G protein-coupled receptor kinase 2 modulates tau pathogenesis in human neurons

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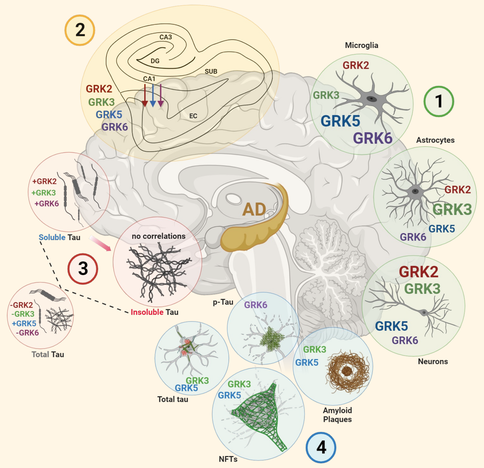
BACKGROUND

Aggregation of tau in neurofibrillary tangles (NFTs) is a pathological hallmark of Alzheimer's Disease (AD)[1]. The pathological phosphorylation of tau reduces its solubility and physiological interaction with microtubules, leading to the progressive aggregation and accumulation of tau in NFTs[2]. The lack of successful kinase-targeted therapeutic approaches for AD[3] along with phosphorylation sites in tau with unidentified causative kinases[4] led us to investigate the putative involvement of G protein-coupled receptor kinases (GRKs)[5] in the pathological phosphorylation and aggregation of tau in human AD brains. **We have shown that GRK2 is highly expressed in neurons, positively correlated with soluble tau levels, and associated with NFTs in the AD brain.**



*All schemes created with BioRender.com

Neuropathol Appl Neurobiol, 2021 Dec;47(7):942-957.
doi: 10.1111/nan.12742.

HYPOTHESIS

GRK2 can directly modify tau phosphorylation and aggregation in AD.

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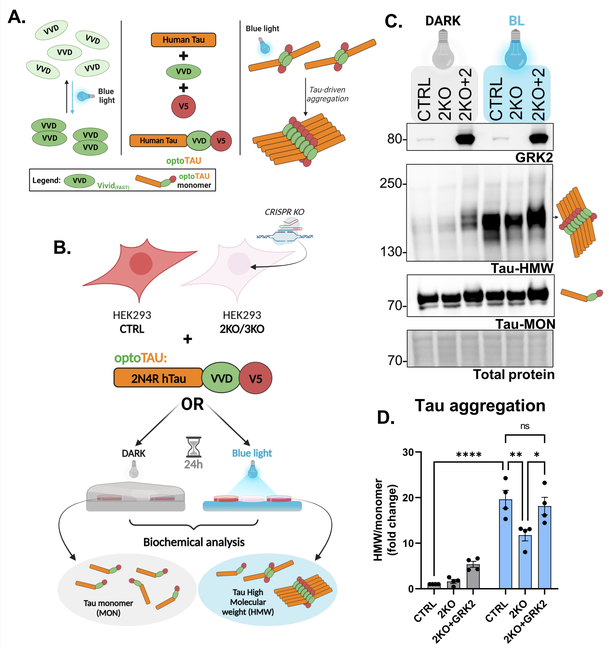
ACKNOWLEDGMENTS

The support and guidance from all the Thathiah lab members have been indispensable for the completion of this work over the years. We would also like to thank the patients and their families for their support and altruism. We also greatly appreciate the gift of human brain samples from the AD Research Center (ADRC) at the University of Pittsburgh. Lastly, we are extremely thankful for the support of our collaborators.

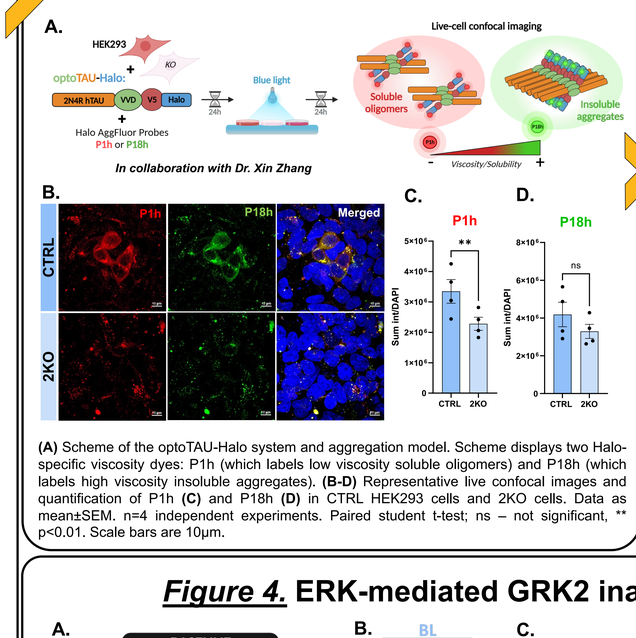
RESULTS

Figure 1. GRK2 modulates tau aggregation in HEK293 cells



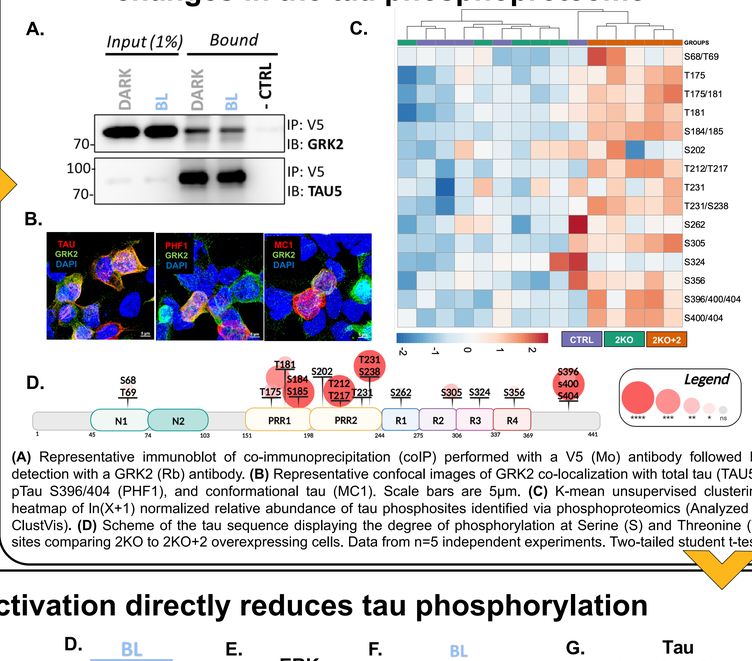
(A) Scheme of the optogenetic tau (optoTAU) aggregation model **(B)** and experimental design. Full-length (2N4R) tau was attached to the blue-light responsive photoreceptor Vivid (VVD), which leads to the formation of optoTAU aggregates upon blue light (BL) stimulation. **(C** and **D)** Representative immunoblots **(C)** and quantification **(D)** of optoTAU high molecular weight (HMW), monomer (MON), and total protein (Ponceau) in HEK293 cells expressing endogenous, CRISPR-mediated *knockout* (2KO), and overexpression of GRK2. **(D)** Data as mean±SEM. n=4 independent experiments. Two-way ANOVA with Sidak's post hoc test. ns – not significant, * p<0.05, ** p<0.01, **** p<0.0001. Molecular weight is in kDa.

Figure 2. GRK2 deficiency specifically decreases soluble tau levels



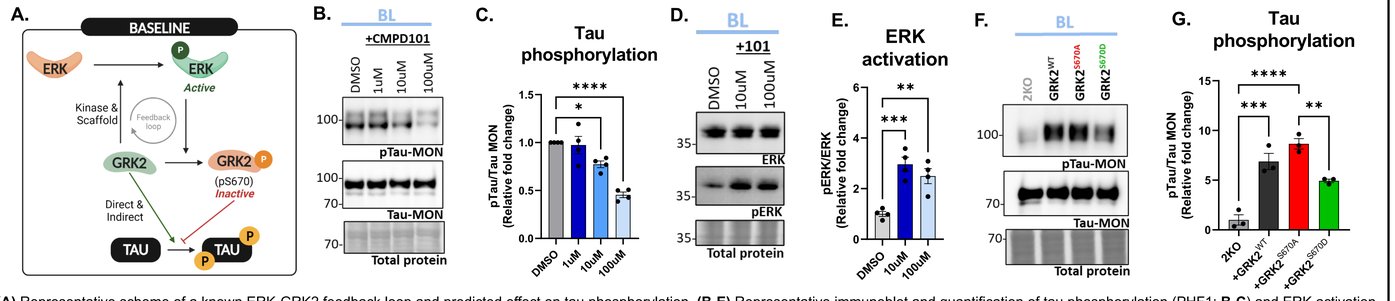
(A) Scheme of the optoTAU-Halo system and aggregation model. Scheme displays two Halo-specific viscosity dyes: P1h (which labels low viscosity soluble oligomers) and P18h (which labels high viscosity insoluble aggregates). **(B-D)** Representative live confocal images and quantification of P1h **(C)** and P18h **(D)** in CTRL HEK293 cells and 2KO cells. Data as mean±SEM. n=4 independent experiments. Paired student t-test; ns – not significant, ** p<0.01. Scale bars are 10μm.

Figure 3. GRK2 overexpression induces global changes in the tau phosphoproteome



(A) Representative immunoblot of co-immunoprecipitation (coIP) performed with a V5 (Mo) antibody followed by detection with a GRK2 (Rb) antibody. **(B)** Representative confocal images of GRK2 co-localization with total tau (TAU5), pTau S396/404 (PHF1), and conformational tau (MC1). Scale bars are 5μm. **(C)** K-mean unsupervised clustering heatmap of ln(X+1) normalized relative abundance of tau phosphosites identified via phosphoproteomics (Analyzed in ClustVis). **(D)** Scheme of the tau sequence displaying the degree of phosphorylation at Serine (S) and Threonine (T) sites comparing 2KO to 2KO+2 overexpressing cells. Data from n=5 independent experiments. Two-tailed student t-test.

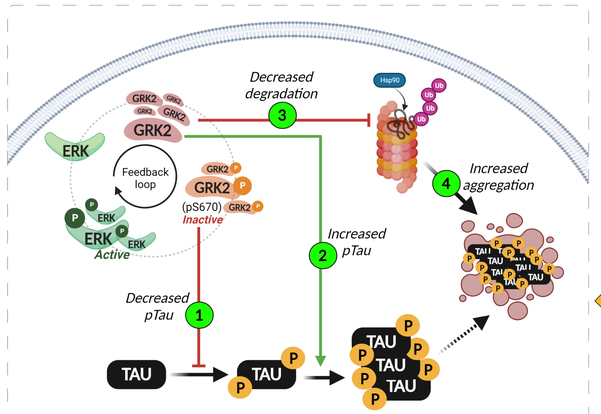
Figure 4. ERK-mediated GRK2 inactivation directly reduces tau phosphorylation



(A) Representative scheme of a known ERK-GRK2 feedback loop and predicted effect on tau phosphorylation. **(B-E)** Representative immunoblot and quantification of tau phosphorylation (PHF1; **B-C**) and ERK activation **(E-F)** after 24h of GRK2 inhibition with CMPD101 in HEK293 cells expressing optoTAU and exposed to BL. **(F-G)** Representative immunoblot and quantification of tau phosphorylation (PHF1) upon overexpression of GRK2 WT and single mutants at S670. Data as mean±SEM. n=3-4 independent experiments. One-way ANOVA with Tukey's post hoc test. * p<0.05, ** p<0.01, ***p<0.001, **** p<0.0001. Molecular weight is in kDa.

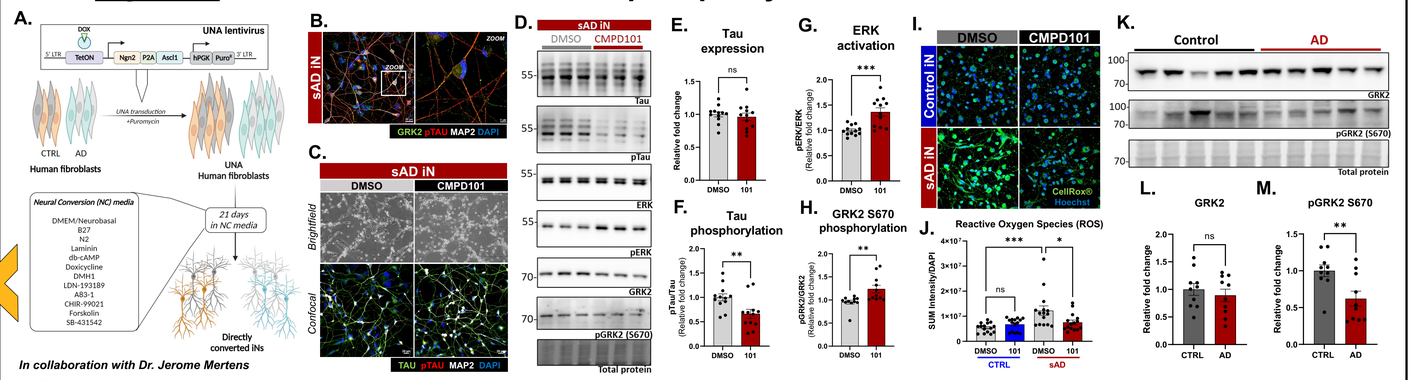
SUMMARY

Our studies causally implicate ***GRK2*** as a ***multifactorial modulator*** of tau pathobiology and support further investigation of GRK-mediated therapeutic intervention strategies for AD.



Sources of support: NIA R01 AG058851 and Clear Thoughts Foundation grant. The ADRC is supported by NIA grants P50 AG005133 and P30 AG066468

Figure 5. GRK2 inhibition attenuates phosphorylated tau and neuronal distress in AD



(A) Representative scheme of the direct conversion protocol (detailed in *Mertens et al., Cell Stem Cell, 2021*) used to produce induced neurons (iNs) from a healthy control and sporadic AD (sAD) patient skin fibroblasts. **(B)** Representative confocal images of sAD iNs showcasing co-localization of GRK2 and pTau (PHF1; S396/404) in mature (MAP2+) neurons. **(C)** Representative brightfield and confocal images of sAD iNs before and after 24h treatment with the GRK2 inhibitor CMPD101 (100μM). **(D-H)** Representative immunoblots **(D)** and quantification **(E-H)** of the expression of tau **(E),** tau phosphorylation **(F)**, ERK activation **(G)** and GRK2 phosphorylation at S670 **(H). (I-J)** Representative live cell imaging of control and sAD iNs stained with the CellRox® dye **(I)** and quantification **(J)** of CellRox intensity. **(K-M)** Representative immunoblot **(K)** and quantification of GRK2 **(L)** and GRK2 pS670 **(M)** in a cohort of control and AD patient brains (n=10/group). Data as mean±SEM. n=4 independent experiments, 3 biological replicates each. Unpaired student t-test. ns – not significant,.* p<0.05, ** p<0.01, ***p<0.001. Molecular weight is in kDa. Scale bars are as represented.