Exploring Differentiation and TEAD Inhibition in NF2-Knockdown NES Cells

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Background: The *NF2* gene is a tumor suppressor encoding gene on chromosome 22 that is a known regulator of the Hippo pathway. When the mammalian version of the pathway is inactive, such as with a loss of NF2, downstream proteins YAP/TAZ remain unphosphorylated, enter the nucleus to form a complex with TEAD 1/2/3/4, and begin transcription. Hyperactivation of the YAP/TAZ-TEAD complex has been observed in many cancers, allowing for targeting with TEAD inhibitors. Here, we assess how the loss of NF2 in human neuroepithelial stem (NES) cells affect their differentiational development. We also seek to understand the effects of TEAD inhibition on wildtype (WT) and NF2-knockdown NES cells.

Materials and Methods: *Differentiation.* WT and NF2-knockdown cells were grown in media without growth factors to differentiate them. *TEAD Inhibition.* Non-differentiating and differentiating WT and NF2-knockdown cells were treated with TEAD Inhibitor 690 (TEADi). During both conditions, cells were harvested at 5 points throughout the growth period.

Results: Decreased NF2 in cells promoted retention of an earlier cell morphology compared to WT, which appeared to develop neuronal features, such as axons. WT cells exhibited elevated expression of genes characteristic of NES differentiation when compared to NF2-knockdown cells. Following the addition of TEADi, cell culture imaging revealed seemingly increased cell death in WT cell populations compared to NF2-knockdown cells. Interestingly, differentiating NF2-knockdown cells adhere to one another to form clusters, but with TEADi, these clusters are formed to a much lesser extent.

Conclusion: Although more experimentation is needed, these are early steps in demonstrating how NF2 loss appears to halt the differentiation of NES cells. Additionally, TEAD inhibition seems to reduce the clustering seen in differentiating NF2-knockdown cells; however, experimental concentrations need to be explored in the future. Further work is needed to understand the effects of TEAD inhibition on NF2-knockdown cells.