The Effects of Growth Factors on Embryonic Stem Cell Differentiation inside of Fibrin Scaffolds

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Statement of Purpose: The goal of this work was to determine which specific growth factors could direct embryonic stem cell derived neural lineage precursor cells (ESNLCs) seeded inside of fibrin scaffolds to differentiate into neurons and oligodendrocytes while minimizing the number of astrocytes produced. Five different growth factors, including neurotrophin-3 (NT-3), basic fibroblast growth factor (bFGF), platelet derived growth factor AA (PDGF), ciliary neurotrophic factor (CNTF), and sonic hedgehog (Shh) were tested over a range of concentrations (2 ng/mL to 25 ng/mL) to determine their effect on cell differentiation into neural phenotypes and overall cell survival. Fibrin scaffolds containing ESNLCs and growth factors could serve as a potential treatment for spinal cord injury.

Methods: RW4 mouse ES cells were passaged as previously described [1] and induced to form embryoid bodies (EBs) containing ESNLCs using the $4^{-}/4^{+}$ retinoic acid differentiation protocol [2]. EBs were then seeded into fibrin scaffolds (10 mg/mL, bottom layer: 300 µL, top layer: 100 µL) and cultured for 14 d as described previously [1]. EBs were grown in complete media containing serum for 3 days and then the media was switched to serum free media containing B27 supplement. Growth factors were added the indicated concentrations to both sets of media.

After 14 days, the cells were dissociated from the fibrin (24 wells per experiment) by trypsinization for 20 min. For SSEA-1 and O4 (undifferentiated ES cell marker and oligodendrocyte marker) staining, cells were washed thoroughly, blocked using goat serum, followed by 1 hr incubations with primary and secondary antibody. For nestin (neural progenitor cell marker), Tuj1 (early neuronal marker), and GFAP (astrocyte marker), cells were washed thoroughly, fixed using 1% formalin for 20 min at 4 ºC , permeabilized with 0.5% saponin, blocked using goat serum, and incubated with primary and secondary antibodies for 30 min each. For Live/Dead staining (6 wells per experiment), cells were incubated with 0.5 mL of phosphate buffered saline containing 0.1 µM calcein AM and 16 µM EthD-1 for 20 min.

Results/Discussion: For each of the five growth factors, dose response studies were performed to determine the percentage of cells staining positive for each cell differentiation marker after 14 d of culture. NT-3 and Shh showed the most promising results for promoting neuronal and oligodendrocyte differentiation (Fig. 1). At higher doses, NT-3 induced formation of more neurons than those found in cultures with no growth factors present, while the percentage of astrocytes produced was reduced. At a dose of 25 ng/mL NT-3 in the media, an increase in the percentage oligodendrocytes produced is also observed. At all three doses of Shh tested, an increase in the percentage of oligodendrocytes produced, along with a decrease in percentage of neural progenitors and astrocytes produced was observed. At a 10 ng/mL dose of Shh, a decrease in percentage of undifferentiated ES cells and an increase in the percentage of neurons produced occured. For PDGF, a 2 ng/mL dose produced an increase in the percentage of oligodendrocytes present while higher doses reduced astrocyte production. At 10 and 25 ng/mL doses of CNTF, an increase in the percentage of neurons was observed. At a 25 ng/mL bFGF dose an increase in the percentage of neurons and oligodendrocytes was observed.

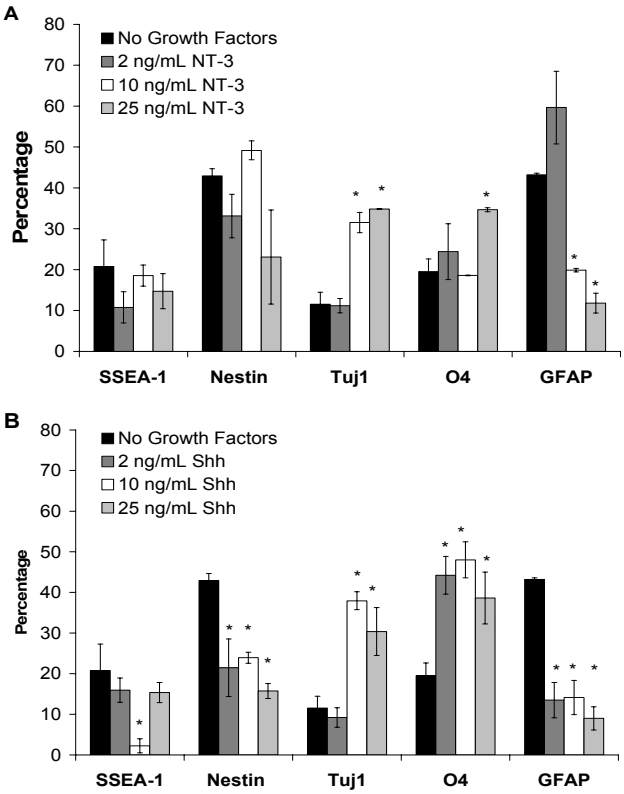

Figure 1: Dose Response of ESNLCs to A) NT3 B) Shh. * indicates $p < 0.05$ versus 14 day culture with no growth factors present. Markers include SSEA-1 (undifferentiated ES cells), nestin (neural precursors), Tuj1 (early neurons), O4 (oligodendrocytes) and GFAP (early astrocytes).

Conclusions: 25 ng/mL of NT-3 and 10 ng/mL of Shh increased the percentage of neurons and oligodendrocytes formed from ESNLCs seeded inside of fibrin scaffolds while reducing the percentage of astrocytes present. Future work will investigate the effect of growth factor combinations and the effects of controlled release of growth factors on ESNLC differentiation inside of fibrin scaffolds.

Funding: NIH R01 NS051454
References: [1] Willerth, SM et al. Biomaterials. 2006: 36: 5990-6003. [2] Bain, G et al. Dev Bio. 1995: 2: 342–357