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Research report

FKBP12-immunopositive inclusions in patients with α-synucleinopathies

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ABSTRACT

α-Synuclein (α-SYN), a presynaptic protein with the tendency to aggregate, is linked to α-synucleinopathies such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). α-SYN is the main component of round intracytoplasmic inclusions called Lewy bodies (LBs), which are the hallmark of PD and DLB. In addition, accumulation of amyloid-β and neurofibrillary tangles as in the pathology of Alzheimer's disease has been found in the DLB brain. Glial cytoplasmic inclusions are an MSA-specific type of inclusion found in oligodendrocytes and mainly comprise α-SYN. FK506-binding protein (FKBP) 12 is a member of the immunophilin family with peptidyl-prolyl isomerase activity that promotes protein folding and is believed to act as a chaperone protein. Previous *in vitro* work indicated that FKBP12 accelerated α-SYN aggregation more than other peptidyl-prolyl isomerases. The enzymatic activity of FKBP12 increases the formation of α-SYN fibrils at subnanomolar concentrations. In this study, we found that FKBP12 colocalized with α-SYN in LBs and neurites in PD and DLB brains. Furthermore, FKBP12-immunopositive neurofibrillary tangles colocalized with phosphorylated tau in DLB and FKBP12-immunopositive glial cytoplasmic inclusions colocalized with α-SYN in MSA. These findings suggest that FKBP12 is linked to the accumulation of α-SYN and phosphorylated tau protein in α-synucleinopathies. FKBP12 may play important roles in the pathogenesis of α-synucleinopathies through its strong aggregation function. Thus, FKBP12 could be an important drug target for α-synucleinopathies.

1. Introduction

α-Synuclein (α-SYN) is a presynaptic protein with the tendency to aggregate. α-SYN is linked to neurodegenerative diseases collectively known as α-synucleinopathies, which include Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) (Baba et al., 1998; Barker and Williams-Gray, 2016; Wakabayashi et al., 1998). In addition, α-SYN is the main component of Lewy bodies (LBs) and glial cytoplasmic inclusions (GCIs) (Baba et al., 1998; Barker and Williams-Gray, 2016; Papp and Lantos, 1994; Wakabayashi et al., 1998). Biochemical, pathological and animal modeling studies have provided considerable evidence showing that aggregation of α-SYN is vitally involved in the pathogenesis of PD, DLB, and MSA (Bandopadhyay, 2016; El-Agnaf et al., 2004; Vaikath et al., 2015).

MSA is a chronic, progressive neurodegenerative disease. Individuals with MSA become bedridden within several years and the disease is fatal. The etiology of MSA is unclear and there are no definitive treatments. GCIs are an MSA-specific type of inclusion that are localized to oligodendrocytes (Papp and Lantos, 1994; Wakabayashi et al., 1998). α-SYN, which is normally found in neurons as a presynaptic protein, becomes abnormally deposited in GCIs. Ultrastructural studies have determined that the central core of GCIs is composed of tubular filaments (Gai et al., 2003).

Abbreviations: AD, Alzheimer's disease; α-SYN, α-Synuclein; AT8, anti-human PHF-tau monoclonal; DAB, 3,3′-diaminobenzidine tetrahydrochloride; DLB, dementia with Lewies; FKBP12, FK506 binding protein 12; GCI, glial cytoplasmic inclusion; MSA, multiple system atrophy; LB, Lewy body; NFT, neurofibrillary tangle; PD, Parkinson's disease; PPIase, peptidyl-prolyl isomerase.

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A hallmark of PD is reduced dopamine production, and the symptoms are due to a decreased number of dopaminergic neurons. LBs, which are the hallmark of PD, are round intracytoplasmic inclusions that mainly consist of α-SYN (Baba et al., 1998; Javed et al., 2016). In addition, abnormal neurites in PD also contain α-SYN (Baba et al., 1998). It has been suggested that alteration of α-SYN homeostasis leads to accumulation and aggregation of α-SYN in LBs and neurites in the PD brain. Important roles of α-SYN have been identified from pathological and therapeutic studies of PD. DLB is characterized by abnormal accumulation of α-SYN in the cerebral cortex, limbic system, and brainstem neurons and neurites (Barker and Williams-Gray, 2016; Goldberg et al., 2017; Iseki, 2004). Previous studies reported that DLB has Alzheimer's disease (AD)-like pathology, namely, accumulation of amyloid-β and neurofibrillary tangles (NFTs) (Iseki et al., 2003, 2004).

FK506-binding protein (FKBP) 12 is a member of the immunophilin enzyme family that supports protein folding via its peptidyl-prolyl isomerase (PPIase) activity (Deleersnijder et al., 2011). It is believed to be a type of chaperone protein. Previous work revealed that FKPB12 is found in human brain and that its levels were changed in PD, DLB, and AD (Avramut and Achim, 2002). Both *in vitro* and cell culture data provided strong evidence that FKBP12 is a crucial PPIase involved in α-SYN aggregation and could be a drug target for α-SYN diseases (Deleersnijder et al., 2011; Gerard et al., 2006, 2008, 2010). In this study, we found that FKBP-immunopositive inclusions in PD, DLB, and MSA colocalized with phosphorylated α-SYN. In addition, NFTs in DLB colocalized with FKBP12 but not with phosphorylated α-SYN.

2. Results

2.1. FKBP12-immunopositive neuronal cells in control brain

In the control specimens, many neurons were immunopositive for the anti-FKBP12 antibody. FKBP12-immunoreactivity was typically observed in the neuronal bodies and dendrites, but nuclei were not stained (Fig. 1A). In addition, the glial cells were FKBP12 immunopositive but were only weakly stained (Fig. 1B).

2.2. FKBP12-immunopositive LBs and neurites in PD

We detected many FKBP12-immunopositive LBs (Fig. 2A) and neurites (Fig. 2B) in the pons of the brain of patients with PD. These FKBP12-immunopositive inclusions were observed in all patients with PD.

2.3. Double staining of FKBP12 and phosphorylated α-SYN in LBs

To confirm the anatomical relationship between FKBP12 and phosphorylated α-SYN, we performed double staining of patients with PD. FKBP12-immunopositive LBs colocalized with phosphorylated α-SYN in the LBs of PD brain (Fig. 3A–D).

2.4. FKBP12-immunopositive GCIs in MSA

In the tissue sections from patients with MSA, we found numerous FKBP12-immunopositive GCIs (Fig. 4A–D). These FKBP12-immunopositive GCIs were observed in all patients with MSA.

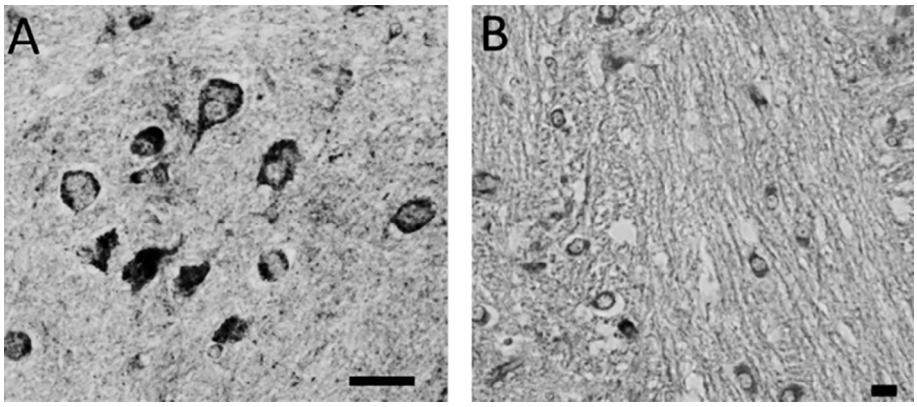

Fig. 1. FKBP12-immunopositive neuronal cells in control brain (pons). (A) In the control specimens, FKBP12 immunoreactivity was typically observed in the neuronal bodies and dendrites, but nuclei were not stained. (B) The glial cells were FKBP12 immunopositive, but they were only weakly stained. Scale bars: A = 20 μm; B = 10 μm.

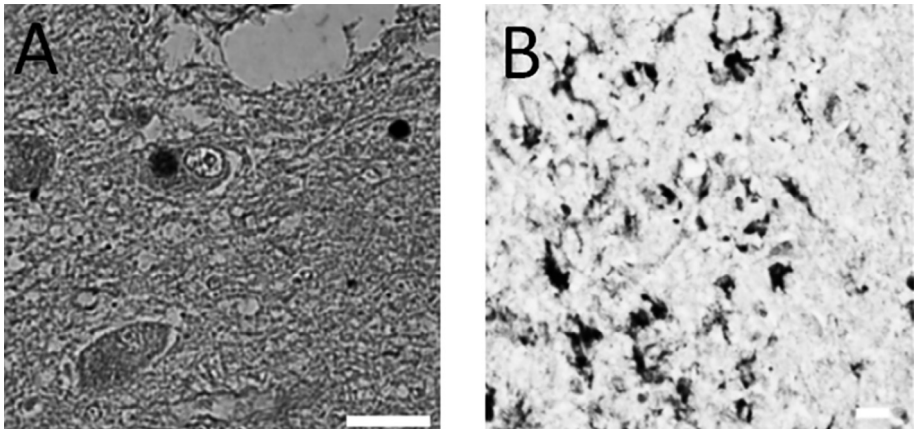

Fig. 2. FKBP12-immunopositive (A) LBs and (B) neurites in the pons of patients with PD. Scale bars: A = 20 μm; B = 10 μm.