Increasing Protein O-GlcNAcylation as a Disease Modification Strategy for Tauopathies

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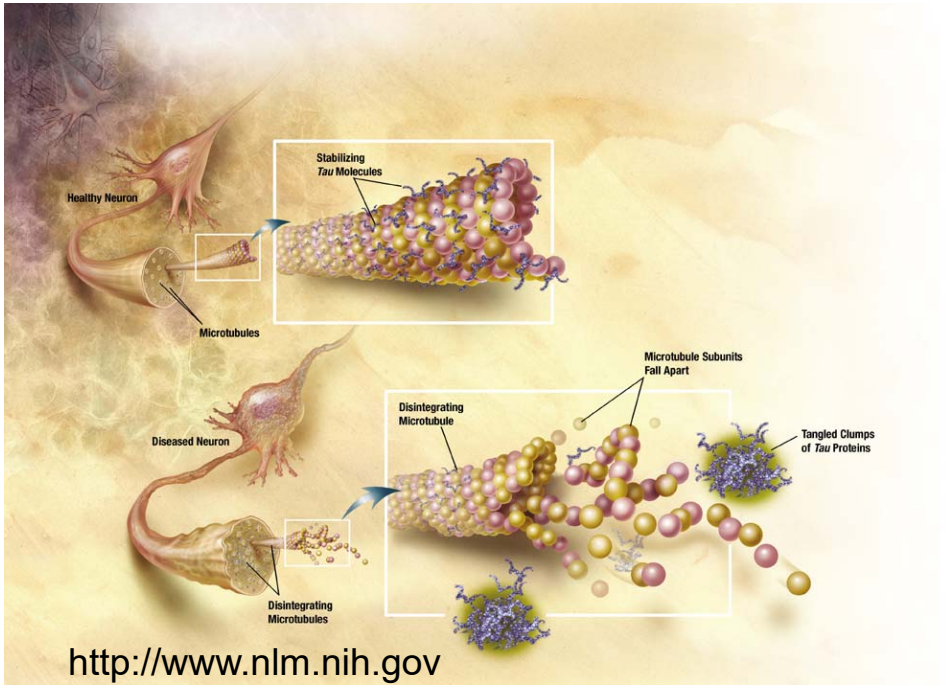
Tauopathies



http://www.nlm.nih.gov

Tauopathies

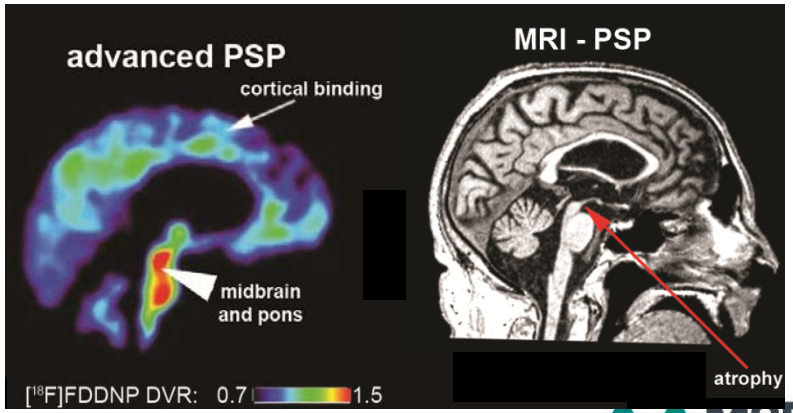
- A class of neurodegenerative diseases characterized by the deposition of abnormal tau proteins in the human brain

Alzheimer's disease (AD)

- Aggregation of hyperphosphorylated tau into neurofibrillary tangles is a pathological hallmark of AD
- The degree of tau pathophysiology is correlated with disease severity

Progressive Supranuclear Palsy **(PSP)**

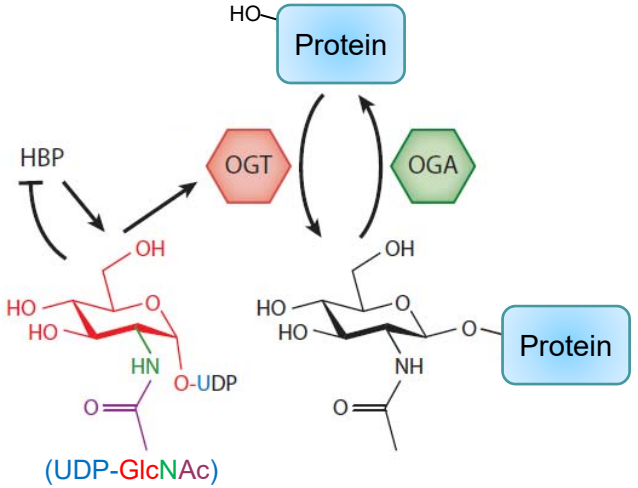
- PSP is an aggressive neurodegenerative disease for which there are no treatments
- The pathology of PSP is predominately associated with tau aggregation
- Clinical syndrome: gait instability and supranuclear gaze palsy



Murray et al. Alzheimer's Research & Therapy 2014

Review of O-GlcNAcylation

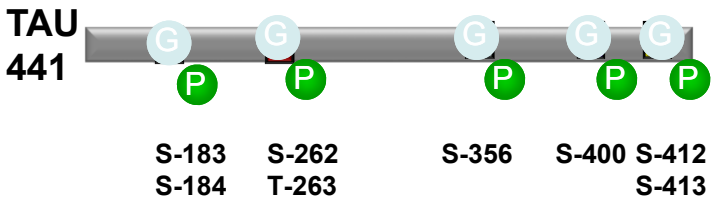
- O-GlcNAcylation is a common post-translational modification that plays a role in regulating protein stability
  - Destabilization of proteins has been implicated in the etiology of several neurodegenerative disorders including AD, ALS, HD, and PD
- O-GlcNAcylation is regulated by two enzymes
  - **O-GlcNAc transferase (OGT)**
  - **O-GlcNAcase (OGA)**
- OGA is highly expressed in the brain and unlike other mechanisms targeting tau is highly amendable to small molecule drug discovery



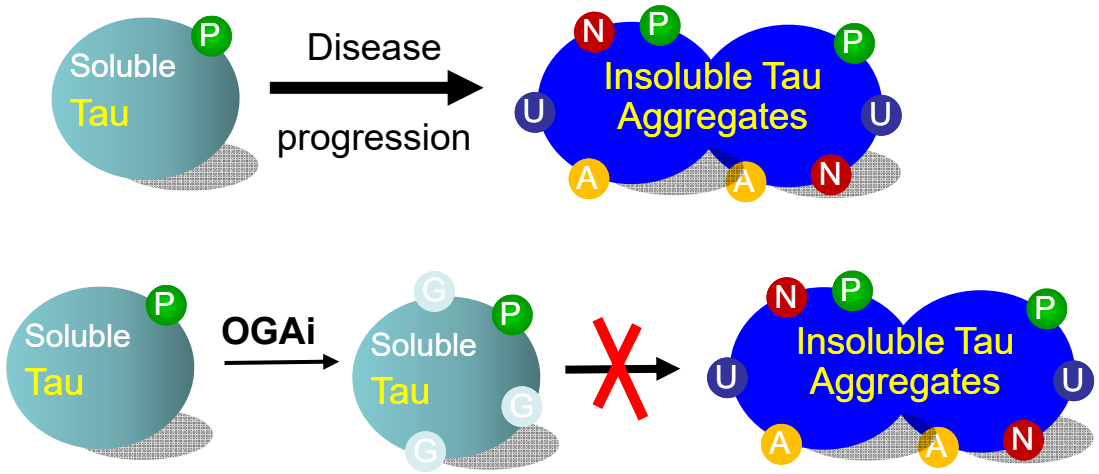
- Postmortem analysis of brain tissue reveals that protein O-GlcNAcylation is reduce in AD consistent with impaired glucose metabolism in AD brain
- Inhibition of OGA leads to an up-regulation of O-GlcNAc modified proteins both *in vitro* and *in vivo*

O-GlcNAcylation and Tau

- In adult brain, tau is O-GlcNAcylated at multiple sites
- Preclinical findings demonstrate that selective OGA inhibitors increase O-GlcNAcylation of tau, reduce the formation of aggregated tau and attenuate neurodegeneration in multiple transgenic models



Directly measure tau modification in human samples

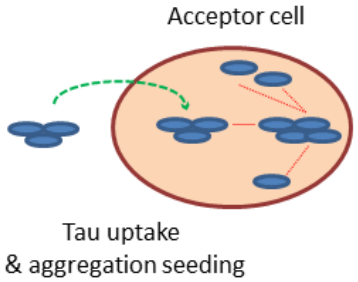


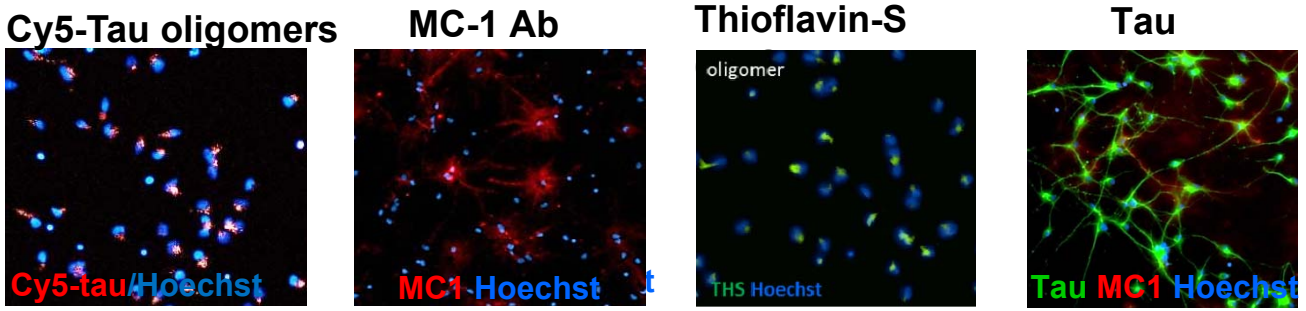
Understanding the OGAi MOA:

- Hyperphosphorylated tau (P) aggregates; contains several translational modifications such as acetylation (A), nitration (N) and ubiquitination (U)
- OGA inhibition increases O-GLcNAcylation (G) of soluble tau and prevents pathological tau formation

OGA Inhibitors reduced pathological tau conformation in iPSC-derived hu-neurons

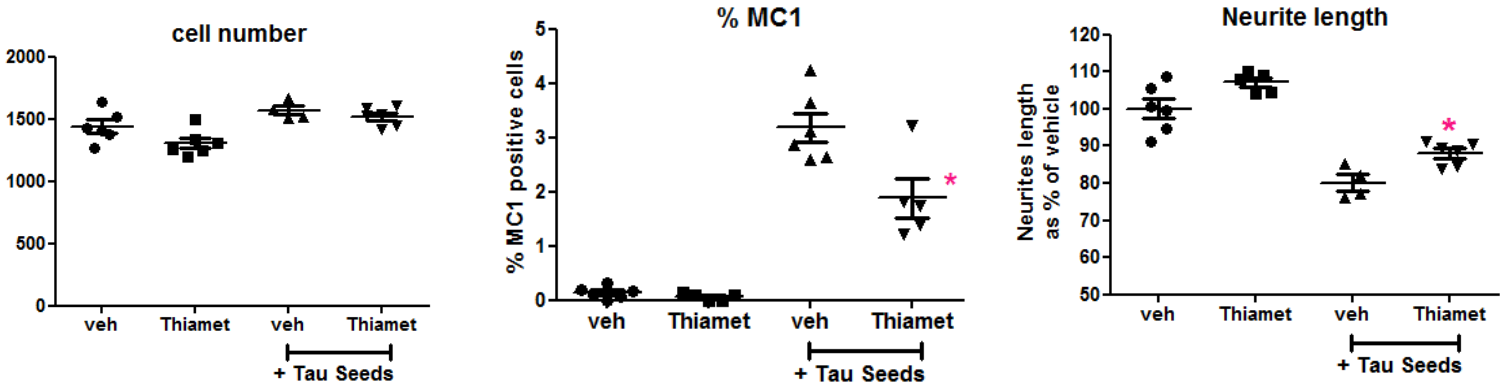
- **Seeding of tau oligomers induced intracellular tau aggregation**





- **Effect of OGAi, Thiamet G at 3 days post-seeding of tau oligomers**

iPSC-derived hu-neurons (DIV 7) were pre-treated with Thiamet-G 24h before seeding

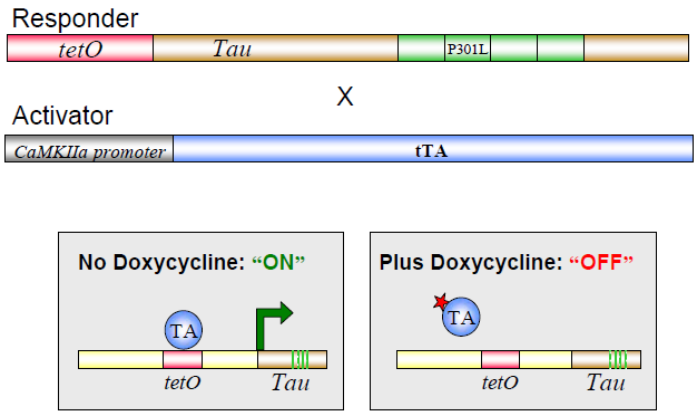


Quantification using High Content Image Analysis

** P<0.05 vs. Veh + Tau seeds ANOVA and Dunett's multiple comparison test*

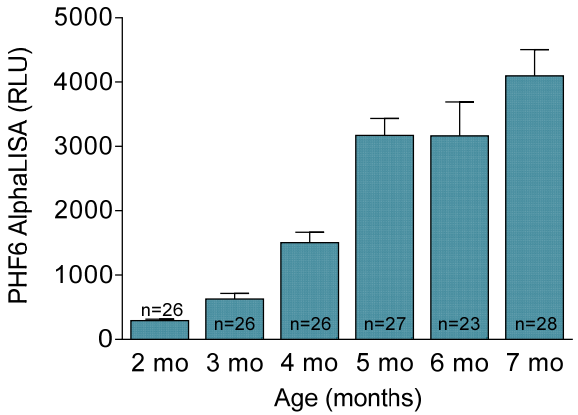
The Tg4510 model: A Transgenic Mouse Model of Brain Tauopathy

Tg4510 mice



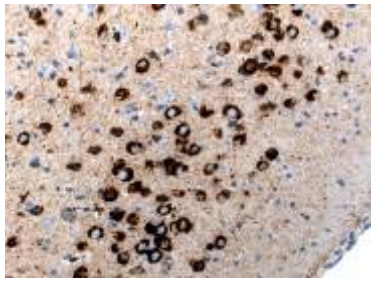
- ✓ Overexpressing human tau with P301L mutation that is link to FTDP-17
- ✓ Mutant tau expression is driven by CaMKII promoter and is regulatable by doxycycline

Tau Pathology



nY29 (NFT)

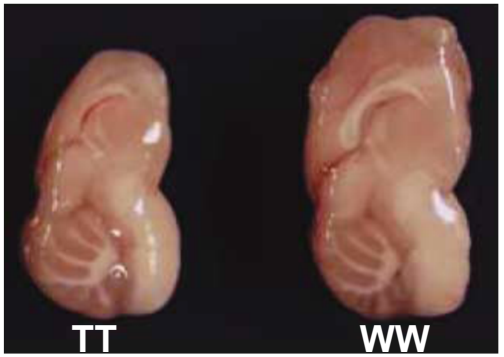
4- 5mo



hyperphosphorylated tau in Tg4510 brain (bottom) and Neurofibrillary tangles (top)

Brain Atrophy

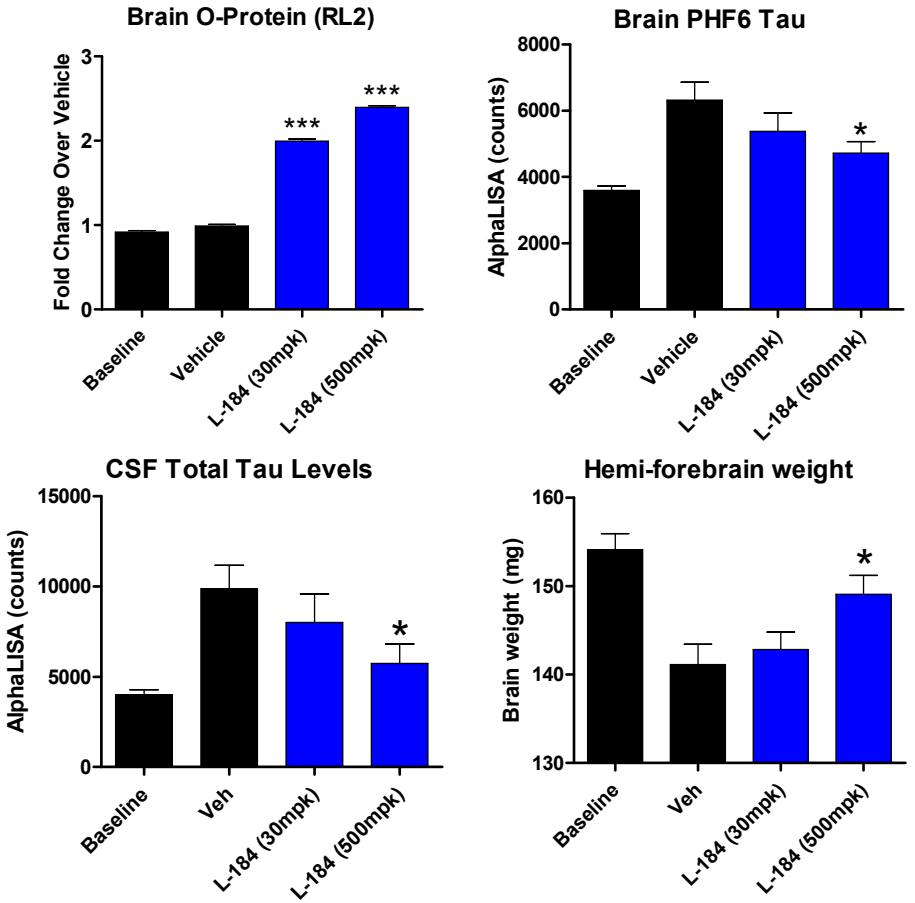
Mutant tau expression

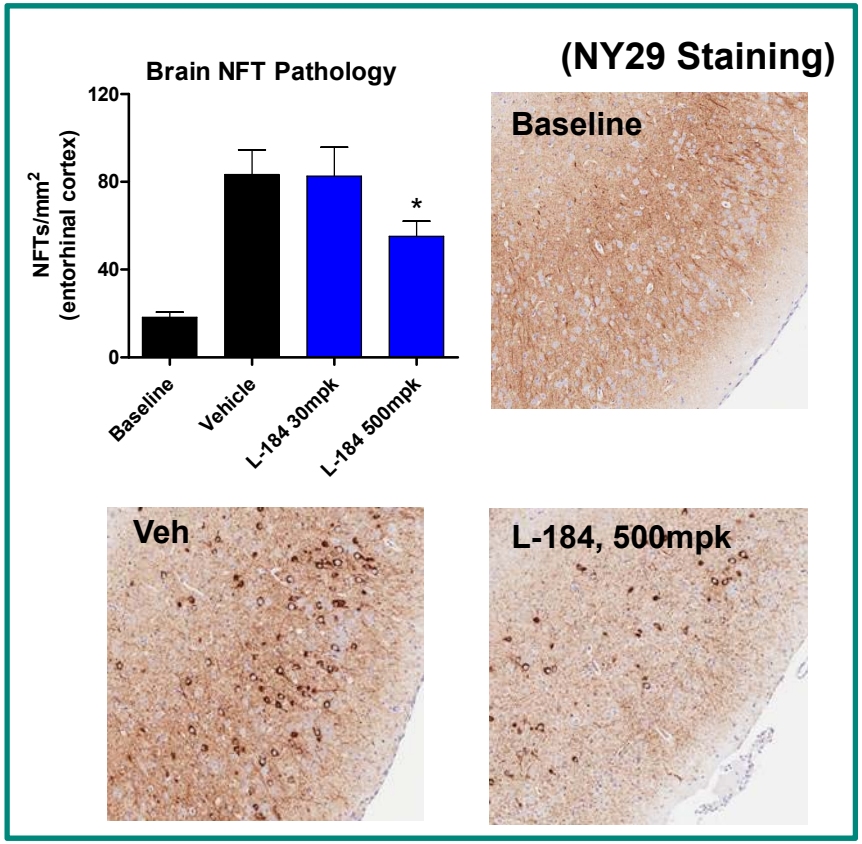


10 mo

Brain atrophy detected in forebrain structures correlates with mutant tau expression

Efficacy of OGA Inhibitors in Tg4510 Mice





- Tg4510 mice were treated (n=25/group) from age 8 week to 16 week with L-184 (30 and 500 mpk, QD, PO)
- L-184 (500 mpk) significantly increased brain o-protein levels, reduced brain PHF6 tau and CSF total tau, prevented brain atrophy and ameliorated NFT pathology

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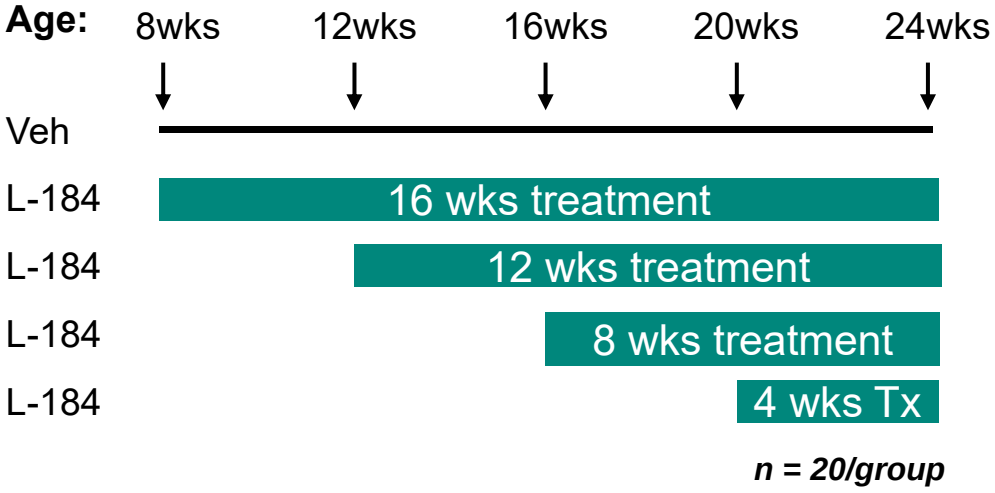
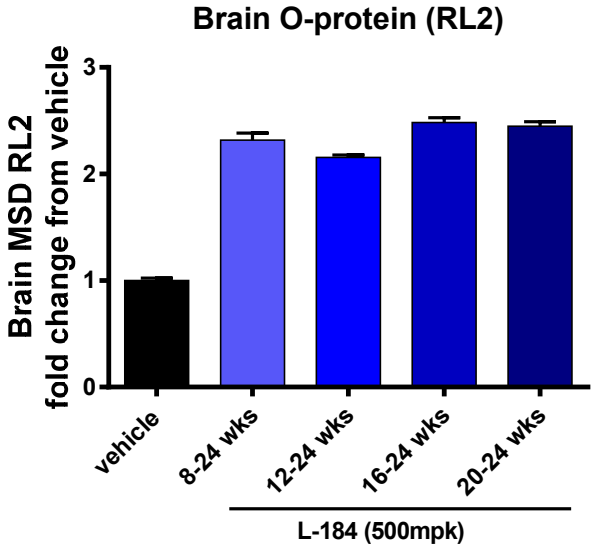
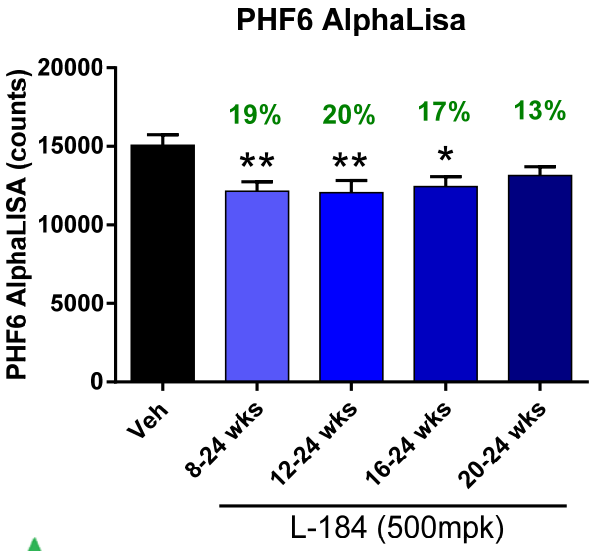
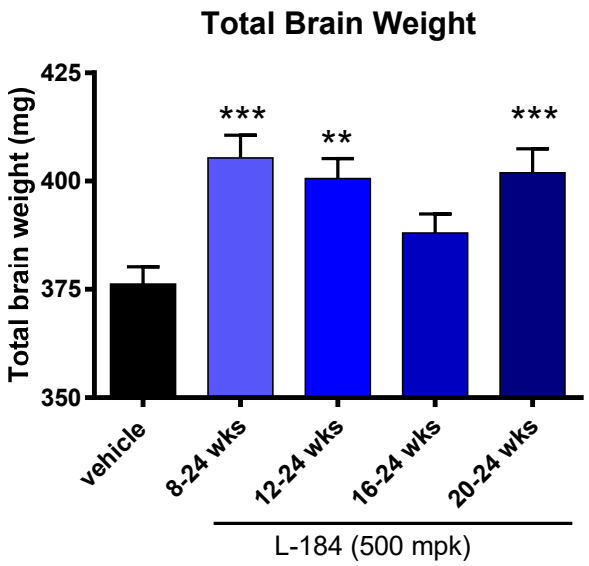
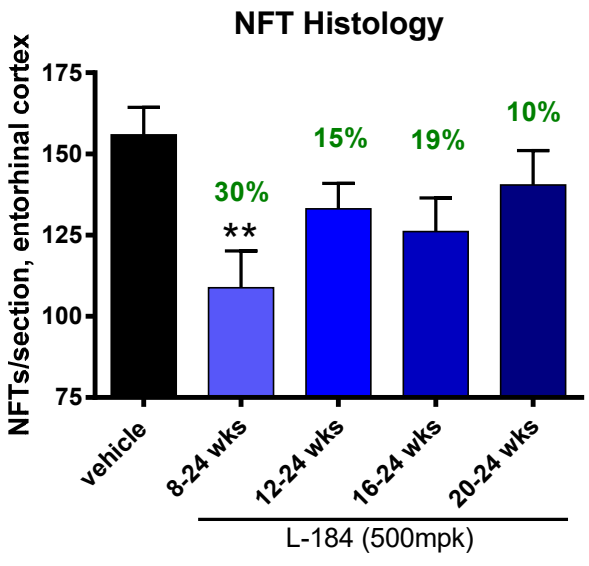
OGA Inhibitors are Efficacious in Tg4510 Mice with Existing Tau Pathology



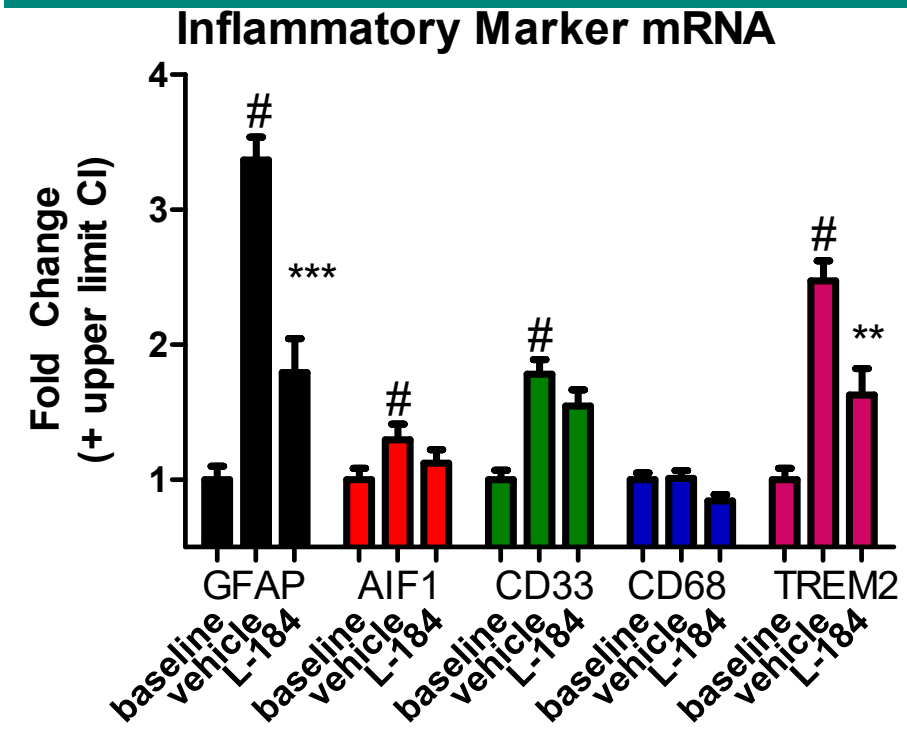






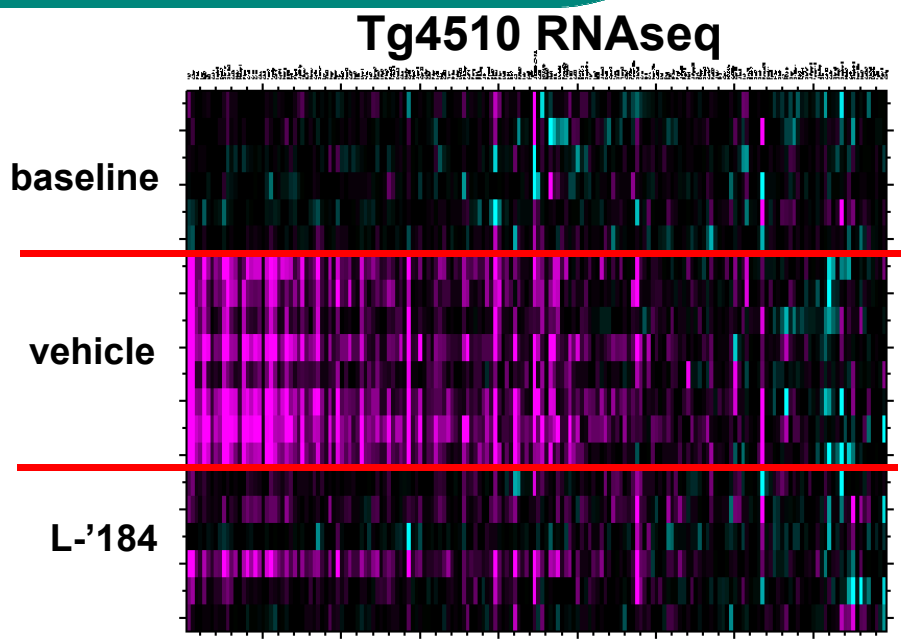


Inflammatory Gene Expression is Reduced in Tg4510 Mice Treated with OGA Inhibitor



 # - significant elevation above baseline
**** - significant reduction from vehicle**
n=10/group

GFAP (astrocytosis) and TREM2 (phagocytic microglia) are significantly reduced in 16wk old Tg4510 mice treated with L-'184 (500mpk) by single gene qPCR



Induction (red) of human brain inflammatory geneset is ameliorated in Tg4510 mice treated with OGA inhibitor

Full transcriptome effects of OGAi in Tg4510 are under analysis.

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OGA Inhibitor Efficacy has Been Reported in Additional Transgenic Mouse Models

Mouse Model	Promoter	Transgene	OGAi Reduces			Reference
			Tangle / Plaque Pathology	Neuro-degeneration	Behavior deficit	
Tg4510	Tet / CamKII	Tau P301L	Yes (tau tangle)	Yes (CSF t-tau)	Yes	Internal Data
JNPL3	Prion	Tau P301L	Yes (tau tangle)	Yes (motor neuron)	No, trend	Yuzwa *et al*. 2012 Nature Chemical Biology Vol. 8, 393–399
Tau.P-301L	Thy-1	Tau P301L	Not determined	Yes (survival)	Yes	Borghgraef et al., 2013 PLOS One Vol. 8(12) e84442
5XFAD	Thy-1	APPSw lLon PSEN* 146L*L 86V	Yes, amyloid plaque	Not determined	Yes	Kim et al. 2013, Neurobiology of Aging Vol. 34 (1), 275 - 285

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Summary

- Inhibition of O-GlcNAcase (OGA) enzyme reduced pathological tau aggregation in iPSC-derived human neurons
- Oral administration of OGA inhibitor produced a sustained elevation in O-Protein levels in the brain
- OGA inhibition significantly reduced pathological tau, brain inflammation, and brain atrophy in Tg4510 transgenic mice
- Increasing protein O-GlcNAcylation is a promising disease modification strategy for tauopathies

Acknowledgments

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