Huntingtin associated protein 1 associates with amyloid precursor protein and regulates its trafficking and Aβ levels

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Amyloid precursor protein (APP) is a type I transmembrane receptor-like molecule involved in the pathogenesis of Alzheimers disease. Following endocytosis, APP is delivered to endosomes, where β- and γ-secretases are localized and Aβ neurotoxic peptide is produced. Huntingtin associated protein 1(HAP1) is a brain-enriched protein and participates in intracellular trafficking in neurons. HAP1 interacts with kinesin light chain and dynactin p150Glued, regulates the anterograde and retrograde transport of a number of proteins including proBDNF and APP (McGuire *et al*,, 2006). However, how HAP1 regulates APP trafficking and the significance of this regulation remain unknown.

Using HEK 293 cells transfected with HAP1-CFP and APP-YFP plasmids, we showed that these two proteins were highly colocalized. Immunohsitochemical data showed that these two proteins are present in a number of brain regions such as cortex, hippocampus and hypothalamus with a similar distribution patterns in the mouse and human brains. Confocal microscopy showed that they also co-exist in a number of subcellular structures. FRET analysis showed that the FRET efficiency between HAP-CFP and APP-YFP was over 20%, much higher than negative and positive controls, indicating these two molecules were close to each other *in vivo*. Immunoprecipitation experiments on over-expressed HEK293 cell lysates or on human brain homogenates showed that HAP1 and APP were present in the immunoprecipitated samples forming a complex. To see whether HAP1 regulates APP subcellular trafficking, we cultured cortical neurons from HAP1+/+ and HAP1-/- neonatal mice and analyzed the co-localization of APP with organelles marker proteins. The results showed that APP has a high co-localization ratio with giantin GM130 (cis-Golgi marker), Golgi97 (trans-Golgi complex marker), EEA1 (early endosome marker) and SEC22b (ER-Golgi intermediate compartment marker) in HAP1-/- cortical neurons, but has no significant difference with GRP78 (endoplasmic reticulum marker), CD71 (recycling endosome marker), Lamp1 (lysosome marker) and VPS35 (retromer marker) in neurons between HAP1+/+ and HAP1-/- mice. However, there was a lower co-localization ratio between APP and the autophagy marker beclin1 in HAP1-/- neurons, compared with wt neurons. These results suggest that APP is retained in cis-Golgi, trans-Golgi complex, early endosome and ER-Golgi intermediate compartment when HAP1 is deleted, and HAP1 may increase the APP trafficking to autophagy vesicles. Sucrose gradient fractionations on wt and HAP-/- brain homogenates showed that the APP distribution is altered. In the normal wt brain there was only one peak of APP, corresponding to membranous organelles such as Golgi and ER, whereas in the HAP1-/- mice, there were two peaks of APP distribution: one is near to the bottom of the gradient and the other was in the cytosol fractions. Interestingly, GM130, EEA1 and GRP78 had a similar distribution pattern to APP in HAP1-/- mice. APP internalization assay using antibody imaging and biotinylation techniques on HAP1-/- neurons showed that significant alteration in APP endocytosis in HAP1-/- neurons and abnormal re-insertion of APP into the cytoplasmic membrane. Live imaging analysis and FRAP assay on APP-YFP vesicles in HAP1-/- neurons showed that the trafficking speed was reduced and the number of motionless particles were increased. To see whether HAP1 regulates Aβ production, we cultured cortical neurons from Alzheimers disease mice and knocked down HAP1 protein with interference RNA. We found that the down-regulation of HAP1 increases Aβ levels.

Taken together, our data suggest that HAP1 associates with APP and regulates APP subcellular trafficking to the non-amyloidogenic pathway. Up-regulation of HAP1 may increase APP re-insertion into cytoplasmic membrane and reduce Aβ production.

McGuire JR, Rong J, Li SH, Li XJ. (2006) Interaction of Huntingtin-associated protein-1 with kinesin light chain: implications in intracellular trafficking in neurons. *Journal of Biological Chemistry* **281:** 3552-3559.