Title: The role of NUB1 in the clearance of tau aggregates in Alzheimer's disease

Background: Intracellular aggregations of abnormal hyperphosphorylated tau are a common marker in Alzheimer’s disease brain patients. Previous data revealed that NEDD8 ultimate buster 1 (NUB1) plays a role in reducing the aggregation and phosphorylation of tau in an in vitro model while the proteasome is inhibited. NUB1 is a ubiquitin-like (UBL)/ ubiquitin-associated (UBA) protein that works as a negative regulator for ubiquitin-like modifier proteins such as NEDD8, FAT10 and neurodegenerative diseases-related proteins such as synphilin-1, huntingtin. The aim of the study is to understand whether NUB1 affects tau aggregation and phosphorylation via autophagy.

Methods: A GFP-tau inducible neuroblastoma cell line was generated and treated with proteasome inhibitor to induce the formation of aggresomes. To define the role of NUB1 on GFP-tau aggregation, GFP-tau cells were transduced with the optimized multiplicity of infection (MOI) of lentiviral vectors expressing NUB1 cDNA, prior to induction and treatment with a vehicle (DMSO) or a proteasome inhibitor. Western blotting, filter trap assays, immunoprecipitation (IP) and immunofluorescence (ICC) were performed to evaluate phosphorylation levels of tau and changes in the autophagy markers.

Results: The western blot data indicated that NUB1 overexpression via lentiviral vectors affected phosphor-tau levels in the GFP-tau inducible cells treated with the proteasome inhibitor. Filter trap assays confirmed that increasing NUB1 level significantly decreased the level of phospho-tau on total tau in a MOI dependent manner. NUB1 affected phospho-tau and tau ratio both treating the cells with DMSO and with an inhibitor of the proteasome.
However western blot revealed that blocking the proteasome induced the upregulation of p62 and an increase in LC3-I/LC3-II ratio. IP data revealed that NUB1 overexpression promotes the interaction between tau and p62. Additionally LC3-II/LC3-I ratio is affected by NUB1 overexpression and ICC revealed an effect of NUB1 also on LC3 puncta. **Conclusion:** These data suggest that NUB1 affects tau phosphorylation by activating the autophagic pathway.