DOCTORAL THESIS

Neuroprotective effects of a novel TFEB activator E4 and its self-carried nanoparticles in MPTP-induced Parkinson's disease models

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ABSTRACT

Parkinson's disease (PD) is one of the most common neurodegenerative diseases characterized by cell death in the substantia nigra pars compacta (SNpc) and the appearance of aggregated α-synuclein (α-syn). Autophagosomes accumulation and lysosomal reduction were discovered in PD patients' brain, which indicated the deficiency of autophagy in the progress of PD.

TFEB (transcription factor EB) is a member of basic helix –loop-helix-leucine-zipper transcription factors (MiT family) and is a key master monitor for autophagy and lysosome biogenesis. Overexpression of TFEB is able to rescue the dopaminergic neurons (DAs) loss and α-syn aggregated in α-syn transgenic mice model and MPTP PD model. Hence, in recent years, many researchers have considered TFEB as a new therapeutic target for PD

In this study, we discovered a novel TFEB activator named E4 by screening synthesized curcumin analogs. We have found that E4 strongly promoted TFEB nuclear translocation and induced autophagy in different cell lines. TFEB is essential for E4-induced autophagy flux. We also demonstrated that the underlying mechanism of E4 activate TFEB is mainly through inhibiting mTORC1 activity . We constitutively activate mTOR by knockdown TSC2 abrogated the increase of LC3-II and decrease of p-TFEB.

We further estimated the protective effects of E4 in overexpressed α-syn model and neurotoxins induced cytotoxicity model. Treated with E4 for 48h in N2a transfected with A53T α-synuclein cells dose-dependently reduce the α-synuclein level. At the same time, we established the MPP^+ model in PC12 cells which is pre-treated cell with E4 for 6 hours and then co-treated cells with MPP^+ for 48 hours. The cell viability results showed that E4 significantly protect PC12 cells against MPP^+ cytotoxicity dose-dependently. E4 had shown good neuroprotective effects in PD *in vitro* models while poor water solubility and low brain permeability restricted its application in PD animal models.

Hence, assembling E4 molecules into self-carried nanoparticles (NanoE4) addressed the issue of poor water solubility and intranasal administration solved the problem of low permeability. In order to track NanoE4 release *in vitro* and *in vivo*, we further investigated the absorption and emission of NanoE4. However, the absorption fluorescence results showed that NanoE4 exhibits the strong aggregation-caused quenching effect (ACQ) due to π-π stacking of the planar molecule within the NPs. NanoE4 have much weak emission compared with E4 molecules. Therefore, we fabricated E4-TPAAQ NPs by co-reprecipitating E4 molecules with the reported fluorescent organic compound TPAAQ (2,6-Bis[4-(diphenylamino) phenyl] anthraquinone).

Next, we developed an intranasal drug delivery system in our lab. After intranasal

co-drop nanodrug E4-TPAAQ NPs for 24 hours, we observed strong fluorescence distributed in the brain which indicated that deliver nanoparticles into the brain successfully through nasal-brain system. Therefore, we examined the protective effect of NanoE4 in MPTP-induced PD mice model. In MPTP models, we found autophagy dysfunction, motor function decrease and increase of α-synuclein as reported previously. Treatment with NanoE4 rescued the motor dysfunction induced by MPTP. NanoE4 also increase TH level in the striatum part of midbrain. NanoE4 treatment also decreased the α-synuclein protein aggregate in both SNpc and striatum. Overall, these results demonstrate the neuroprotection NanoE4 against PD.

Collectively, our findings 1) discovered a novel TFEB activator E4 that inhibited the mTOR pathway 2) indicated *in vitro* and *in vivo* experimental evidence for TFEB activator as the anti-PD drug candidate 3) provide a novel drug develop and delivery system for potential PD that limited by water solubility and BBB (blood-brain barrier) obstacle.

KEYWORDS: TFEB; MPP^+/MPTP; self-carried nanoparticles; intranasal; Parkinson's disease; curcumin

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