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Degradation of alpha-synuclein by dendritic cell factor 1 delays neurodegeneration and extends lifespan in *Drosophila*

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ABSTRACT

Parkinson's disease (PD) is a common neurodegenerative disease associated with the progressive loss of dopaminergic neurons in the substantia nigra. Proteinaceous depositions of alpha-synuclein (α-syn) and its mutations, A30P and A53T, are one important characteristic of PD. However, little is known about their aggregation and degradation mechanisms. Dendritic cell factor 1 (DCF1) is a membrane protein that plays important roles in nerve development in mouse. In this study, we aimed to show that DCF1 overexpression in a PD *Drosophila* model significantly ameliorates impaired locomotor behavior in third instar larvae and normalizes neuromuscular junction growth. Furthermore, climbing ability also significantly increased in adult PD *Drosophila*. More importantly, the lifespan dramatically extended by an average of approximately 23%, and surprisingly, DCF1 could prevent α-syn–induced dopaminergic neuron loss by aggregating α-syn in the dorsomedial region of *Drosophila*. Mechanistically, we confirmed that DCF1 could degrade α-syn both in vivo and in vitro. Our findings revealed an important role of DCF1 in PD process and may provide new potential strategies for developing drugs to treat neurodegenerative diseases.

1. Introduction

Parkinson's disease (PD), which affects approximately 4% of the world population over 80 years of age (Twelves et al., 2003; de Lau and Breteler, 2006), is the second most common age-associated neurodegenerative disease after Alzheimer's disease. It is characterized by dopaminergic (DA) neuron loss in the substantia nigra and by the accumulation of proteinaceous intraneuronal inclusions known as Lewy bodies, which mainly consist of the protein *alpha-synuclein* (α-syn) (Jiang et al., 2013; Karuppagounder et al., 2014). The clinical and pathological symptoms of familial PD are resting tremor, muscular rigidity, bradykinesia, hypokinesia/akinesia, small handwriting, flexed posture, and postural instability (Wolters, 2008). α-syn is a soluble, natively unfolded protein that is highly enriched in the presynaptic terminals of neurons in the central nervous system (Iwai et al., 1995). Two missense mutations in α-syn, A30P and A53T, have been identified as genetic lesions in familial PD (Polymeropoulos et al., 1997), which increase the potential for protein misfolding, oligomerization, and aggregation (Li et al., 2001; Olanow and McNaught, 2006). Both mutations oligomerize more rapidly than the wild-type α-syn (Dev et al., 2003). The mechanisms for this may be related to the phosphorylation of α-syn at serine 129 (Arawaka et al., 2006; Basso et al., 2013; Smith et al., 2005), tyrosine 125(Chen et al., 2009), and other gene regulation pathways, such as Rab11 (Breda et al., 2015), GRKs (GRK2, GRK3, GRK5, and GRK6), (Pronin et al., 2000) and LRRK2 (Qing et al., 2009). Therefore, blocking α-syn aggregation would represent a breakthrough in the prevention and treatment of PD.

Dendritic cell factor 1 (DCF1), also known as TMEM59, is a membrane protein that plays a role in selective autophagy (Boada-Romero et al., 2013). It also affects amyloid precursor protein shedding and Aβ generation in Alzheimer's disease (Ullrich et al., 2010). In a previous study, we showed that DCF1 was involved in neural stem cell differentiation (Wen et al., 2002), brain development, and dendritic spine formation (Liu et al., 2018). More recently, we showed dopamine system dysfunction in DCF1 gene knockout mice, (Liu et al., 2017) which suggested an association with PD.

Here, we used a PD *Drosophila* model to explore the effect of DCF1 expression on PD prevention and processes. Results suggested that DCF1 significantly ameliorates PD-related phenotypes including extending lifespan via degradation of α-syn, which suggested a pivotal role of DCF1 in the onset and development of neurodegenerative diseases.

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2. Materials and methods

2.1. Flies and α-syn mutants

The tyrosine hydroxylase-GAL4 driver, elav-GAL4 driver, UAS-α-syn, UAS-α-syn-A30P, and UAS-α-syn-A53T were donated by Dr Aike Guo from the Institute of Neuroscience, Shanghai Institute for Biological Science, Chinese Academy of Sciences. UAS-DCF1 was created in our lab. The F1 generation was collected and subjected to further experiments. *Drosophila* raised at 25 °C were cultured in standard cornmeal medium and harvested at the same time.

2.2. Immunocytochemistry

Larva or adult fly brains were dissected in 0.01 M phosphate-buffered saline (PBS) and fixed in 4% formaldehyde for 30 minutes followed by 30 minutes in 0.4% PBS with 0.4% Triton X-100. The brains were washed for 3 × 5 minutes in 0.01 M PBS and then incubated in a blocking solution of 10% goat serum for 1 hour. Samples were incubated overnight at 4 °C with horseradish peroxidase (1:100, Santa Cruz), and small size synaptic boutons were defined <1.5 μm, rabbit tyrosine hydroxylase antibody (1:200, Millipore Bioscience Research Reagents), and mouse α-syn antibody (1:200, Santa Cruz LB509). Unbound antibodies were removed by 3 × 5 minute washes in 0.01 M PBS before samples were incubated for 2 hours at room temperature with secondary antibody (1:200, antirabbit and antimouse; Santa Cruz). After 3 × 5 minute washes with 0.01 M PBS, brains were mounted in 3% n-propylgallate + 80% glycerol PBS solution.

2.3. Electrophysiology

At 30 days after eclosion, giant fibers (GF) system neurons in the brains of adult flies (n ≥ 10) were directly stimulated and recordings were obtained from the output muscles of the GF system (dorsal longitudinal muscles [DLMs]). Flies were anesthetized with CO_2. The fly's legs and wings were removed, and the rest of the body was fixed in wax. A ground glass electrode was placed into the posterior end of the abdomen, and 2 glass stimulating electrodes were placed through the eyes into the brain. A glass recording electrode was placed into the left (or right) DLM fiber. During the 100 Hz frequency stimulation, the stimulation intensity was 30–60 V with duration of 0.1 ms, and 150% of the threshold stimulation intensity was at 0.5 Hz. A sudden potential drop of 20–60 mV indicated intracellular penetration into muscle. The muscle identity (DLM) was determined by electrode placement. Signals were amplified by Multiple Clamp 700B (Molecular Devices) and digitized at 20 kHz by Digidata 1440A (Molecular

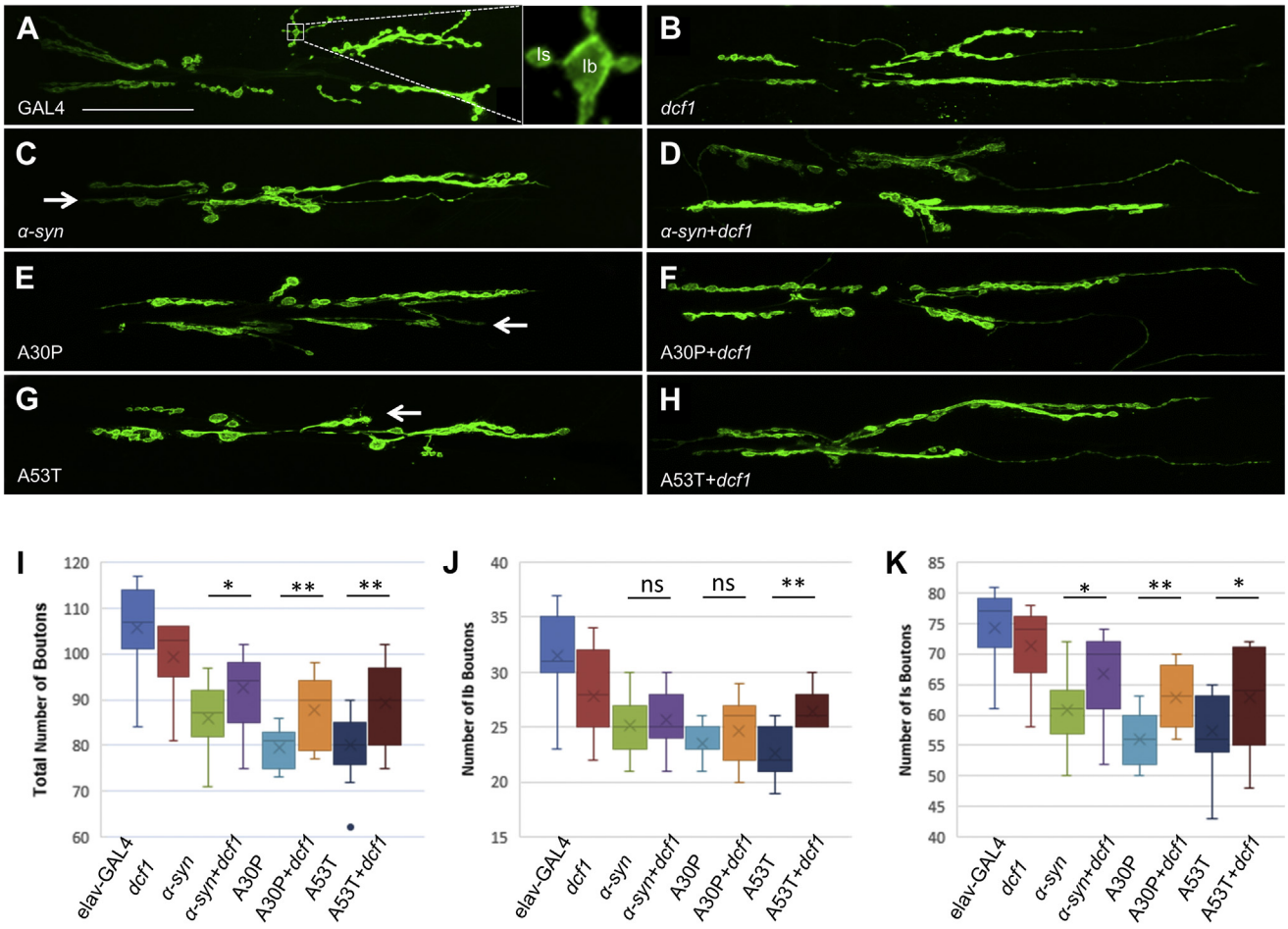

Fig. 1. DCF1 overexpression intensifies NMJ expansion in PD *Drosophila* larvae. Confocal image of *Drosophila* NMJ 6/7 stained with neuronal marker, HRP. (A) elav-GAL4/+; +/+ heterozygous larvae. (B) elav-GAL4/DCF1; +/+ heterozygous larvae. (C, E, and G) elav-GAL4/+; α-syn/+, elav-GAL4/+; A30P/+ and elav-GAL4/+; A53T/+ display a decrease in the total number of synaptic boutons. (D, F, and H) Nervous system overexpression of DCF1 in PD *Drosophila* larvae partially restores the number of synaptic boutons. (I) Total number of boutons. (J) Number of type Ib boutons. (K) Number of type Is boutons. Data were analyzed by multivariant ANOVA with supplementary Newman–Keuls test and presented as mean ± SEM (n = 15) with * $p < 0.05$, ** $p < 0.01$. Scale bar = 50 μm. Abbreviations: DCF1, dendritic cell factor 1; HRP, horseradish peroxidase; NMJ, neuromuscular junction; PD, Parkinson's disease; type Ib, type I big; type Is, type I small.