"Behavioral and cell-based complementary screens to identify druggable targets modulating tau protein levels"

Brief introduction:

The ultimate goal of this proposal is to identify "druggable" gene networks and inhibitors that attenuate Alzheimer's Disease (AD) pathogenesis by reducing the steady-state levels of the tau protein.

Our specific aims are:

Aim-1. Perform a druggable genome screen using a behavioral assay in Drosophila.

Aim-2. Conduct a FACS-based siRNA screen to ascertain which suppressor genes decrease tau levels in human cells.

Aim-3. Validate top candidates on endogenous tau levels and mouse primary cultures.

The modifier screen is performed in an in vivo model (fruit fly). The subsequent cell-based screen has two functions: 1- pin point which of the suppressor genes can decrease tau protein levels and 2- validate the fruit fly hits in human cells. The genes passing these two filters will have high translational value and will be ready for preclinical assessment using pharmacological inhibitors.

Major activities

The major activities of the funding period have conformed to the proposed timeline. We have performed most of the *Drosophila* based RNAi screen to identify genes that can ameliorate tau-induced neurodegeneration (aim-1) and we also are well advanced in identifying which mammalian homologs of the *Drosophila* hits can decrease tau levels in human cells (aim-2) using a combination of fluorescence activated cell sorting (FACS) followed by confirmation using anti-tau Elisa. **Importantly, we have already identified four potentially druggable genes whose knockdown suppresses tau-induced behavioral deficits in *Drosophila* and lowers tau levels in two different mammalian cell types and *in vivo* in the fruit fly**.

Specific objectives and significant results

Aim-1

Objective: to perform an RNAi screen targeting ~3,700 *Drosophila* genes considered potentially druggable (based on *in silico* curation) to identify those whose knockdown ameliorates tau-induced neurodegeneration *in vivo*.

Significant results: at the moment I write this report we have screened ~3,700 genes and have identified 222 primary hit genes that when knocked down can attenuate tau-induced degeneration (two examples of hit genes identified using our automated behavioral screening platform are shown in Figure 1A).

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Aim-2

Objective: to ascertain which suppressor genes from the *Drosophila* screen decrease tau levels in human cells. Using lentiviral-shRNAs we have began targeting the human homologs of the hits from Goal-1 to identify those whose reduction lowers the levels of tau in human cells.

In order to strengthen our confidence in the modifiers identified in this goal, we developed a two tier approach. First we screened using FACS analysis, to identify which of the candidate shRNAs could decrease the levels of a tau:EGFP reporter (DsRed-IRES-tau-EGFP) in a human cell line of neuronal lineage (Daoy cells) as described in our research plan. Second, we tested the hits from the primary FACS screen for their ability to decrease endogenous tau levels by Elisa in HEK293T cells.

Significant results: At the moment this report is written, we have targeted the first 100 hits that resulted from Goal-1. We have identified 51 genes whose knockdown decreases the levels of the tau:EGFP reporter in Daoy cells by FACS analysis. Next we tested the ability of these 51 genes to decrease the levels of endogenous tau in HEK293T, and uncovered 25 genes that when knocked down where able to decrease endogenous tau levels by Elisa (examples shown in Figure 2B). As an additional confidence measure, we asked whether knockdown of these 25 genes could decrease tau levels also in *Drosophila*. Interestingly, inducible RNAis targeting four of these genes were also able to lower tau in *Drosophila* by western blot (Figure 1C and D shows two of these genes), thus providing us very high confidence targets, due to the robust cross-species validation level.

Key outcomes

1- We have completed most of the *Drosophila* based genetic screen and we are confident the whole screen will be finalized in the upcoming funding period. The 222 modifiers we have identified so far are providing the backbone for advancing aims 2 and 3. In addition, these 222 modifiers can be mined beyond the goals of this proposal to learn about the complex biology of tau and uncover additional therapeutic avenues and causative mechanisms for AD and tauopathies.

2- Our second goal has resulted in the identification of four highly validated genes (full datasets for two of these genes shown in Figure 1A-D) that exemplify the standard for therapeutic targets that we seek in this project: 1- they suppress a phenotype based on neuronal function (Figure 1A), 2- they decrease the levels of the causative protein tau, but they do not completely eliminate it (Figure 1B-D), thus avoiding potential secondary effects from tau complete loss of function. 3- they target the wild type tau holoprotein, therefore offer the potential to treat the most common sporadic AD cases. Our focus on the potential druggable genome increases the chances that we will find pharmacological inhibitors for our hits.

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Figure 1. Two modifier genes identified in our screen whose knockdown ameliorates tau-induced behavioral deficits and causes a decrease in tau levels in all the endpoints we have established in our platform. (A) Expression of human tau in the *Drosophila* nervous system leads to motor performance deficits that can be quantified using our automated data acquisition platform. One of the outputs measured is speed. Notice the decreased speed of fruit flies expressing tau (black line) compared to age matched controls (blue line). Knockdown of two *Drosophila* genes identified as modifier-1 and modifier-2 (red lines) leads to an improved motor performance and increased climbing speed compared to animals expressing tau alone. Error bars indicate s.e.m and stars $p<0.05$ compared to "tau/no modifier". (B) Knockdown of the human homologs of the genes shown in A, leads to a decrease in tau levels measured by anti-tau Elisa in HEK293T cells. KHB0041 total tau elisa kit (ThermoFisher) was used. BCA was used to control for loading and quantification was normalized to scramble-sh1. An shRNA targeting tau was used as positive control. Error bars are s.e.m. and stars indicate $p<0.05$. (C) Knockdown of the same two genes as A and B in *Drosophila* causes a decrease in tau levels. Quantification in (D). Error bars are s.e.m and stars indicate $p<0.05$ compared to control. Significant differences in A, B and D where established using Anova followed by Tukey-Kramer's hsd.