Epitope mapping experiment using deletion mutants revealed that A5201A recognizes multiple regions in NCT, implicating that a mode of recognition of A5201A is conformation-dependent manner. Next we generated a scFv based on the variable region of A5201A. Intriguingly, the overexpression of A5201A-based scFv as an intrabody in mammalian cells suppressed the γ-secretase activity. Biochemical analyses revealed that the scFv disrupted the proper folding and the appropriate glycosyl maturation of NCT, which are required for the stability of the γ-secretase complex and the intrinsic proteolytic activity, respectively. **Conclusions:** These results provide compelling evidence that the extracellular domain of NCT can be a therapeutic target for Alzheimer's disease through suppression of Aβ generation.

O1-05-04 EXPLORING THE EFFECT OF A CHANGE IN PLASMA MEMBRANE CHOLESTEROL ON THE LIPID RAFTS-PARTITIONING OF APP USING FLUORESCENCE CORRELATION SPECTROSCOPY (FCS)

Catherine Marquer[1], Jack-Christophe Cossec[1], Sandrine Lécart[2], Géraldine Liot[3], Sandrine Humbert[3], Frédéric Saudou[3], Charles Duyckaerts[1], Sandrine Lévêque-Fort[2,4], Marie-Claude Potier[1], *[1]CRICM Inserm UMR-S 975; CNRS UMR 7225, Paris, France; [2]CLUPS, Université Paris-Sud 11, Orsay, France; [3]Institut Curie-UMR146 du CNRS, Orsay, France; [4]CNRS-Laboratoire de Photophysique Moléculaire, Orsay, France. Contact e-mail: catherine.marquer@espci.fr*

Background: One of the histopathological hallmarks of Alzheimer's disease (AD) is the presence in the brain of AD patients of amyloid plaques resulting from the accumulation and the deposition of the amyloid peptide (Aβ). Aβ derives from the clivage of the transmembrane amyloid precursor protein (APP) by two enzymes, the β- (Bace1) and γ- secretases. Recent converging data point to an important role of cholesterol in AD pathogenesis both in vivo and in vitro. Since both APP and the secretases are transmembrane proteins and cholesterol is enriched in membrane microdomains called lipid rafts, we investigated the effect of modifying the membrane cholesterol concentration on the lipid rafts-partitionning of APP using both biochemical and biophysical techniques. **Methods:** Detergent-resistant membrane fractions (DRMs) were isolated by ultracentrifugation and analysed by western blotting using specific antibodies directed against APP, Bace1 and flotillin. Diffusion coefficients of APP-YFP, transferrin-Alexa555 and cholera toxin-Alexa 488 were measured using fluorescence correlation spectroscopy (FCS). Membrane cholesterol was modulated in HEK cell lines and in embryonic rat hippocampal neurons using either MβCD or a MβCD-cholesterol complex. **Results:** Using biochemical techniques, we showed that when cholesterol concentration at the plasma membrane is increased, APP concentrates in DRMs and that this leads to an increased Aβ production. Additionnally, our FCS results indicate that changes in membrane cholesterol concentration have no effect on the lipid rafts-partitionning of transferrin-Alexa 555, a marker of disordered (non-raft) phase, and cholera toxin-Alexa 488, a marker of ordered (raft) phase. However, the partitionning of APP-YFP is strongly influenced by cholesterol concentration. More cholesterol increases the proportion of APP located in the ordered (raft) phase while less cholesterol increases the part of APP located in the disordered (non-raft) phase. **Conclusions:** Here we show for the first time in live cells the impact of a change in cholesterol on the rafts-partitionning of APP, providing new insight into a possible link between concentration in lipid rafts and increased endocytosis. Similar studies concerning the β-secretase Bace1 are currently underway.

O1-05-05 STRUCTURAL AND FUNCTIONAL GOLGI DEFECTS IN ALZHEIMER'S DISEASE

Yanzhuang Wang, Youjian Chi, Yi Xiang, Henry Paulson, *University of Michigan, Ann Arbor, MI, USA. Contact e-mail: yzwang@umich.edu*

Background: The accumulation of amyloid beta (Aβ) peptides and β-amyloid plaques are central events in the development of Alzheimer's disease (AD). Aβ is produced by proteolysis of the amyloid precursor protein (APP). Trafficking and maturation of APP and its processing enzymes require proper functioning of the Golgi apparatus, a highly organized, stacked structure in the cell that plays a central role in protein trafficking, processing and maturation. It has been observed that the Golgi structure is fragmented abnormally in neurons from AD patients. However, the mechanisms underlying Golgi fragmentation and the consequences for APP trafficking and Aβ production are poorly understood. **Methods:** Using cell biological and biochemical methods we established for studying Golgi biogenesis, we sought to define Golgi defects in AD and determine the relationship between APP processing and Golgi perturbation. We used AD tissue culture (e.g. CHO cells) and transgenic mouse models in which both APPswe (APP "Swedish" mutation) and PS1ΔE9 (presenilin 1 with exon 9 deleted) are expressed. **Results:** APP expression and Aβ accumulation were shown to induce Golgi fragmentation in both tissue culture and transgenic mouse models. Golgi fragmentation in turn was found to accelerate intracellular protein trafficking and alter glycosylation of cell surface membrane proteins. Fragmentation of the Golgi is mediated via phosphorylation of specific Golgi structural proteins (e.g. GRASP65) by kinases whose activity increases when Aβ accumulates. Taking advantage of new insights into the molecular regulation of Golgi integrity, we further demonstrate that Golgi structure can be rescued either by kinase inhibitors (e.g. roscovitine) or by overexpression of non-regulatable Golgi structural proteins (e.g. GRASP65). Ongoing studies will test whether the principal kinase inhibited by roscovitine is cdk5 and whether rescue of Golgi structure in the tissue culture model reduces Aβ production. **Conclusions:** Our results support a model of AD pathogenesis in which Aβ accumulation perturbs Golgi function, which in turn further augments Aβ production in the late secertory pathway. As rescue of the Golgi structure may reduce Aβ production and slow the amyloid cascade, the current results suggest potential novel therapeutic strategies in Alzheimer's disease.

O1-05-06 NOVEL DOWNSTREAM MEDIATORS OF APP SIGNALLING

Dale E. Bredesen[1], Qiang Zhang[1], Andrzej Swistowski[1], Mark Orcholski[1], Alexei Kurakin[2], *[1]Buck Institute for Age Research, Novato, CA, USA; [2]Beth Israel Deaconess Medical Center, Harvard Medical School., Boston, MA, USA. Contact e-mail: dbredesen@buckinstitute.org*

Background: The prevailing view of AD is that the Aβ peptide causes toxicity through chemical and physical mechanisms, such as metal binding, ROS production, and membrane damage. Our data suggest an alternative view of AD as an imbalance in physiological signaling mediated by APP: in this model, Aβ functions physiologically as an anti-trophin, competing with trophic ligands for APP. Aβ binding to APP induces the formation of a peptide, APP-C31, mediating neurite retraction and cell death (Nat Med 6:397). Thus preventing APP-C31 in transgenic mice renders them resistant to AD (PNAS 103:7130). Competing with Aβ is a novel APP ligand, netrin-1, which mediates neurite extension through APP. The intracellular domain of APP (AICD) interacts with several adaptor proteins, including Fe65, Mint1/X11 and JIP1. Interestingly, AICD, Fe65 and Tip60 form a transcriptionally active triple protein complex (Science 293:115). **Methods:** TAIS, Co-IP, Confocal Microscopy, Gal4 transactivation assay. **Results:** In an effort to identify novel mediators of APP signaling, we employed target-assisted iterative screening (TAIS) and identified 40 novel proteins that potentially associate with APP through the PDZ domains of Mint1/X11. Surprisingly, 14 turned out to be transcriptional regulators. We first focused on two of these, TAZ and YAP. TAZ was found to co-IP with both Mint1 and Mint3. In the presence of Mint1 or Mint3, TAZ was also found to co-IP with APP. In confocal microscopy colocalization studies, TAZ and YAP colocalized with both Mint1 and Mint3. While Mint3 did not significantly affect the nuclear and cytoplasmic distribution of TAZ and YAP, co-expression of Mint1 relocated TAZ and YAP to cytoplasm, excluding them from the nucleus. In APP-Gal4 transactivation assays, TAZ and YAP induced potent transactivation in the presence of Mint3, while co-expression of Mint1 or treatment with the γ-secretase inhibitor DAPT abolished this effect. In Mint3-Gal4 transactivation assays, TAZ and YAP induced potent transactivation. This effect was reduced by co-expression of APP and further