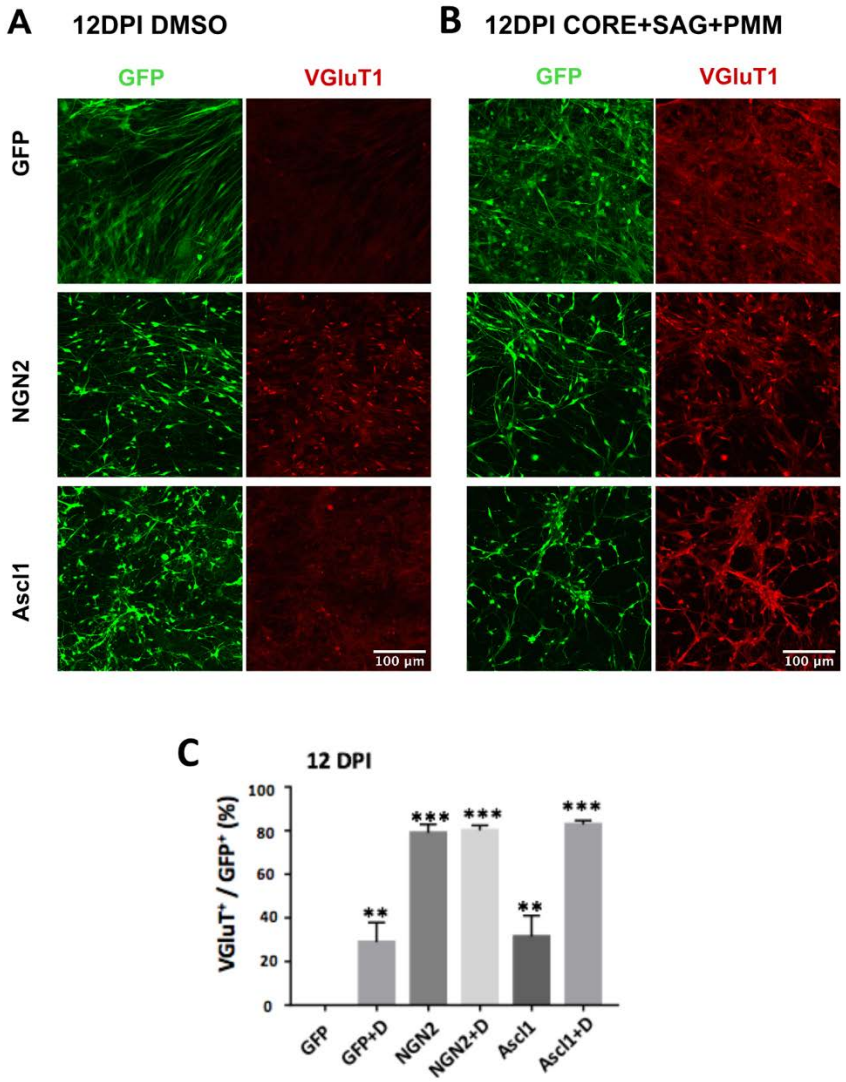

Figure 5: Single neuronal transcription factors NGN2 or Ascl1 along with Core+SAG+PMM converts U251 human GBM cells to glutamatergic neuron.
A) NGN2-GFP or Ascl1-GFP retrovirus infection in U251 human GBM cells led to a massive number of neuron-like cells compared with GFP control retrovirus at 12 DPI. NGN2, Ascl1converted cells were immunopositive for glutamatergic neuron markers VGluT1 (red). **B**) Ascl1+D retrovirus infection in U251 human GBM cells led to a massive increase of neuron-like cells compared with GFP control retrovirus at 12 DPI and were immunopositive for VGluT1 as well. **C**) Quantitative analyses of conversion efficiency at 12 days' post infection revealed by the percentage of $GABA^+$ cells over the total infected cells labeled by GFP (GFP, 0; GFP+D, 28.9% ± 4.0%; NGN2, 79.0% ± 4.0%; NGN2+D, 80.1% ± 4.0%; Ascl1, 31.4% ± 4.0%, Ascl1+D, 82.9 ± 4.0%). Data are represented as mean ± SEM, and were analyzed by one-way ANOVA followed with Dunnett's test. **, $p < 0.01$; ***, $p < 0.001$; $n > 250$ cells quantified from triplicate samples.

Determining the Conversion of GBM cells to Inhibitory Neurons

To further characterize the subtypes of inhibitory GABAergic neurons generated by the single transcription factor and small molecule combination, the transcription factor and small molecule induced neurons were immunostained using parvalbumin (PV), somatostatin (SST), Calretinin, and Calbindin which are all calcium binding protein or neuropeptide that are used to classify inhibitory GABAergic neurons (Xu et al., 2006). The small molecule and single transcription factor induced neurons were also stained by DARPP-32 which is a kinase/phosphatase inhibitor and plays a vital role in striatal signaling (Bateup et al., 2008).

At 12DPI, the NGN2 treated cells in DMSO were immunopositive for both Calbindin and Calretinin (see Figure 6A). The Calbindin and Calretinin signals co-localized with each other and expressed in elongated neuron-like cells. GFP in DMSO was immunonegative for both Calbindin and Calretinin. Ascl1treated cells showed few cells immunopositive for Calretinin and Calbindin. The addition of small molecules, Core, SAG, and PMM, along with the single transcription factors Ascl1 or NGN2 increased the expression of both Calbindin and Calretinin in comparison to the cells just treated with a single transcription factor. (See Figure 6 A-B). The morphology of the Calbindin and Calretinin positive cells were very elongated, neuron-like and clustered, when treated particularly with Ascl1 and Core, SAG and PMM. There was significant increase in Calbindin expression when treated with Ascl1 and small molecules (Ascl1+D, 78.9 ± 4.9% SEM; n = 3 batches) when compared with cells treated with Ascl1 only (Ascl1, 28.6% ± 4.9% SEM; n = 3 batches) (see Figure 6C). Similarly, there was significant increase in Calretinin expression when treated with Ascl1 and small molecules (Ascl1+D, 81.0 ± 4.9% SEM; n = 3 batches) when compared with cells treated with Ascl1 only (Ascl1, 42.8% ± 5.9% SEM; n = 3 batches) (see Figure 6D). There was, however, no significant difference in Calbindin (NGN2,

63.1% ± 4.9% SEM; versus NGN2+D, 69.2% ± 4.9% SEM; n = 3 batches) or Calretinin (NGN2, 67.1% ± 4.9% SEM; NGN2+D, 86.3% ± 4.9% SEM; n = 3 batches) expression when the cells were treated with NGN2 and small molecules versus just with NGN2, DMSO. This may be due to NGN2, DMSO already giving a high conversion efficiency.

The reprogrammed U251 human GBM cells immunostained with DARPP-32 revealed that the Ascl1 and NGN2 converted cells were immunopositive DARPP-32, while the GFP treated cells were immunonegative (see Figure 7A). The addition of small molecules, Core, SAG, and PMM, along with the single transcription factors Ascl1 particularly increased the expression of DARPP-32 in both groups. There was also more neuron-like morphology evident in the small molecule treated GFP group compared to the GFP, DMSO control (see Figure 7B). There was no significant difference in expression of DARPP-32 between GFP, DMSO treated cells and GFP with Core, SAG, and PMM treated cells.

Human U251 GBM cells treated with single neuronal transcription factors, Ascl1 or NGN2, significantly increased the expression of DARPP-32 compared to GFP, DMSO (see Figure 7C). There was significant increase in DARPP-32 expression when treated with Ascl1 and small molecules (Ascl1+D, 70.1 ± 7.1%, SEM; n = 3 batches) when compared with cells treated with Ascl1 only (Ascl1, 22.5% ± 7.1%, SEM; n = 3 batches) (see Figure 7C). Similar to the expression of Calbindin and Calretinin, there was no significant difference in DARPP-32 expression when the cells were treated with NGN2 and small molecules Core, SAG and PMM, (NGN2+D, 71.5% ± 7.1%, SEM; n = 3 batches) versus when cells were just treated with NGN2, DMSO (NGN2, 51.3% ± 7.1% SEM; n = 3 batches). This may be due to NGN2, DMSO already having a high conversion efficiency.

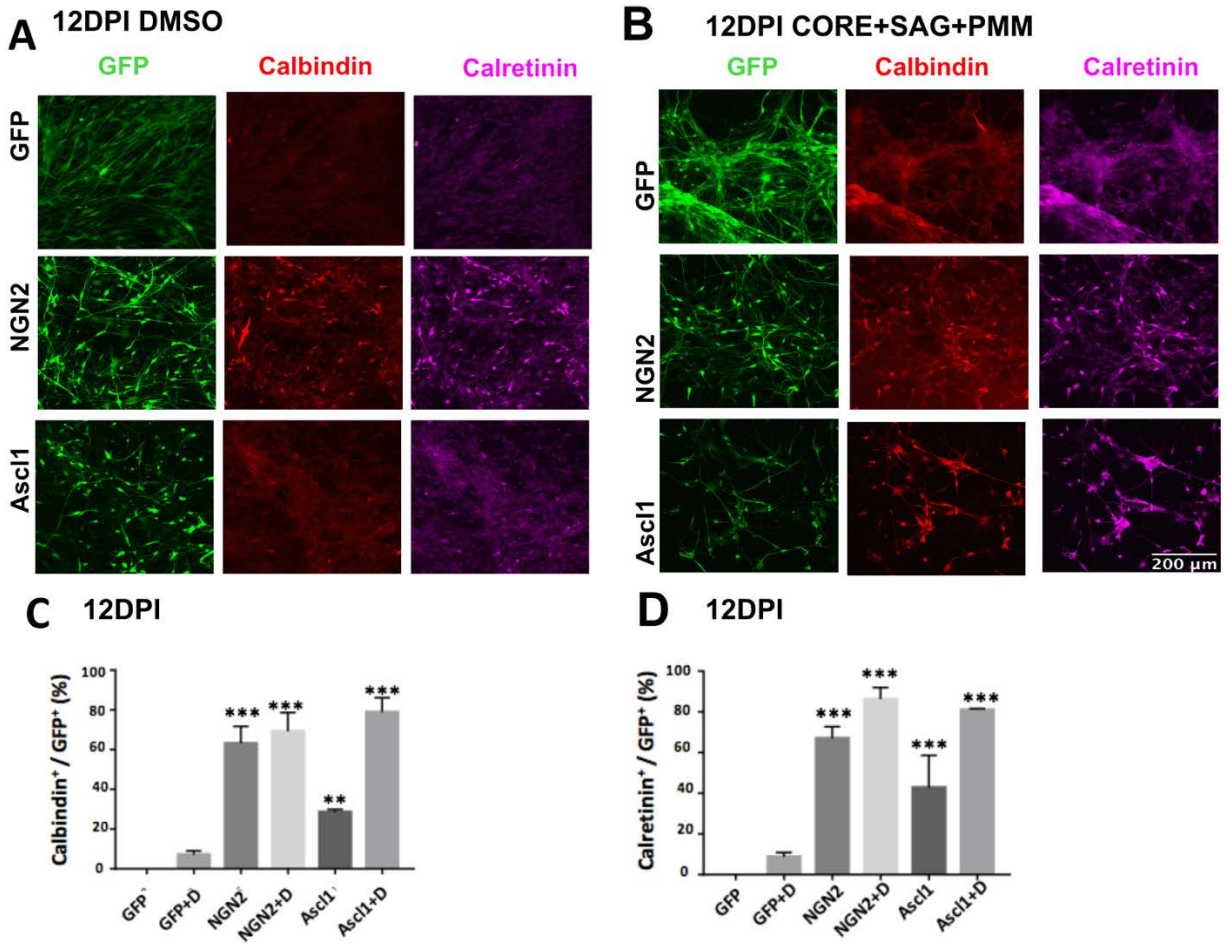

Figure 6: Increased calcium binding protein, Calbindin and Calretinin, expression when Ascl1 is combined with the small molecules Core+SAG+PMM combination.
A) The NGN2 converted cells were immunopositive for both Calbindin (red) and Calretinin (magenta). **B**) NGN2+D, Ascl1+D converted cells were immunopositive for both Calbindin (red) and Calretinin (magenta). **C**) Quantitative analyses of conversion efficiency at 12 days' post infection revealed by the percentage of Calbindin$^+$ cells over the total infected cells labeled by GFP (GFP, 0; GFP+D, 7.2% ± 4.9%; NGN2, 63.1% ± 4.9%; NGN2+D, 69.2% ± 4.9%; Ascl1, 28.6% ± 4.9%, Ascl1+D, 78.9 ± 4.9%). **D**) Quantitative analyses of conversion efficiency at 12 days' post infection revealed by the percentage of Calretinin$^+$ cells over the total infected cells labeled by GFP (GFP, 0; GFP+D, 8.8% ± 4.9%; NGN2, 67.1% ± 4.9%; NGN2+D, 86.3% ± 4.9%; Ascl1, 42.8% ± 5.9%, Ascl1+D, 81.0 ± 4.9%). Data are represented as mean ± SEM, and were analyzed by one-way ANOVA followed with Dunnett's test. **, $p < 0.01$; ***, $p < 0.001$; n > 250 cells quantified from triplicate samples.

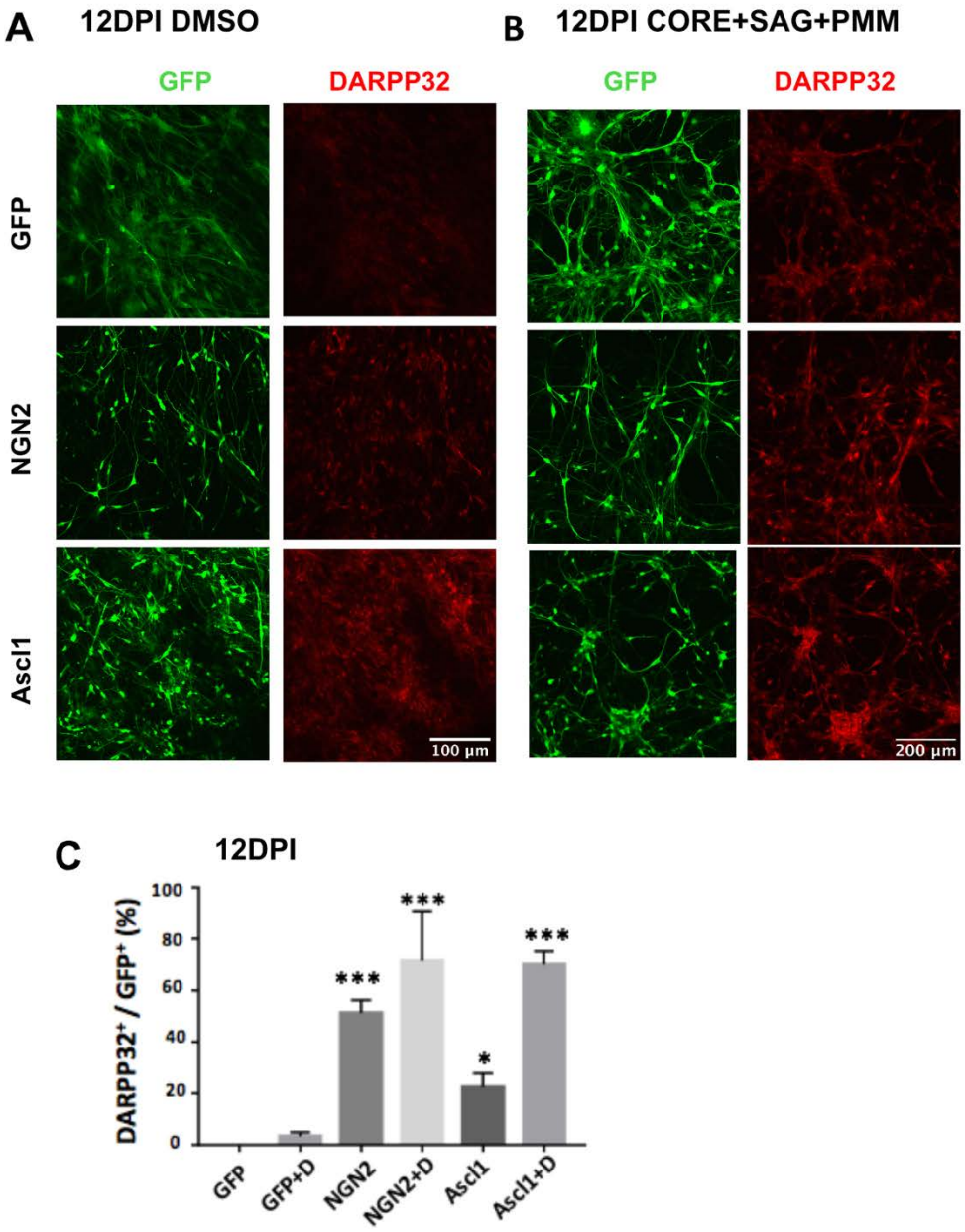

Figure 7: Increased expression of GABA subtype marker in U251 human glioblastoma cells treated with transcription factors Ascl1 or NGN2 with small molecules Core+SAG+PMM. **A**) The Ascl1 converted cells were immunopositive DARPP-32 (red). **B**) NGN2+D, Ascl1+D converted cells were both immunopositive for DARPP-32 (red). **C**) Quantitative analyses of conversion efficiency at 12 days' post infection revealed by the percentage of DARPP-32 $^{+}$ cells over the total infected cells labeled by GFP (GFP, 0; GFP+D, 3.4% ± 7.1%; NGN2, 51.3% ± 7.1%; NGN2+D, 71.5% ± 7.1%; Ascl1, 22.5% ± 7.1%, Ascl1+D, 70.1 ± 7.1%). Data are represented as mean ± SEM, and were analyzed by one-way ANOVA followed with Dunnett's test. **, $p < 0.01$; ***, $p < 0.001$; $n > 250$ cells quantified from triplicate samples.

At the same 12DPI time point, reprogrammed U251 human glioblastoma cells were immunostained with other inhibitory GABAergic neuron subtype markers such as parvalbumin (PV) and somatostatin (SST). However, all the experimental groups (GFP, GFP+D, NGN2, NGN2+D, Ascl1, Ascl+D) were immunonegative for both the markers, indicating negative results for the particular subtypes inhibitory GABAergic neurons.

Analyzing the Effects of Transcription Factors and Small Molecule on Cancer Proliferation

After analyzing the effects of the single transcription factor and small molecules on the reprogramming efficiency of the glioma cells to neuron cells, we studied the transcription factor and small molecules treatment's impact on reducing cancer proliferation. The proliferating cells were indicated by Ki67 and Glycogen Synthase Kinase 3 (GSK3β) immunostaining. Ki67 is a cell proliferation marker associated with cancer cell proliferation and GSK3β is involved in energy homeostasis to proliferation and apoptosis. When staining with both markers, the cell morphology was very much elongated and neuron-like, consistent to what was seen with previous conversion.

In this study, we found that there was significant reduction in Ki67 expression in NGN2, DMSO and Ascl1, DMSO retrovirus infected U251 human glioblastoma compared to GFP, DMSO control at 12DPI (GFP, 100; NGN2, 78.4% ± 6.4%; Ascl1, 56.7% ± 6.4%, SEM, n = 3 batches) (see Figure 8C). However, when treated with small molecule Core, SAG and PMM, there was no significant reduction of Ki67 expression in comparison to GFP, DMSO control and the single transcription treated groups (see Figure 8B). The small molecule did not seem to have any effect on the U251 human glioblastoma proliferation reduction, whereas, the single

transcription factor alone reduced the glioblastoma proliferation more efficiently than the combination of small molecules and transcription factor (see Figure 8).

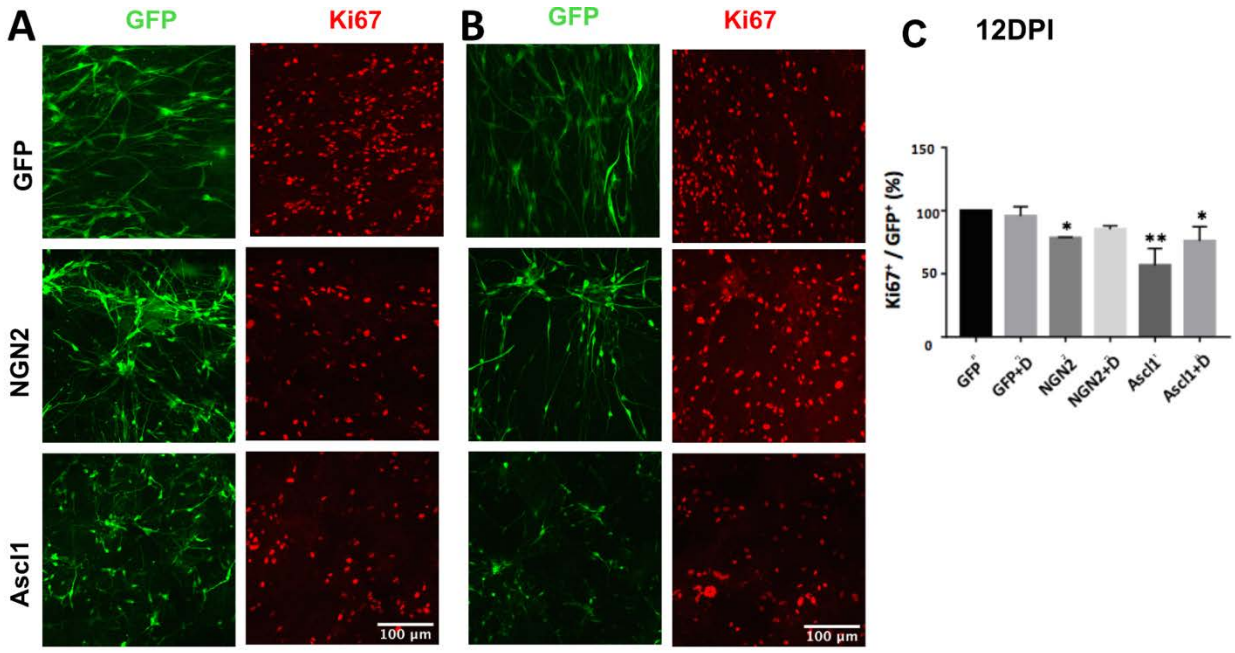

Figure 8: Neuronal conversion of GBM cells inhibits proliferation when stained with Ki67. **A**) NGN2-GFP, Ascl1-GFP retrovirus infected U251 human glioblastoma cells showed revealed reduced expression of proliferation marker Ki67 by 12DPI even when small molecules are not added. **B**) Ascl1+D infected U251 reveals reduced expression of Ki67 by 12dpi. **C**) Quantitative analyses of conversion efficiency at 12 days' post infection revealed by the percentage of $Ki67^+$ cells over the total infected cells labeled by GFP (GFP, 100; GFP+D, 95.7% ± 6.4%; NGN2, 78.4% ± 6.4%; NGN2+D, 85.2% ± 6.4%; Ascl1, 56.7% ± 6.4%, Ascl1+D, 75.9 ± 6.4%). Data are represented as mean ± SEM, and were analyzed by one-way ANOVA followed with Dunnett's test. **, $p < 0.01$; ***, $p < 0.001$; $n > 250$ cells quantified from triplicate samples.

At 12DPI, the reprogrammed neurons were also immunostained with GSK3β, However, all the experimental groups (GFP+D, NGN2, NGN2+D, Ascl1, Ascl+D) along with the GFP, DMSO control were immunonegative for the marker.

Chapter 4

Discussion

Implications from this Study

Our results demonstrate that the application of a cocktail of six small molecule compounds (SB431542, LDN193189, CHIR99021, DAPT, SAG, and PMM) along with the single transcription factors (Ascl1 and NGN2) can efficiently reprogram human glioblastoma cells into neurons *in vitro*. The single transcription factor Ascl1 or NGN2 neurons were immunopositive for neuronal markers DCX, TUJ1, and MAP2 and the addition of the small molecules with the single transcription factors showed increased efficiency in the reprogramming of overall neuronal conversion. Introducing the small molecules with NGN2 and Ascl1 also increased the number of GABAergic neurons generated significantly in comparison to just using the single transcription factors. NGN2 alone had a reprogramming efficiency of 13% for converting human glioblastoma cells to GABAergic neurons; NGN2 with the six small molecules increased the efficiency of GABAergic neuron generation to 52%. Similarly, the six small molecules with Ascl1 increased the GABAergic neuron generation efficiency to 72% from 33% with just Ascl1. This demonstrated how adding the small molecules with transcription factors could potentially increase not only overall neuronal reprogramming, but also the number of GABAergic neurons when paired with Ascl1. The single transcription factors, Ascl1 or NGN2, showed increased efficiency in the reprogramming of GABAergic neuron subtypes indicated by Calbindin, Calretinin, and DARPP-32 markers. There was no difference in the GABA subtype marker expression when compared with NGN2 and small molecules treated cells versus just NGN2 treated cells most likely due to NGN2, DMSO treated cells already having a

very high expression of GABAergic subtype neuronal markers. The small molecules combined with Ascl1, however, increased the expression of the GABAergic subtype markers significantly when compared to cells only treated with Ascl1.

This reprogramming of human glioblastoma cells could potentially inhibit the glioma cells from their proliferative state, especially if they are converted to GABAergic neurons and could potentially not only reduce tumor growth but also reduce the epileptic symptoms that are often associated with brain tumor growth by restoring the excitation-inhibition balance.

The efficiency of glioma conversion to excitatory glutamatergic neurons was also quantified where adding the small molecule to Ascl1 increased the efficiency from 31% to 83%. However, the small molecules did not increase the efficiency of glutamatergic neuron conversion when added with NGN2 significantly. This is due to NGN2 alone having a very high efficiency for glutamatergic neurons. The addition of small molecules when combined with Ascl1 demonstrated significant increase in GABAergic neuron and glutamatergic neuron conversion, while small molecules combined with NGN2 increased GABAergic neuron conversion.

The single transcription factors were also capable of reducing the cell proliferation. Ki67 was downregulated when Ascl1 or NGN2 retrovirus infection was administered to the U251 glioblastoma cells. However, the small molecules do not seem to have any direct impact reducing the proliferation when compared to transcription factors alone since there was no major difference in downregulation of proliferation marker, Ki67, when comparing treatments with transcription factor and small molecules versus transcription factors alone. In fact, there was no significant difference in Ki67 expression when comparing cells treated with single transcription factors with small molecules and GFP, DMSO control group.

Overall, through this study, the results demonstrate that there may be some effect of combining our small molecule cocktail with Ascl1 or NGN2 to increase the efficiency of glioma cell conversion to GABAergic neuron *in vitro,* but more has to be done to reduce the overall proliferation, increase the survival rates, and determine the functionality of the generated neurons.

Future Focuses and Improvements

One of the major concerns about the experiment revolves around reducing apoptosis and increasing the survival rate of the neurons. Through the preliminary data, we demonstrated that the induced neurons were able to survive until 24 days' post infection. The overall neuronal conversion was almost completed by 12DPI, and therefore, the drug treatment was reduced from 16 days to 12 days. However, it is necessary to determine how long these cells can survive and how to reduce apoptosis to increase farther efficiency of the conversion. Somehow, we have to reduce apoptosis as well as cellular proliferation in *in vitro* and then be able to translate it to *in vivo*. In a recent study done in our lab to convert fetal astrocytes into neurons through multiple signaling pathways, focused on narrowing down to a few small molecules that impacted the overall efficiency by narrowing down and modulating four signaling pathways in order to reduce the number of small molecules thus reducing the toxicity and increasing the efficiency (Yin, et al., 2019). Their induced neurons were able to survive over five months. Following a similar method of trial and error where the effects of each of the six small molecules on the efficiency of reprogramming human glioblastoma cells to GABAergic neuron could potentially be a solution to elongate the longevity of the generated neurons.

On the other hand, another approach may be to administer the drugs sequentially instead of all at once from day one. This similar approach was taken by the recent study from our lab as well as a previous study focusing on using small molecules to convert human astroglial cells into functional neurons (Guo et al., 2014; Li et al., 2015; Zhang et al., 2015). A combination of nine small molecules were used by them, six of which are the same ones used in this study; however, they divided the nine drugs to be added at different time points based on their functionality and influence on different signaling pathway (Li et al., 2015; Zhang et al., 2015). During the beginning of this project, the sequential approach with all nine drugs was administered, however, almost all the cells died when replicating the procedure onto the glioma cell-line. This study also focuses on using a combination of single transcription factors such as Ascl1 along with small molecules to increase the efficiency of GABAergic neuron generation from human glioma cells. It would be more beneficial to combine the sequential approach of adding the small molecules alongside narrowing down the small molecules to the most essential ones when combined with each of the different transcription factors. Not only would this approach possibly reduce the cells from being overwhelmed with too many drugs at once, but it could also increase the efficiency by targeting different signaling pathways at specific time points to drive the conversion more efficiently.

Another focus of the future study would be to be able to detect the functionality of the newly generated neurons before being translated to *in vivo*. This could easily be addressed by conducting an electrophysiology experiment. A representative trace of repetitive action potentials can be recorded in small molecule and transcription factor induced neurons at different time points during the experiment, but particularly post drug treatment of mature neurons.

Inhibitory GABAergic events could potentially be revealed in the human glioblastoma converted GABAergic neurons (Zhao et al., 2012, Li et al., 2015).

Current Treatments and Our Goals

In a 2018 study done by Ozdemir et al. current advances in glioblastoma treatment, particularly focusing on nanoparticle therapy. The standard procedure for glioblastoma is currently surgery, the efficiency of which is highly limited due to the fact of glioblastoma's aggressive nature and infiltration into its surrounding tissue, almost making it impossible to remove the tumor completely, and therefore contributing to the high recurrence rate. The evolution of chemotherapy with efficacy of certain drugs to effectively cross the blood brain barrier and combining it with chemotherapy both before and after surgery have allowed patients to live longer (Ozdemir-Kaynak et al., 2018). However, chemotherapy to glioblastoma is very limited to a few compounds, and despite with the improved understanding of glioblastoma pathways and focus on targeting the genes that may be involved to improve the specificity, chemotherapy is essentially injecting patients with a chemical concoction to fight off cancer cells. Despite some improvements, often time these chemical concoctions fail to discriminate between healthy and malignant cells. As so, through the use of chemotherapy, patients are putting their own bodies through a lot of stress making the outcome of curing the disease being very slim.

As much as improving delivery of drugs through the blood brain barrier and improving the specificity is crucial and may posit a potentially improved cure for glioblastoma, using transcription factors and small molecules involved in terminally differentiating rapidly proliferating cell, such as glioblastoma cells, would prove as a potentially better treatment due to

limiting the invasive nature of potentially toxic drugs as well as a better way of addressing certain neurophysiological symptoms such as neuronal loss and epilepsy. The goals through this study aims to provide a better alternative option and counter come of the faults of current methods. Through this approach, we hope to induce the glioma cells to be reprogrammed to GABAergic neurons to reduce the proliferation as well as tackle symptoms such as epilepsy. Through our approach, we eliminate the chances of killing any cells and by simply terminally differentiating the glioma cells. However, at this stage we have barely started. More time, effort, and discovery has to be done in the future to make this objective come true.

Chapter 5

Appendix A

Supplemental Information

Table 1: Antibody List

Primary Antibody	**Concentration**	**Company**
chicken-GFP	1:1000	AVES
rabbit-GABA	1:1000	Sigma
rabbit-VGluT1	1:800	SYSY
rabbit-DARPP-32	1:800	Millipore
rabbit-Calbindin	1:1500	Swant
goat-Calrectinin	1:2000	Millipore
goat-DCX	1:1000	ABCam
rabbit-MAP2	1:1000	Millipore
chicken-MAP2	1:1000	ABCam
mouse-Tuj1	1:1000	Biolegend
rabbit-Ki67	1:800	ABCam
rat-SST	1:800	Santa Cruz
rabbit-GSK3β	1:800	CST
mouse-PV	1:800	Santa Cruz

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ACADEMIC VITA

AASMA HOSSAIN

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EDUCATION

Pennsylvania State University—University Park, PA Aug. 2015-May. 2019
Eberly College of Science, Schreyer Honors College
B.S. in Biology and Minor in Psychology

Upper Darby High School, Drexel Hill, PA 2011-2015

RESEARCH EXPERIENCE

Chen Lab, Charles H. "Skip" Smith Life Sciences Laboratory March 2016- May 2019
PI: Dr. Gong Chen, Professor and Verne M. Willaman Chair in Life Sciences

- Conduct research on liver cancer (2015-2019) and glioma (2017-2019)
- Investigate conversion of tumor cells to healthy liver cells using transcription factors suitable for both *in vivo* and *in vitro* models
- Developed and conducted new research project of converting high-grade glioma cells to GABAergic neurons using transcription factors and drug treatment
- Collect and present weekly data to lab mates and mentors and train new lab members on a variety of lab techniques

WORK EXPERIENCE

Penn State Undergraduate Teaching Intern Jan. 2019-May 2019
*Teaching Assistant for BIOL/BBH 470: Functional Neuroscience***,** Jan 2019-Present

- Assist students with understanding course material and administer review session

Penn State Alternative Breaks Associated with Break Away Jan 2019-May 2019
Spring Alternative Break Addressing Urban Revitalization in Cleveland Ohio

- Planned and organized the urban revitalization service trip in Cleveland, Ohio
- Coordinated activities addressing refugees, tackling children education, homelessness and food insecurity

Penn State Learning Assistant Program Aug 2018- Dec 2018
Learning Assistant Biology Lab 424: The Use of Plants/ Plant Medicine

- Assisted and motivated students to learn the course material in class
- Coordinated and headed discussion sessions and graded exams, homework and assignments

Penn State Teaching Assistant Program Jan 2018- May 2018
Assistant TA for Functional and Developmental Biology Lab 240W

- Assist students conduct experiments and assist TA to prepare for class and clean-up

Penn State Schreyer Career Development Program mentor Oct 2017- Present
Schreyer Mentor

- Motivated and navigated freshmen honors students with honors classes and thesis guidance

Undergraduate Research Society (URS) & URISE Apr 2016 – Dec 2018
Treasurer and mentor for Undergraduate Research Society and URISE

- Coordinated and directed the financial planning for basic lab training.
- Reorganized and improved URISE to help students learn basic laboratory skills to aid in finding research labs
- Secured funding for continuation of URISE program and educational trips to Hershey Medical Center, National Institute of Health, and the Smithsonian Museums

Network of Excellence in Undergraduate Sciences (Nexus) Jun 2016- Present
Orientation Leader

- Conducted orientation for the Eberly College of Sciences and provided academic guidance for freshmen

VOLUNTEER EXPERIENCE

Fall Alternative Break Aug 2018- Present
Addressing Mental Health at Niagara

- Assisted at mental health relief homeless shelter and involved in the soup kitchen

A. E. D.: National Health Professions Honors Society Apr 2018- Present
Service Member

- Involved in services related to healthcare such as volunteering at hospitals and participating in healthcare related services

Mount Nittany Medical Center
Volunteer and trainer, Jun 2017- Present

- Volunteer in the patients' floor including ICU, and maternal ward
- Discharge patients, send and retrieve items from sterile processing, assist nurses, pharmacist and same day surgery, interact and address patients and visitors' needs

Upper Darby Township Welcome Center May 2015-Dec 2015
ESL teacher at 7000 Walnut St, Upper Darby, PA 19082

- Teach English in ESL classes to adults and children
- Advised immigrant students and administered classes according to their need

Delaware County Memorial Hospital
Professional Field Experience Program Jan 2015- May 2015

- Shadow doctors, surgeons, EMT and nurses in weekly rotations for a semester, five days a week, over 300 hour

EXTRACURRICULAR ACTIVITES

Penn State Alternative Breaks Aug 2018-present
A. E. D.: National Health Professions Honors Society Apr 2018- present
BKind: World Kindness Apr 2018- Present

URS: Undergraduate Research Society	Apr 2016- Present
Nexus: Eberly College of Science Orientation Group	Jun 2016- Present
KPMD: KPOP Music and Dance	Aug 2016- Aug 2017

HONORS/AWARDS

National Dean's List	Aug 2015- May 2019
John K. Tsui Scholarship	Aug 2015- May 2019
David S. Rocchino Family Foundation Scholarship	Aug 2017- May 2018
Wheeler P. Davey Scholarship	Aug 2016- May2019
Academic Excellence Scholarship	Aug 2016- May2019
Rhodes and Gluck Trustee Scholarship	Aug 2015- May 2019
Doris N. McKinstry Scholarship	Aug 2018- May 2019

INTERNATIONAL EDUCATION

International Healthcare Experience in Costa Rica
Study Abroad in Costa Rica, 7-14 May 2018

- Experienced healthcare at Costa Rica and examining their three level healthcare system for a week as well as experiencing the rich Costa Rican culture

Presented at the **Undergraduate Research Exhibition,** *An Examination of Factors that Pushed Life Expectancy in Costa Rica to Surpass Life Expectancy in the United States of America***,** Spring 2018

LANGUAGE PROFICIENCY

English: Native Speaker
Bengali: Native Speaker