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Research Paper

Urate promotes SNCA/α-synuclein clearance via regulating mTOR-dependent macroautophagy

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ABSTRACT

Serum urate levels are reported to be significantly lowered in patients with Parkinson's disease (PD) and inversely correlated to the risk and progression of PD. However, the mechanism by which urate affects PD is poorly understood. Here we showed that treatment with uric acid (UA) resulted in an autophagy activity enhancement in PC12 cells in dose- and time-dependent manners, as indicated by LC3-II increase and P62 decrease. Moreover, UA was still able to increase the LC3-II level and the number of LC3 puncta in the presence of Bafilomycin A1, a lysosomal inhibitor. These changes of autophagic markers were preceded by mTOR inhibition and ULK1 activation. Co-treatment with 3-benzyl-5-((2-nitrophenoxy) methyl)-dihydrofuran-2(3H)-one (3BDO), an mTOR activator, abolished the UA-induced LC3-II increase. More importantly, UA reduced SNCA/α-synuclein accumulation in PC12 cells that overexpress wildtype or A53T mutant SNCA, and this was blocked by Bafilomycin A1 co-treatment. The *in vivo* study showed that UA administration was able to modulate the levels of autophagy markers, increase the autophagosome/autolysosome formation, and reduce SNCA accumulation in the midbrain of *SNCA*^{A53T} transgenic mice. Taken together, our findings suggest that UA could induce autophagy activation via an mTOR-dependent signaling and ameliorate SNCA accumulation. This implicates that urate-elevating agent may become a potential strategy for PD therapy.

1. Introduction

Parkinson's disease (PD) is an age-related neurodegenerative disorder, probably caused by complicated interactions between genetic and environmental factors. The exact pathogenesis of PD remains elusive. Pathologically, it is featured by the progressive loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and appearance of SNCA/α-synuclein-containing Lewy bodies and neurites (Dauer and Przedborski, 2003; Dickson et al., 2009). SNCA is a key pathogenic protein in PD, although its physiological function is yet to be determined. The multiplication or point mutations in *SNCA* gene is linked to familial PD (Hashimoto and Masliah, 1999). Additionally, the polymorphisms in *SNCA* gene are major risk factors for sporadic PD (Simon-Sanchez et al., 2009). In recent years, SNCA aggregates are found to spread via prion-like mechanisms through neural networks, and the spreading increases with age (Luk et al., 2012; Recasens et al., 2014). Therefore, promoting the clearance of misfolded or aggregated SNCA is proposed to be a potential approach that may halt or delay the progression of PD.

Autophagy-lysosome pathway is a catabolic process that recruits dysfunctional organelles and protein aggregates into autophagosomes and delivers them to lysosomes for degradation and recycling. Mutant SNCA and its aggregates are degraded via macroautophagy (referred to as autophagy thereafter). Autophagy impairment results in SNCA accumulation. Aberrant alterations in autophagy-related proteins, accompanied with SNCA aggregation, were observed in the patients with PD (Alvarez-Erviti et al., 2010; Anglade et al., 1997). Conditional knockout of autophagy-related gene leads to protein aggregates formation and progressive losses of dopaminergic neurons in the SN (Ahmed et al., 2012). Compelling evidence suggests that autophagy impairment contributes to PD pathogenesis. However, the factors that impede autophagy induction or autophagy flux are poorly understood.

Urate, the anionic form of uric acid (UA), is the end product of purine metabolism due to the *urate oxidase gene* mutation during

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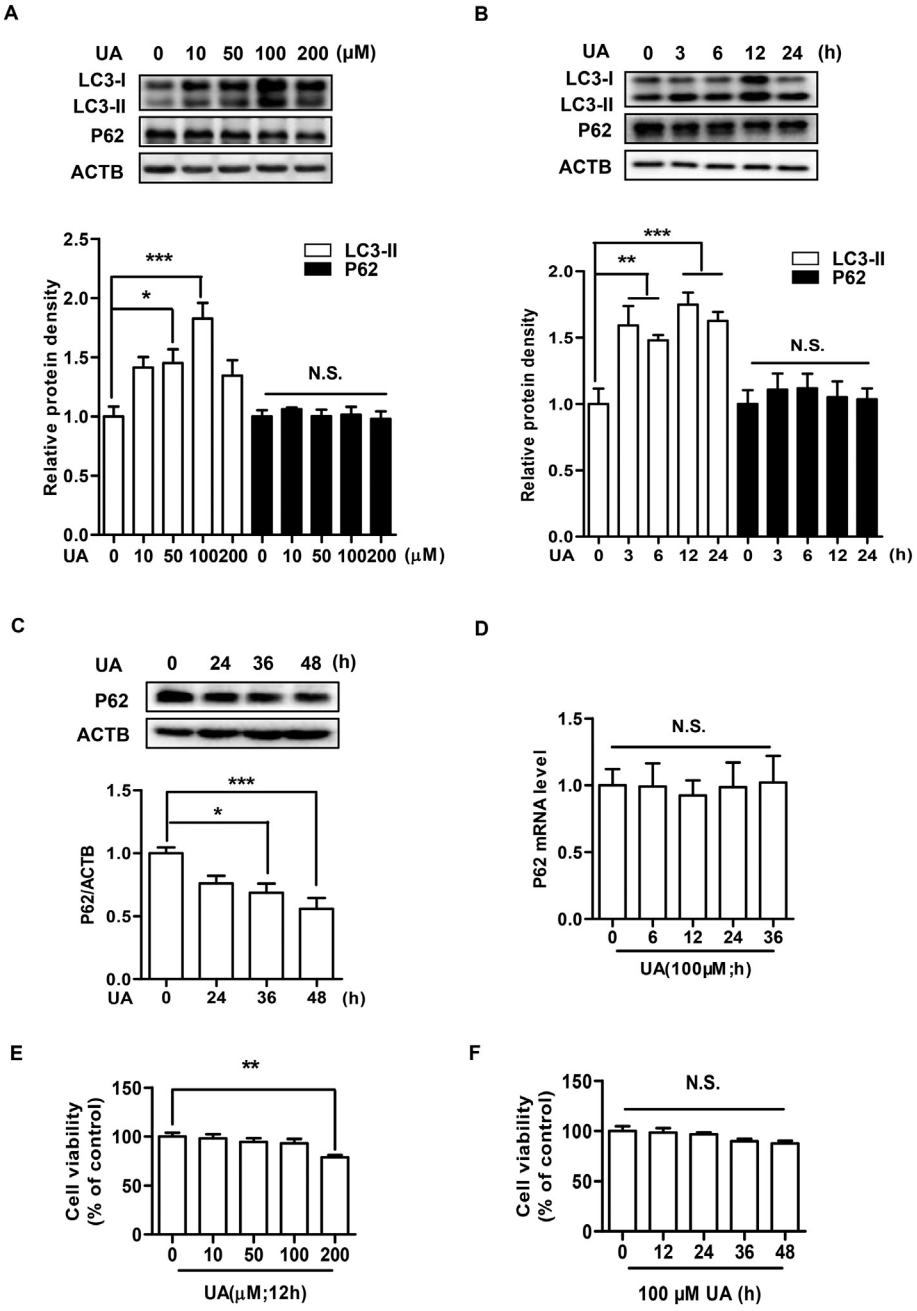

Fig. 1. Dose- and time-dependent effects of UA on autophagy-related proteins (LC3-II and P62) levels in PC12 cells. (A–C) Dose- and time-dependent effects of UA on LC3-II and P62 levels. Treatment with UA for a short period (< 24 h) had little effect on P62 level (n = 5–6), but did reduce P62 protein level after a prolonged (36 h and 48 h) treatment (C, n = 5). Actin/ACTB served as loading controls. (D) UA did not affect P62 transcription. Relative values were obtained after calibration to 18S levels, and the values in control group were normalized to 1, n = 3. (E and F) At the concentrations no more than 100 μM UA had little impact on cell survival for up to 48 h treatment (n = 4). Cell viability was expressed as the percentage of control group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. One way ANOVA followed by Dunnett's analysis. N.S., not significant.

evolution. It is a potent antioxidant and acts as the scavenger of superoxide, peroxynitrite and other free radicals (Davies et al., 1986). Serum urate levels were reported to be lower in patients with PD compared to healthy controls, and inversely related to the risk and progression of PD (Gao et al., 2016). We previously reported that intraperitoneal administration with UA exerted neuroprotection to midbrain dopaminergic neurons and alleviated the motor deficits in 6-hydroxydopamine-lesioned rats (Gong et al., 2012; Zhang et al., 2014). However, whether or not UA affects SNCA aggregation is unknown. Therefore, in this study we aimed to explore the effect of UA on SNCA aggregation and the underlying mechanism. Our findings demonstrate that treatment with UA promoted the clearance of SNCA by enhancing an mTOR-dependent autophagy activity.

2. Materials and methods

2.1. Reagents and antibodies

Uric acid (UA, U2625), rotenone and cycloheximide (CHX) were purchased from Sigma-Aldrich (St Louis, MO, USA). UA was dissolved in sterile water, pH-adjusted to 7.4 with sodium hydroxide. Bafilomycin A1 (BafA1) was purchased from Abcam (New Territories, Hong Kong). 3-Benzyl-5-((2-nitrophenoxy) methyl)-dihydrofuran-2(3H)-one (3BDO) was gifted by Prof. JunYing Miao from Shandong University.

2.2. Cell lines and cell culture

Rat pheochromocytoma PC12 cells were purchased from Institute of Cell Biology (Chinese Academy of Sciences, Shanghai, China). To establish SNCA overexpressing cells, PC12 cells were infected with the lentivirus (titer: 2 × 10ˆ8 TU/ml, Genechem Shanghai, China)