**UNCOVERING THE ROLE OF NOTCH SIGNALING IN EARLY HYPOTHALAMIC FATE CHOICES USING PRIMARY NEUROSPHERES AND MICROENVIRONMENT ARRAYS**

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The hypothalamus is a key regulator of homeostatic function within the body. The neurons containing distinct neuropeptides and glial cells each arise from a common early progenitor to carry out these complex processes. During embryogenesis, both intrinsic and extrinsic signals instruct early progenitor cells to differentiate into their respective mature cell types. Dysregulation of this developmental process can result in physiological consequences persisting into adulthood including obesity and reproductive deficits.Previous *in vivo* work from our lab has shown that the Notch signaling pathway acts as a critical molecular switch during early development of the hypothalamus. Interestingly, active Notch signaling not only maintains early progenitors as SOX2-positive progenitors of the hypothalamic ventricular zone, but also appears to promote expression of Glial Fibrillary Acidic Protein (GFAP), a marker tanycytic stem/progenitor cells. Notch also appears to select the Kisspeptin neuron fate at the expense of dopaminergic neurons or neurons of the feeding circuit within the hypothalamus. However, the interaction between Notch signaling and extrinsic signals including growth factors and the extracellular matrix is unknown.

In our current study, we developed a hypothalamic progenitor cell culture in which we can activate or inhibit Notch signaling in a controlled extracellular environment. We hypothesized that inhibition of the Notch signaling pathway as well as removal of growth factors may promote primary hypothalamic progenitor cells to adopt specific differentiated fates. To address our hypotheses, primary neurospheres were treated with the γ-secratase inhibitor of Notch (DAPT) in the presence or absence of fibroblast growth factor (FGF) and epidermal growth factor (EGF) to assess their lineage bias. We determined that acute treatment with DAPT is sufficient to significantly reduce the downstream Notch target genes *Hes1* and *Hey1* and induce the proneural gene *Mash1*. Additionally, removing the growth factors in the presence of DAPT induces expression of the immature neuronal marker TUJ1 and the dopaminergic marker TH. Taken together, these data provide *in vitro* evidence for the direct role of Notch signaling in cell fate choices in the developing hypothalamus. Next,, we utilized a Notch ligand array coupled with multiple extra-cellular matrix (ECM) proteins to determine which Notch ligands may direct the fate of cultured neurospheres. We find that hypothalamic progenitors prefer to adhere to the ECMs laminin and fibronectin, as opposed to collagens. We saw no effect of the Notch ligands with respect to proliferation or the progenitor marker SOX2. However, in the presence of growth factors, DAPT treatment reduces proliferation and reduces GFAP expression. These ongoing studies will help further elucidate the role of Notch signaling in hypothalamic progenitor fates.

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