A CRISPR-Cas9 screen to identify genetic modifiers of APP/BACE-1 interactions

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Research Abstract

? DESCRIPTION (provided by applicant): The overall goal of this proposal is to discover molecules along trafficking pathways leading to the convergence of two key proteins in Alzheimer’s disease (AD) pathogenesis – Amyloid Precursor Protein (APP) and ?-site APP-cleaving enzyme-1 (BACE-1). This convergence, and consequent enzymatic ?-cleavage of

APP, is the rate-limiting step of amyloid beta (A?) production – a pathological hallmark of AD brains and a prevailing focus in AD research. Visualizing APP/BACE-1 trafficking in hippocampal neurons, we recently found that after synthesis, APP and BACE-1 are sorted into distinct vesicles, with BACE-1 selectively routed into recycling endosomes. At steady state, APP and BACE-1 convergence is a low-frequency event – producing A? at basal levels (Das et al., Neuron 2013; PMID: 23931995). Following up on these studies, we reasoned that ascertaining molecular pathways leading up-to this seminal convergence event would allow: 1) identification of the repertoire of trafficking pathways by which APP and BACE-1 meet to initiate the amyloidogenic cascade; and 2) discovery of novel “”druggable targets”” that can be manipulated to diminish APP/BACE-1 convergence and A? production. Towards this we developed an in-cellulo Optical assay to visualize Convergence of APP and BACE-1 (OptiCAB). Based on fluorescence complementation, this assay reports APP/BACE-1 interactions as a simple on/off readout, correlates with APP ?-cleavage, and is suitable for large-scale analyses. Combining this assay with a newly-developed powerful genome-scale screen using CRISPR-Cas9 knockout (GeCKO) library (collaboration with Feng Zhang, MIT), our goal is to discover genes involved in `trafficking-related’ upstream pathways that eventually lead to APP/BACE-1 convergence and A? production. Notably, CRISPR-Cas9- based screens are not limited by incomplete protein depletion and confounding off-target effects that have historically limited the utility of RNAi. Secondary validation of `hits’ (i.e. genes that attenuate APP/BACE-1 interactions) will be done in human induced pluripotent stem cells (iPSC’s); where APP-cleavage products will be analyzed after relevant CRISPR-knockout. Our aims are: Aim #1: Discover pathways leading to APP and BACE-1 convergence using OptiCAB and GeCKO; and Aim #2: Validate `hits’ from Aim 1 in human neuronally-differentiated iPSCs. Our experiments will not only provide insights into the physiologic “”amyloid-pathway”” in humans, but may also offer new targets for AD. Finally, note that our focus on the repertoire of trafficking pathways leading up-t APP/BACE-1 approximation stems from our own live-imaging studies; and is different from the current narrow focus on enzymatic activity of the secretases.

Further information available at:

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