Functional and Structural Characterization of the IgLON Protein Family

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According to the CDC, one in every 68 children is diagnosed with autism in the US. A subfamily of the immunoglobulin superfamily, the IgLON family, has recently been classified as potential candidate genes implicated with autism. The IgLONs is a family of cell adhesion proteins found in the central nervous system and consists of five members- OPCML, NTRI, LAMP, NEGRI, and IGLON5. During a high-throughput ELISA-based binding assay that detects protein-protein interactions, I identified the interaction of two IgLON family members, NTRI and NEGR1. This interaction was also confirmed by a literature search. In this project, I investigated the interactions among all IgLON family proteins and started characterizing their in vivo functions. The IgLON genes were first cloned into two desired vectors and the proteins were expressed using suspension adapted HEK293F cells. The protein-containing media were then collected and used during the ELISA-based binding assay to test for homophilic and heterophilic interactions across all five members of the IgLON family. One interaction, between NTRI and NEGR1, was further examined with the BioLayer Interferometry (BLI) and cell-aggregation assay. I also started characterizing its function in neuronal growth using a neuron-HEK co-culture experiment. The ELISA assay indicated both homophilic and heterophilic interactions across the IgLON family proteins. Moreover, the interaction between NTRI and NEGR1 was identified to be a trans interaction with the cell-aggregation assay. Furthermore, through the BLI experiment, we determined that the first Ig-like domain of NTRI is the active interacting domain that binds to NEGR1. Additionally, the neuron-HEK293 cell co-culture experiment indicated that both NTRI and NEGR1 have synaptogenic activities, raising the possibility that they may do so in the brain. Given the potential importance of the IgLON family in autism, the characterization of interactions and their functions is critical in understanding neurodevelopmental disorders like autism.