Glycosaminoglycan sulfation differentially regulates FGF-2 mediated human neural stem cell (hNSC) proliferation and cell cycle progression

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Statement of Purpose – Chondroitin Sulfate Proteoglycans (CSPGs) are key constituents of the brain extracellular matrix. They possess O-linked sulfated glycosaminoglycan (GAG) side chains that exhibit 'sulfation code' dependent regulation of growth factor binding and signaling (Gama C. et al, 2006). We hypothesized that site-specific and sulfation-dependent FGF2 interactions with sulfated GAG motifs will: a) stabilize and prolong FGF2 activity; and b) enhance human neural stem cells (hNSCs) proliferation by promoting FGF2-FGFR signaling. Here, we investigated the effects of differential GAG sulfation on potentiating FGF2 mediated hNSCs proliferation *in vitro*. The investigation of GAG sulfation specific regulation of growth factor binding and signaling can lead to the development of tailored sulfated GAG glycomaterials that possess desirable structure-function attributes.

Methods – Standard culture conditions for hNSCs incorporated 20 ng/ml FGF2 to maintain their stemness. Since sulfated GAGs are known to potentiate FGF2 activity, we conducted a dose response study with hNSCs cultured in the presence of heparin (2,4, N-sulfated), CS 4-O-sulfated (CS-A), and regioselectively oversulfated CS 4, 6 -O-sulfated (CS-E) GAGs, and were treated with different doses of FGF2 - No FGF2, 1ng/ml, 5 ng/ml and 20 ng/ml, to determine the critical concentration required for maintaining hNSC proliferation. An EdU (5-ethynyl-2'-deoxyuridine) cell proliferation assay was used to quantify GAG mediated cell proliferation. Cells were co-stained with nuclear dye, Hoechst 33342 and flow cytometry was performed at 48 hours. The percentages of EdU+/Hoechst+ cells were computed across all treatments and plotted. Cell cycle progression was assessed using nuclear stain, Draq5, across different FGF2 doses without GAG at 48 hours and percentages across different cell cycle phases were plotted. Data was analyzed using FlowJo_v10.8.1 software.

Results – A linear increase in cell proliferation was observed across all range of doses of FGF2 with sulfated GAGs **(Fig. 1A).** Results at each FGF2 dose indicated that heparin had the most potentiating effect on proliferation followed by CS-E and then CS-A. We observed that as the extent of sulfation increases, FGF2 dose dependency decreases. Heparin and CS-E in the presence of No FGF2 and 1ng/ml FGF2, respectively, were able to match the proliferation rate of hNSCs grown in standard CGM culture without GAGs, as compared to monosulfated CS-A, which required 20 ng/ml FGF2. Our results indicate that, at low FGF2 doses, sulfated GAGs mediate higher cell proliferation **(Fig 1B)**. Cell cycle data trended towards an increase in the S-phase and G2/M-phase with an increase in FGF2 concentration. This data correlated with overall

GAG-FGF2 mediated cell proliferation and cell cycle analysis of hNSCs

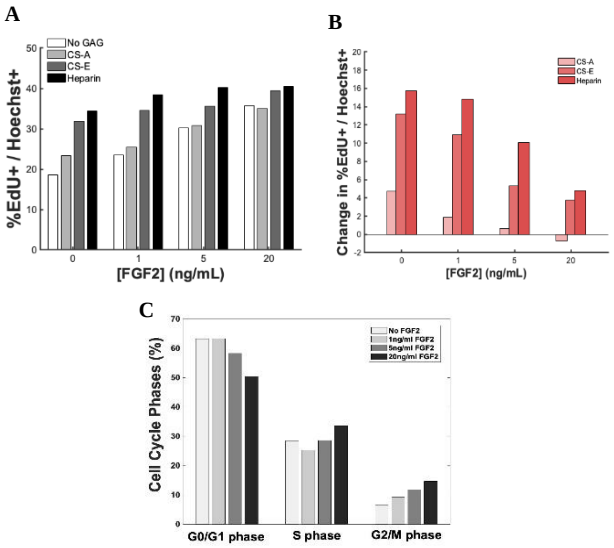

Figure 1- a) Flow Cytometry analysis of proliferating (EdU+) hNSCs mediated by sulfated GAGs - heparin, CS-A and CS-E compared to No-GAG across different FGF2 concentrations. b) Change in EdU+ cell proliferation rate normalized to No GAG control. c) Cell cycle progression across FGF2 doses without GAG.

reduction in the G1 phase across the FGF2 dose range **(Fig 1C).** Ongoing studies are focused on evaluating the influence of GAG sulfation on cell cycle progression. Future mechanistic studies will focus on specific binding affinity, function blocking, and cell signaling assessments.

Conclusion – Our study provides proof-of-concept data demonstrating that the GAG sulfation influences potentiation of FGF2 mediated hNSC proliferation and is expected to regulate cell cycle progression *in vitro*. Future investigations will focus on the design and optimization of functionalized sulfated GAG glycomaterials that can support endogenous NSC homeostasis and differentiation *in vivo.*

References –

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