Modulation of GPRC6A Signaling to Mitigate Tauopathies

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Background

Microtubule associated protein named tau, often becomes hyperphosphorylated and aggregates to form pathological neurofibrillary tangles in several age-associated neurodegenerative diseases known as tauopathies. Clinical phenotypes of tauopathies manifest as cognitive impairment, behavior disturbances and motor impairment. The aggregation of tau remains a central target for drug discovery, but currently no disease-modifying treatments exist. Our group has recently uncovered a unique interaction between L-arginine metabolism and tauopathies. We found that the depletion of L-arginine by overexpressing the metabolizing enzyme arginase 1 and arginine deiminase significantly reduced tau pathology in transgenic mouse models. We speculate that these effects associate with increased autophagy through amino acid sensing. G protein-coupled receptor (GPCR), family C, group 6, member A (GPRC6A) was deorphanized by binding to basic L-α amino acids, like L-arginine. We hypothesize that decreased GPRC6A signaling inhibits mTORC1 activation and thus promoting autophagy to induce tau clearance in tauopathies. We posit that GPRC6A remains tonically activated and senses extracellular amino acids sufficiency of L-α amino acids, especially becomes more sensitive to L-arginine in tauopathies.

The Hypothetical GPRC6A Sensing Pathway

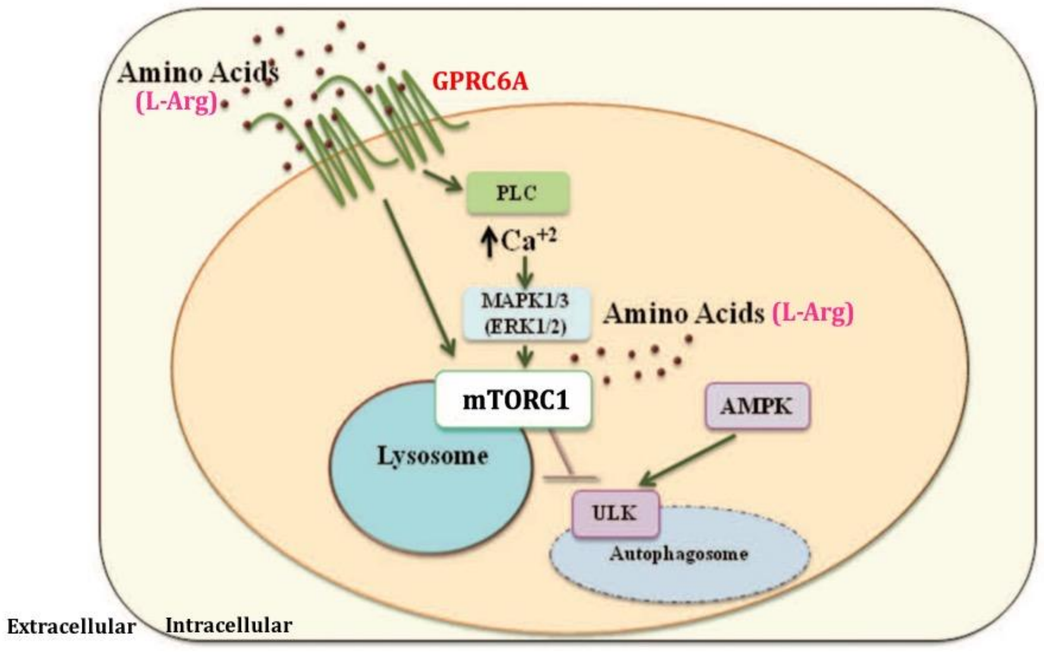

Figure 1: Hypothesized Amino Acid Sensing Through GPRC6A.

GPRC6A directly detects L-α amino acids (L-Arg) leading to the activation of mTORC1 pathway and inhibition of autophagy. This receptor may activate mTORC1, in part, through the activation of phospholipase C (PLC), the increase in intracellular calcium, and the activation of MAPK1-MAPK3. GPRC6A is required for the amino acid-induced mTORC1 localization to the lysosome, a necessary step in mTORC1 activation. (This figure is modified on the Figure 1 from *Amino acid regulation of autophagy through the GPCR TAS1R1-TAS1R3* Wauson E.M. et al. *Autophagy*. 9(3):418-9 (2013))

Methods

Drug Incubation:

All drugs were dissolved in DMSO and used as indicated concentration to incubate with cells plated on 6-well plates for 72 hours in 37 ˚C incubator with 5% CO_2. Tauopathy *in vitro* cell model is C3 cell line which is human HeLa cells expressing human wild type full length 4R0N tau. Nontransgenic E17 mouse primary neurons were harvested and plated for experiments on DIV4.

siRNA/shRNA Transfection:

Transfection of siRNA/shRNA was used Lipofectamie RNAiMax/2000 Reagent from ThermoFisher Scientific following official instructions respectively. Cells were harvested for western blotting after 72 hours.

GPRC6A Allosteric Antagonists Decreased Tau Expression

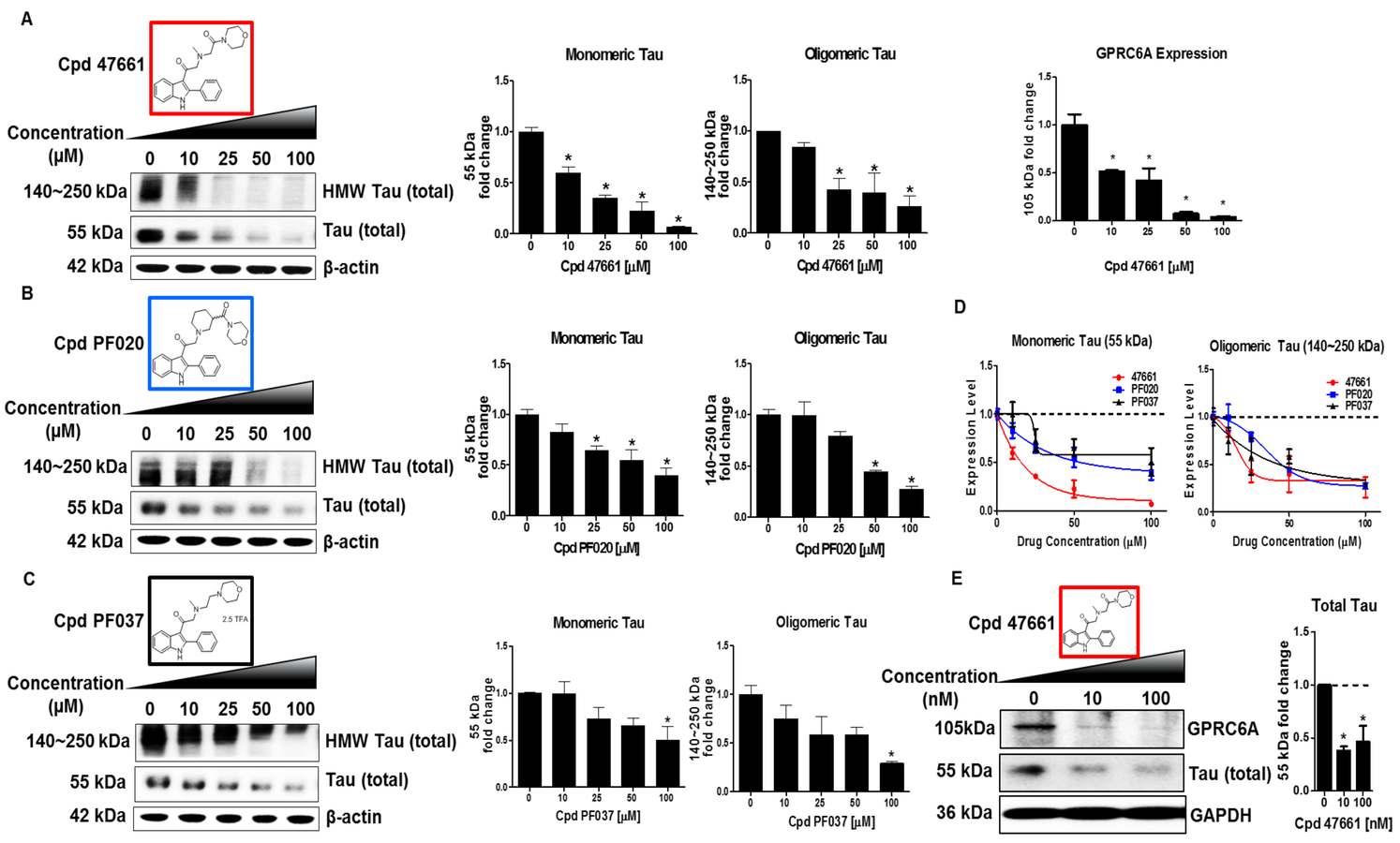

Figure 2. GPRC6A allosteric antagonists induce tau clearance in tauopathy cell line and nontransgenic mouse primary neurons.

[A/B/C]: Three GPRC6A allosteric antagonists (Cpd 47661, Cpd PF020, Cpd PF037) decrease both monomeric tau (55 kDa) and high molecular weight of oligomeric tau in C3 cells. Cpd 47661 significantly induced degradation of GPRC6A. [D]: Summarized of monomeric and oligomeric tau expression level curves correspondent to the three different drugs. [E]: GPRC6A allosteric antagonist (Cpd 47661) decreases endogenous tau expression in nontransgenic mouse primary neurons and induces degradation of GPRC6A. All quantification of western blots represents one way ANOVA and Fisher's LSD as post-hoc analysis. (Mean + SEM, n=3, * p <0.05).

Downregulation of GPRC6A Decreased Tau Expression

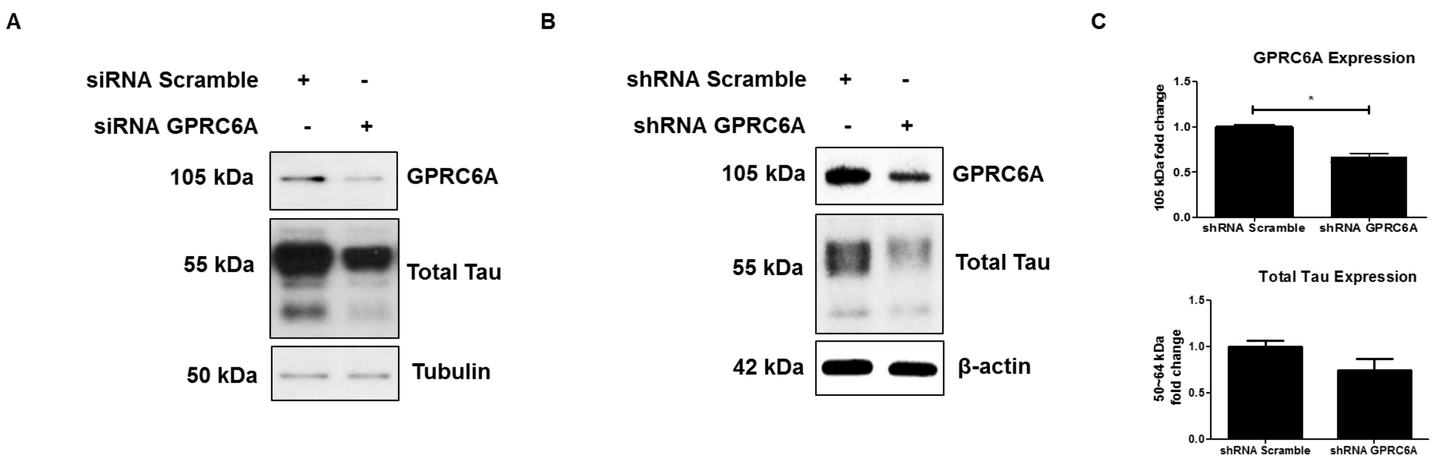

Figure 3. Downregulation of GPRC6A decreases tau expression in tauopathy cell line model.

[A]: Western blots indicate GPRC6A siRNA transfection to C3 cells decreased total tau expression level compared to siRNA scramble control. [B]: Western blots show that GPRC6A shRNA transfection to C3 cells decreased total tau expression level compared to shRNA scramble control. [C] Quantification of western blots data from [B] shows a significant downregulation of GPRC6A and decreased total tau expression level. Statistical analysis represents one way ANOVA and Fisher's LSD as post-hoc analysis. (Mean + SEM, n=3, * p <0.05).

Conclusions

1: From pharmacological perspective, GPRC6A allosteric antagonists selectively blocked GPRC6A signaling and induced tau degradation.

2: From genetic perspective, GPRC6A siRNA/shRNA downregulation decreased total tau expression level.

Future Directions

The mechanistic role of GPRC6A in central nervous system is currently unknown. The L-arginine amino acid sensing pathway through GPRC6A to activate mTROC1 pathway will be further validated *in vitro* and *in vivo*.

Acknowledgements:
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