Influence of Tethered Cadherin on Human Induced Pluripotent Neural Stem Cells

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Introduction: Neurodegeneration affects millions of people worldwide[1]. Although there are no cures for neurodegeneration, human induced pluripotent neural stem cells (hiNSC) can provide insight for the development of possible therapies. The NSCs reside in a region of the brain called the subventricular zone (SVZ)[2]. Their niche is a complicated environment in which the NSCs are affected by many factors, one of which is the the cadherin. N and E cadherin are the two main cadherin types found in the NSC niche[3]. These two cadherin types have been known to play a role in embryonic development[4], to bind homophilically and heterophilically[5], and to play a role in mesenchymal stem cell determination[6]. However, little is known about the role of N and E cadherin in determining hiNSC fate. Therefore, in this study we examine the effect of N and E cadherin on hiNSC to gain baseline 2D knowledge for future 3D experiments.

Methods: Cell culture: hiNSCs were obtained from Alstem and cultured on Matrigel as directed by the manufacturer. Cadherin protein analysis was done via Jess protein electrophoresis to examine baseline cadherin protein expression. Multipotency of hiNSC was evaluated via flow cytometry to check for Nestin expression. Surface Functionalization: borosilicate glass surfaces were functionalized via Fc tagged recombinant N and E cadherin to examine mechanotransduction. Cells were seeded at the normal seeding density (10,000 cells/ cm^2) on cadherin functionalized surfaces. After 60 hours cell viability was assesed via Calcein A and Ethidium homodimer staining. Next, hiNSCs were cultured for 24 hours to obtain cultures of single cells for examination of "surface to cell" cadherin mechanotransduction only. Integrin activation was assesed via immunocytochemistry (ICC) to ensure final evaluation of cadherin interaction only. Students t-test was used to see differences between groups with at least n = 3 samples per group . A significance value of p = 0.05 was used.

Results and Conclusions: Baseline N and E cadherin protein expression evaluation showed that the hiNSC population expressed significantly less E cadherin compared to N cadherin (1A). Next, surface functionalization (1B) was carried out and cell viability was tested. Calcein A/ Ethidium homodimer staining showed viable cells on N cadherin substrates and no viable cells on E cadherin substrates (1C). Here we see that cells did not express E cadherin and did not bind to E cadherin surfaces giving some insight into their ability to act homophilically versus heterophilically. Next, cells seeded on substrates show single cells on the positive control (Matrigel) and N cadherin substrates; however, again E cadherin substrates were not able to allow cells to adhere (1D). Initial investigation into integrin activation showed punctuated talin (magenta) on Matrigel control surfaces and no punctuated talin on N cadherin surfaces (1E). Thus, we show that N cadherin surfaces support cell growth similar to the positive control and that homophilic binding is supported. Our future work will include examination of the effect of 2D surfaces on NSC fate evaluated via qPCR and protein analysis. Also, we will further investigate integrin activation, cadherin mechanotransduction and the effect on gene expression in our hiNSCs.

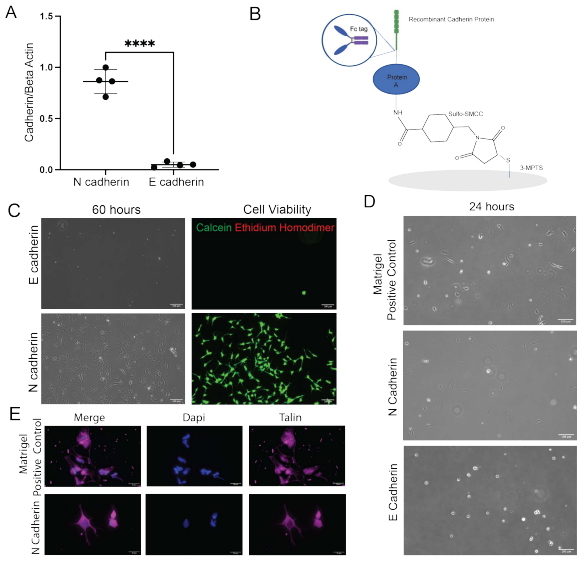

Figure 1: A. N and E cadherin expression in hiNSCs n = 4, $p < 0.0001$. B. Schematic of surface functionalization. C. hiNSCs adhesion to substrates and cell viability testing after 60 hours of growth via Calcein (green) and Ethidium homodimer (red). Scale bar 100 micrometer. D. Single hiNSCs after 24 hours on cadherin surfaces show single cells. Scale bar 100 micrometer. E. Integrin activation via Talin (magenta) shows punctuated Talin in positive control and no punctuations on N cadherin samples. Dapi in blue. Scale bar 20 micrometer.

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