P3-151 GAMMA-SECRETASE INHIBITION INDUCES LIPID DROPLET ACCUMULATION VIA APP-CTF ACCUMULATION

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Background: Abnormal cholesterol metabolism is suspected as one of the factors contributing to Alzheimer disease (AD) pathogenesis. We and others have previously shown that γ-secretase dysfunction, which appears to be a main consequence caused by clinical *presenilin* mutations relevant to familial AD, increases cholesterol level in non-neuronal cells [1, 2]. Additionally, we proposed that increase of one of the γ-secretase substrates, amyloid precursor protein C-terminal fragments (APP-CTFs), is a possible mediator of the cholesterol increase [2]. In this study, we examined the involvement of APP-CTFs in the metabolism of cholesterol and lipid droplets [3] in neuronally differentiated SH-SY5Y (nSY5Y) cells and in mouse embryonic fibroblasts lacking APP expression (MEFs-APPKO). **Methods:** nSY5Y cells differentiated by retinoic acid or MEFs-wild type (MEFs-WT) or MEFs-APPKO were treated with a γ-secretase inhibitor, N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT). To suppress APP-CTF accumulation in nSY5Y cells upon DAPT treatment, cells were co-treated with inhibitors of α-secretase or β-secretase. Levels of lipid droplets and cholesterol were measured by oil red-O staining and enzymatic assay, respectively. **Results:** γ-Secretase inhibition in nSY5Y cells by DAPT significantly increased levels of lipid droplet and cholesterol and affected the expression profile of the proteins involved in cholesterol metabolism, such as ABCA1, NPC1, sterol regulatory element-binding protein 2, and LDLR. Suppression of the DAPT-induced APP-CTFs accumulation completely rescued lipid droplet accumulation; however, cholesterol accumulation and abnormal expression profile of the proteins were not rescued by suppression of the APP-CTFs accumulation. Additionally, γ-secretase inhibition induced lipid droplet accumulation only in MEFs-WT but not in MEFs-APPKO in contrast to cholesterol accumulation, which was detected in both of them upon DAPT treatment. **Conclusions:** These results indicate that γ-secretase inhibition has complex effects on cellular lipid metabolism in neuronal and non-neuronal cells, partly involving accumulated APP-CTFs. References: 1) Grimm MO, et al. Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. Nat Cell Biol. 2005;7(11):1118-1123. 2) Tamboli IY, et al. Loss of gamma-secretase function impairs endocytosis of lipoprotein particles and membrane cholesterol homeostasis. J Neurosci. 2008;28(46):12097-12106. 3) Area-Gomez E, et al. Upregulated function of mitochondria-associated ER membranes in Alzheimer disease. EMBO J. 2012;31(21):4106-4123.

P3-152 INVESTIGATING THE ROLE OF UBIQUILIN2 IN AGE-RELATED NEURODEGENERATION

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Background: Ubiquilins (UBQLNs) are a family of highly homologous proteins that shuttle polyubiquitinated proteins to the proteasome for degradation and clearance. The more widely studied UBQLN1 has been shown to co-precipitate in protein deposits found in brains of patients with Alzheimer's disease (AD). Likewise, UBQLN2 accumulates in protein deposits in the brains of patients with AD, as well as Frontotemporal Dementia (FTD), Huntington's disease (HD) and Lewy body dementia (LBD). The involvement of UBQLN2 and the broader ubiquilin family in a wide variety of neurodegenerative diseases reflects the fact that this family of ubiquitin receptor proteins normally helps maintain neuronal protein homeostasis. Their function in health and disease, however, remains poorly understood. **Methods:** To explore UBQLN2-mediated disease we employed protein biochemistry, cell-based studies, longitudinal fluorescence microscopy, fluorescence recovery after photobleaching (FRAP), and transgenic mouse models. **Results:** *In vitro* aggregation studies demonstrate that wild-type UBLQN2 is intrinsically aggregation-prone, and this aggregation propensity is enhanced by a familial ALS/FTD pathogenic mutation (P506T). UBQLN2 aggregation and solubility are modulated by the protein's ubiquitin-binding and ubiquitin-associated domains, respectively. *In vivo* FRAP studies performed on UBQLN2 puncta in transfected cells establish that UBQLN2 readily self-assembles into dynamic structures with liquid-like properties. Moreover, the P506T mutation impairs liquid droplet dynamics and favors amyloid-like aggregation. UBQLN2 aggregation propensity is recapitulated in the brains of transgenic mice over-expressing WT or P506T-UBQLN2. When expressed at high levels, WT-UBQLN2 begins to form uniform, spherical cytoplasmic puncta in neurons that resemble the liquid-like droplets identified in HEK cells. In contrast, P506T-UBQLN2 accumulates within larger, irregular cytoplasmic inclusions, leaving no detectable diffuse protein. Employing longitudinal fluorescence imaging in primary neuronal cultures transiently expressing WT or mutant UBQLN2, we were able to correlate UBQLN2 aggregation propensity with neurotoxicity. **Conclusions:** Our results demonstrate that UBQLN2 is intrinsically prone to self-assemble into higher order complexes, including liquid-like droplets and amyloid aggregates. The P506T mutation enhances aggregate formation associated with neurotoxicity. Ongoing studies are aimed at determining the composition, identity and behavior of UBQLN2 assemblies, and the impact of disease-associated *UBQLN2* mutations upon aggregate dynamics, which will be critical for assessing their consequences for neurodegenerative disease.

P3-153 VALIDATING LXRS/ABCA1/APOE AXIS INTERVENTION AS A POTENTIAL THERAPEUTIC TAGET TO PREVENT AMYLOID BETA CLEARANCE IMBALANCE

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