Effect of GFP Expression and DiR Labeling on DiR Fluorescence and Cytotoxicity of iNSCs In Vitro

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INTRODUCTION

Currently, stem cell therapy is one of the most promising approaches for the treatment of neurological injury and neurodegenerative diseases. Induced pluripotent stem cell-derived neural stem cells (iNSCs) are a novel stem cell type that may possibly be used as a regenerative therapy. iNSCs are able to differentiate into all three neural lineages: neurons, astrocytes, and oligodendrocytes. Research groups have shown evidence that iNSCs are able to integrate into damaged brain tissue, leading to the replacement of lost neural tissue as a result of traumatic brain injury or ischemic stroke. Although there is evidence that these neurons are integrating into existing neural circuitry, it is unclear exactly what types of neurons these cells are differentiating into. As a way of detecting transplanted cells, iNSCs can be induced to express green fluorescent protein (GFP). Additionally, in order to find the cell engraftment site, iNSCs can be labeled with DiR, a lipophilic dye that laterally diffuses into the cell membrane. DiR and GFP are used in a wide variety of transplantation and migration studies. However, GFP has been shown to be cytotoxic and affect the viability of transduced cells. DiR can also have an effect on cell proliferation. Our aim is to determine the effect of GFP expression and DiR co-labeling on iNSC viability and DiR fluorescence emission in an in vitro model.

OBJECTIVES

- Determine which MOI produces the highest GFP expression in iNSCs
- Assess how GFP expression affects cell survivability in iNSCs
- Assess how GFP expression combined with DiR labeling affects DiR fluorescence and cytotoxicity of iNSCs

MATERIALS & METHODS

Transduction of iNSCs with GFP

iNSCs were transduced using an EF1α-GFP lentivirus at MOIs of 0, 5, 10 and 20. Cells were plated and transduced the following day. Transduction was performed at 37 degrees and 5% CO2 for 24hrs in the presence of polybrene. The following day the existing media was aspirated out, cells were washed with PBS and normal NGM media was added. Cells were then incubated.

Labeling of Cells with DiR

Cells were expanded and FACS sorted to obtain a pure population of GFP+ cells. GFP-iNSCs transduced at an MOI of 10, as well as non-transduced iNSCs were labeled with DiR. Non-labeled iNSCs were utilized as a control. Cells were plated into respective treatment groups and also in a 96 well plate to image on IVIS. Cells were then imaged on IVIS to determine the effect of the GFP on DiR fluorescence by looking at radiant efficiency in each well, or region of interest (ROI). Additionally, A CytoTox-ONE assay was performed to determine percent cytotoxicity of the iNSCs after transduction and labeling.

RESULTS

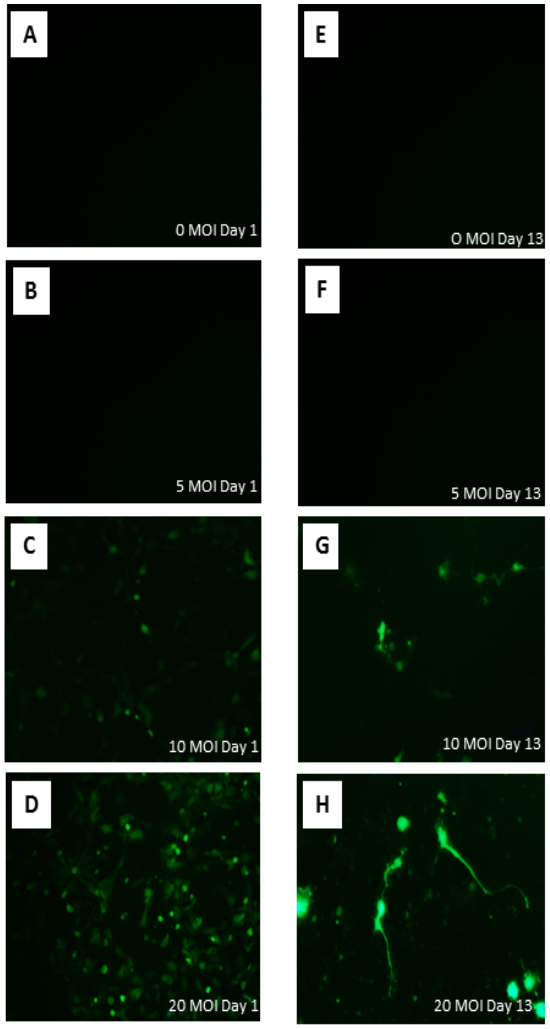


Figure 1. Transduction with higher MOIs leads to increased numbers of GFP+ iNSCs at Day 1 and 13. iNSC populations transduced with an MOI of 10 and 20 (**C** and **D**; **G** and **H**) had larger numbers of GFP+ cells than cells transduced at an MOI of 0 and 5 (**A** and **B**; **E** and **F**) at Day 1 and 13. We observed a decrease in the number of GFP+ iNSCs at Day 13 (**G** and **H**) relative to Day 1 (**C** and **D**). However, we observed higher levels of differentiation in GFP+ iNSCs in 20 MOI cultures (**G**) relative to 10 MOI cultures (**H**) suggesting increased MOIs may lead to enhanced differentiation.

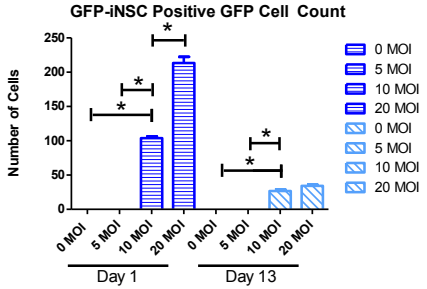


Figure 2. Higher MOIs lead to higher numbers of GFP+ cells, but survivability decreases over time. Specific to Day 1, cells transduced at an MOI of 20 yielded the highest number of GFP+ cells, There is also a significant decrease in the number of GFP+ cells at Day 13 relative to Day 1 at both 10 and 20 MOIs. *=p-value < 0.05; # =p-value < 0.05.

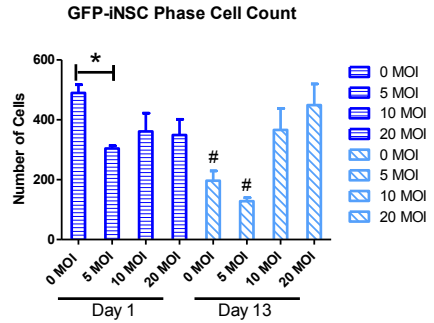


Figure 3. Changes in survivability due to GFP expression. Cell counts of phase images reveal a significant decrease between 0 and 5 MOIs respective to Day 1. After 13 days in culture, cells transduced at 0 and 5 MOIs were significantly different relative to Day 1. Day 13 cell counts revealed increased numbers of cells in both the 10 and 20 MOI.

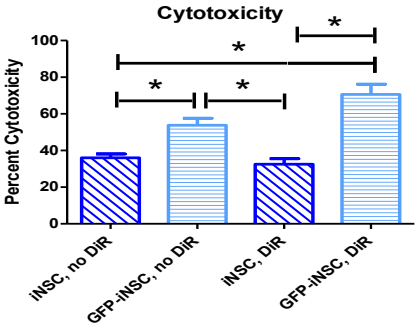
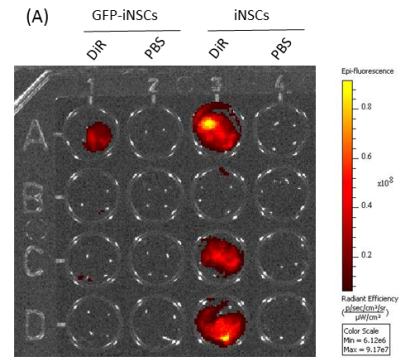
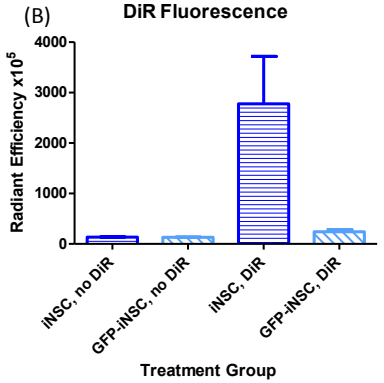


Figure 4. DiR labeling combined with GFP produces increased cytotoxicity, but DiR alone is not cytotoxic. There is a significant difference between non-expressing, non-labeled cells (iNSC, no DiR) and cells that have both combined GFP and DiR (GFP-iNSC, DiR) labeled.





Panel A. Fluorescence recorded from IVIS imaging. iNSCs expressing GFP and labeled with DiR reveal low fluorescence, while cells labeled with DiR but not expressing GFP revealed high fluorescence. This suggests that GFP expression combined with DiR labeling decreases overall fluorescence.

Panel B. Low fluorescence in GFP-iNSC, DiR cells. iNSCs labeled with DiR but not expressing GFP reveal the highest fluorescent value. GFP-iNSCs labeled with DiR reveal extremely low fluorescence, suggesting that combined expression and labeling has a detrimental effect on DiR fluorescence.

CONCLUSIONS

In conclusion, cells transduced at higher MOIs produce more GFP+ cells. It was found that GFP expression causes increased cell death as evident by decreased fluorescence and decreased GFP+ cell counts after 13 days in culture. Furthermore, GFP expression and DiR labeling in combination have a detrimental effect on cells by leading to high levels of cytotoxicity. Significant differences were found between our control of iNSCs, no DiR and our combined labeled cells of GFP-iNSCs with DiR. This supports our theory that GFP and DiR are cytotoxic to cells, so caution should be taken when utilizing these systems for cell tracking studies.

ACKNOWLEDGEMENTS

This material is based upon work supported by a National Science Foundation Research Experiences for Undergraduates (REU) site program under Grant No. 1359095. I would also like to acknowledge the West Lab and Regenerative Bioscience Center in completing this project.