

Addition of Exogenous α -Synuclein Oligomer up-regulates Divalent Metal Transporter-1 and Ferroportin-1 in BV2 Glial Cell Lines

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Abstract: It has been confirmed that α -synuclein (α -syn) aggregation in neurons or glia and formation of Lewy bodies is the remarked pathognomonic hallmark of PD. Neuropathology study have found α -Syn in abnormal aggregation often accompany with iron deposits. At present, a lot of research confirmed that divalent metal transporter 1 (DMT1), is up-regulated and ferroportin-1 is downregulated in the SN of PD and accumulate of iron in this region. Therefore understanding exogenous α -Syn oligomer whether arise this phenomenon in BV2 glial cell lines is necessary.

Keywords: Microglia cell; α -Synuclein oligomer; Iron; Membrane Transport Proteins

INTRODUCTION

Parkinson's disease (PD) is a degenerative disease of central nervous system. The physiological performance is muscle rigidity, resting tremor and posture reflection problems and characterized by the loss of dopaminergic neurons in the substantia nigra and form acidophilous inclusion body (Lewy body) composed mainly with aggregates α -syn^[1-3]. Although heredity, environment and oxidative stress play a certain role, but the exact pathogenesis of Parkinson's disease is not fully understood.

α -syn, as the primary component of Lewy bodies (LBs), forms insoluble aggregates that accumulated in neuron and glia of the substantia nigra and cause neurotoxicity^[3].

α -syn was found by Maroteaux in 1988^[4] and in 1997, the protein began to widely research because of the identification of Lewy bodies (LBs) and Lewy neurites (LNs)^[5]. α -Synuclein (α -Syn) is a 140-residue abundant cytosolic neuronal protein enriched in synaptic terminals where it has been reported to be involved in synaptic vesicle trafficking^[6], regulation of synaptic vesicle pool^[7-8]. α -syn is abundantly expressed in neurons^[9] and its amino acid sequence can be divided into three parts: (i) N-terminal domain (1–60), which contains a multi-repeated consensus sequence (KTKEGV) and forms alpha-helical; (ii) central domain (61–95), named as the non-amyloid-beta component (NAC), this region is involved in α -syn aggregation; (iii) C-terminal

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domian(96-140), enriched in negative charged and enriched in negative charged(**Figure 1**). In α -syn, the NAC domain is the most aggregation prone region but it is mainly protected by the positive and negative charges of the N- and C-termini^[10]. The expression of α -syn in neuron are easier to oligomeization, but not fibrillization, indicate that oligomeric species might be the most toxic forms of α -syn. therefore α -syn oligomers are being notoriety in pathological process of PD^[11-12]. Neuropathology study have found α -syn in abnormal aggregation often accompany with iron deposits, it is prominent indicate that α -syn aggregation is related with iron deposits^[13]. Our lab study in recent years have been proved real high iron can lead to dopaminergic neuron death in substantia nigra par compacta. In brain, iron transport is connected with two transferrins divalent metal transporter 1(DMT1), the mainly protein of iron import; Ferroportin-1 (FPN1), the only known transmembrane protein for cellular iron efflux^[14]. The increasing of intracellular iron due to the high expression of DMT1 and low expression of FPN1. In this paper, the dominating objective is to know whether aggregates α -syn oligomer can change two transferrins DMT1 and FPN1, consequently cause the iron deposits, thereby lead to dopaminergic neuron death and PD.

MATERIALS AND METHODS

Design

In vitro cytological study.

Time and setting

Experiments were completed at the National Key Laboratory of Physiology, Qingdao University Medical School, China in August 2016.

Materials

BV2 glial cell lines were provided by Shanghai Cell Bank of the Chinese Academy of Sciences. Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co. (St. Louis., MO, USA). The

primary antibodies against FPN1 and DMT1 were separately from the Sigma Chemical Co. and the Alpha Diagnostic (ADI, San Antonio, TX, USA). Dulbecco's modified Eagle's medium (DMEM) was from Gibco (Grand Island, NY, USA). α -syn monomer and α -syn antibody(3D5) were provided by the capital university of medical sciences of Xuanwu hospital .

Methods

Cell cultures

BV2 glial cell lines were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μ g/mL streptomycin at 37 °C, in a humid atmosphere of 5% CO₂ and 95% air. For experiments, cells at 70%-80% confluence were sub-cultured and seeded at a density of 105 cells/cm².

α -syn oligomer manufacture

recombinant α -syn monomer dissolve in 0.01mol/L phosphate buffer (PBS, PH=7.4), filtration sterilization. 37°C constant temperature table oscillation incubation 2d~5d, oscillation frequency 280r/min^[15].

Western blot analysis

Cells were treated with 1 μ mol/L、3 μ mol/L、5 μ mol/L α -syn oligomer for 24 hours, then collected after supernatant removal. After three washes with TBST, cells were digested directly on culture plates with RIPA lysis buffer (50 mmol/L Tris-HCl, 150 mmol/L NaCl, 1% Nonidet-40, 0.5% sodium deoxycholate, 1 mmol/L ethylene diamine tetraacetic acid, 1 mmol/L phenylmethylsulfonyl fluoride) with protease inhibitors (pepstatin 1g/mL, aprotinin 1g/mL, leupeptin 1g/mL) for 30 minutes on ice. The insoluble material was removed by centrifugation (12 000 r/min, 20 minutes, 4 °C). 30 μ g total proteins were extracted and separated using 10% sodium dodecyl sulfate-polyacrylamide gels and then transferred to polyvinylidene fluoride membranes. Blots were probed with anti-DMT1 antibody (1:800, ADI) and anti-FPN1 antibody (1:800, Sigma). Blots were also probed with anti- β -actin

monoclonal antibody (1:10 000, Sigma) as a loading control. Cross-reactivity was visualized using UVP gel imaging system and photographed. The DMT1 and FPN1 expressions were represented as the ratio of DMT1/ β -actin and FPN1/ β -actin. The experiment was repeated six times.

Main outcome measures

Changes in DMT1 and FPN1 expressions after α -syn oligomer intervention.

Statistical analysis

Data are presented as mean \pm SEM. SPSS 17.0 software was applied for statistics analysis. The intergroup comparisons were made by one-way analysis of variance and Tukey test. $P < 0.01$ and $P < 0.001$ were considered to be statistically or greatly statistically significant.

RESULTS

The preparation of α -syn oligomer was detected by 10% sodium dodecyl sulfate-polyacrylamide gels and then transferred to polyvinylidene fluoride membranes. Blots were probed by anti- α -syn (3D5) antibody (Figure 2).

Expression of DMT1 protein in 1 μ mol/L, 3 μ mol/L, 5 μ mol/L α -syn oligomer-treated BV2 glial cell line. After treated with 3 μ mol/L α -syn oligomer for 24 h, there was a 1-fold increase in DMT1 protein level in BV2 glial cell lines ($P < 0.001$; Figure 3).

Expression of FPN1 protein in 1 μ mol/L, 3 μ mol/L, 5 μ mol/L α -syn oligomer-treated BV2 glial cell lines. After treated with 3 μ mol/L α -syn oligomer for 24 h, there was a 1-fold increase in FPN1 protein level in BV2 glial cell lines ($P < 0.001$; Figure 4).

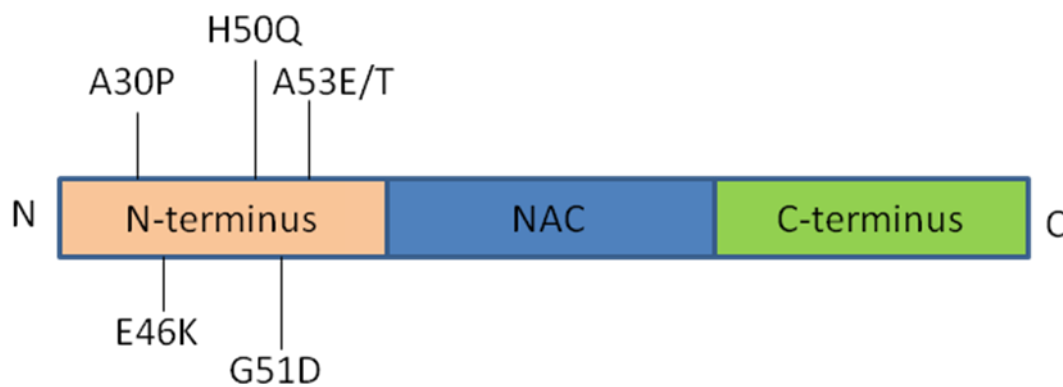


Figure 1 α -syn Protein structure

α -syn is an intrinsically disordered protein with six synucleinopathy-related point mutations described so far.

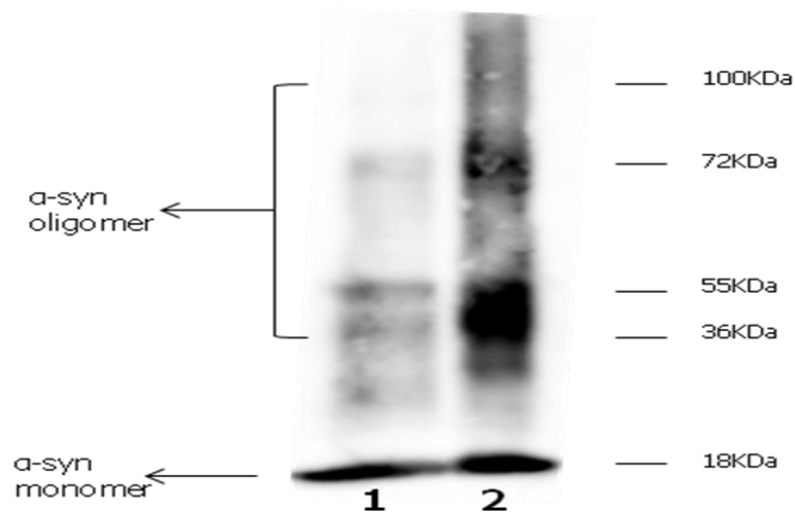


Figure 2 α -syn oligomer detection result by western blot

1: α -syn monomer was detected by western blot assay, which at 18 kDa were higher. 2: α -syn oligomer was detected by western blot assay, which at 36 kDa, 55 kDa and 72 kDa were higher, α -syn oligomer grey value analysis is 3-fold of monomer.

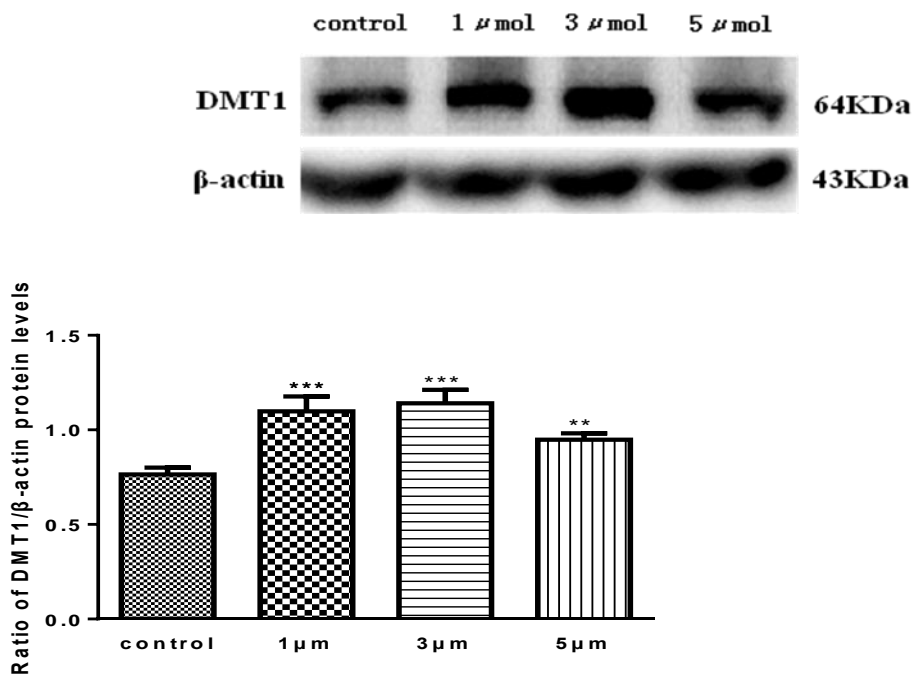


Figure 3 Protein level of DMT1 in α -syn oligomer -treated BV2 glial cell lines

DMT1 expression was detected by western blot assay, which at 64 kDa were higher in α -syn oligomer group than in the control group. *** $P < 0.001$, vs. control group.

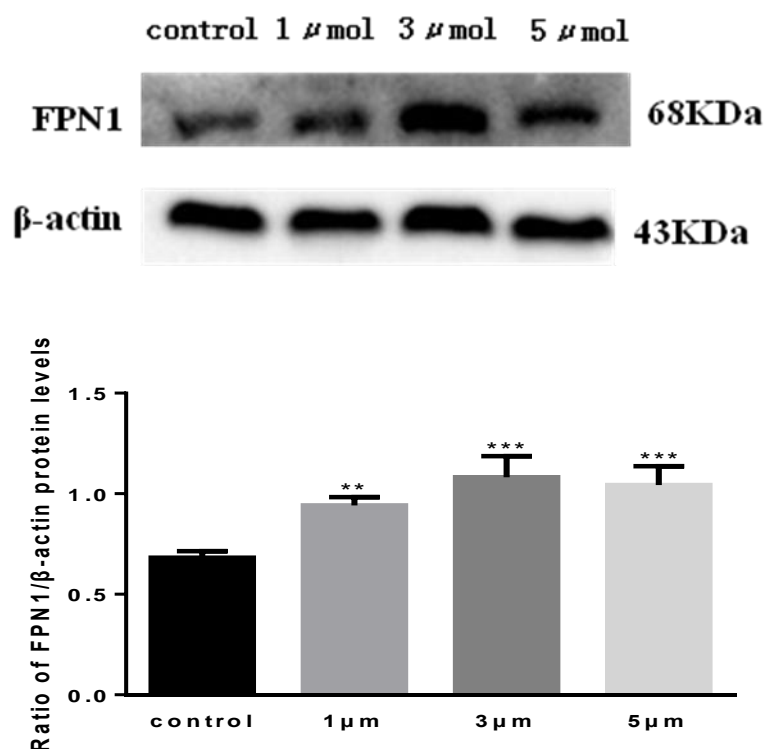


Figure 4 Protein level of FPN1 in α -syn oligomer -treated Bv2 glial cell lines

FPN1 expression was detected by western blot assay, which at 68 kDa were higher in α -syn oligomer group than in the control group.*** $P < 0.001$, vs. control group.

DISCUSSION

Parkinson's disease(PD) is the second most frequent neurodegenerative disorder and the most famous α -synucleinopathy, which is increased the iron levels in the substantia nigra of Parkinson's disease patients, but the potential interlink between both molecule changes has not been fully understood^[16]. The accumulation of iron in the substantia nigra has been associated with oxidative stress, by means of Fenton reaction, iron increases the production of free radicals and can thus contribute to neurotoxicity^[17]. Interestingly, the presence of increased iron levels may not only enhance radical burden but also foster aggregation of α -synuclein^[18]. Several investigations have focused on the role of iron to promote α -syn oligomerization and aggregation^[19]. Several observations support the hypothesis that mismetabolism of iron in the SN

promotes aggregation of α -syn. Levels of iron are significantly higher in the SN relative to other brain regions, increasing the susceptibility of this region to iron-mediated oxidative stress^[20]. Elevated levels of total iron and a shift in the equilibrium of iron to the oxidized state have been reported in the SN of sporadic and familial cases of PD^[21]. Redox-active iron has been detected in association with α -synuclein aggregates in Lewy bodies^[22], a phenotype that is likely to promote oxidation and further aggregation of α -syn and other proteins^[23]. Aggregation of α -syn is inhibited by the iron chelator desferrioxamine, supporting this hypothesis^[24]. α -syn over-expression in the brain led cell more sensitive to the toxic effects of iron, therefore, speculated that excessive α -syn may add the iron level of cell. Recent studies have found that α -syn is a ferrireductase, can restore the Fe^{3+} of the

cell into Fe²⁺, therefore it is provide the possibility that α -syn involved in the iron metabolism, that is say itself a kind of iron metabolism protein, its abnormal expression inevitably involved in the iron metabolism of cell [25]. Our study provides a better understanding of the exogenous α -syn oligomer role in the iron metabolism and the expression of transferrin in the BV2 glial cell lines. Glial cells are important in supporting neuronal survival, synaptic functions and local immunity [26], therefore glial cells might be crucial for the initiation and progression of different neurodegenerative diseases, including PD. We could conclusively demonstrate that α -syn oligomers is capable to increase transmembrane transferrin protein DMT1 and FPN1 expression by a dose-dependent manner in the BV2 glial cell lines. As a consequence, we guess that exogenous α -syn oligomer may be a ferrireductase, regulate the Fe³⁺ into Fe²⁺. Due to the higher expression of iron import protein DMT1, the level of Fe²⁺ is elevated, causing the iron deposits and oxidative stress and eventually generation cytotoxicity, therefore increase the expression of iron export protein FPN1 and export iron out of cell. So, we hypothesize exogenous α -syn oligomer involved in the microglia cell iron metabolism as well as the pathological process of PD, although precise molecule mechanism remain unclear.

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