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# Fasudil, a Rho kinase inhibitor, promotes the autophagic degradation of A53T α-synuclein by activating the JNK 1/Bcl-2/beclin 1 pathway

Feng-Tao Liu[a,b,1], Yu-Jie Yang[a,b,c,1], Jian-Jun Wu[a,b], Shan Li[c], Yi-Lin Tang[a,b], Jue Zhao[a,b], Zhen-Yang Liu[a,b], Bao-Guo Xiao[a,b], Ji Zuo[c], Wen Liu[c,*], Jian Wang[a,b,**]

[a]Department of Neurology, Huashan Hospital, Fudan University, Shanghai 200040, China
[b]Department & Institute of Neurology, Shanghai Medical College, Fudan University, Shanghai 200040, China
[c]Department of Cellular and Genetic Medicine, School of Basic Medical Sciences, Fudan University, Shanghai 200032, China

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ABSTRACT

Accumulation of α-synuclein (α-syn) is pivotally implicated in the pathogenesis of Parkinson's disease (PD), and enhancing its clearance might be a promising strategy in PD treatment. It has recently been shown that Rho kinase (ROCK) activation is involved in many neurodegenerative diseases, and some ROCK inhibitors might promote the degradation of abnormal protein aggregates. However, it is not known if fasudil, the only ROCK inhibitor available in clinical setting, could promote the degradation of α-syn, and ameliorate the α-syn induced neurotoxicity. In this regard, we investigated the effect of fasudil on neurite injury caused by A53T α-syn overexpression and the implicated pathway it might mediate. In the current study, we found that under the condition of A53T α-syn overexpression, the neurite outgrowth decreased significantly with the increasing expression of ROCK2. Fasudil, the ROCK inhibitor, ameliorated such neurotoxicity and promoted the clearance of A53T α-syn. Its underlying mechanism was supported by that fasudil could increase the macroautophagy activation via JNK 1 and Bcl-2 phosphorylation and beclin

Abbreviations: α-syn, α-synuclein; PD, Parkinson's disease; ROCK, Rho kinase; WT, wild-type; LBs, Lewy bodies; UPS, ubiquitin proteasome system; BSA, bovine serum albumin; CMA, chaperone-mediated autophagy; 3-MA, 3-methyladenine; Co-IP, Co-Immunoprecipitation; TEM, Transmission Electron Microscope; PI3K, phosphoinositide 3-kinase; JNK 1, c-Jun N-terminal kinase 1.

*Corresponding author. Fax: +86 21 54237311.
**Corresponding author at: Department of Neurology, Huashan Hospital, Fudan University, 12 Wulumuqi Middle Road, Shanghai 200040, China. Fax: +86 21 52888163.
E-mail addresses: liuft110@aliyun.com (F.-T. Liu), yujieyang1015@me.com (Y.-J. Yang), jungliw@126.com (J.-J. Wu), shanli1121@163.com (S. Li), tangyilin10@163.com (Y.-L. Tang), orangerange_zj@hotmail.com (J. Zhao), besuretowin@gmail.com (Z.-Y. Liu), bgxiao@shmu.edu.cn (B.-G. Xiao), jzuo@shmu.edu.cn (J. Zuo), liuwen@shmu.edu.cn (W. Liu), wangjian336@hotmail.com (J. Wang).
[1]Contributed equally to this work.

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1/Vps34 complex formation. Taken together, fasudil might be able to provide a novel and promising strategy for PD treatment by enhancing α-syn clearance and activating the JNK 1/Bcl-2/beclin 1 pathway.



## 1. Introduction

α-Synuclein (α-syn), especially the aggregated forms, has a significant pathological role in both familial and idiopathic Parkinson's disease (PD). The missense mutations, such as *A53T*, *A30P*, and *E46K* in α-syn found in some genetic studies, have been associated with familial parkinsonism (Polymeropoulos et al., 1997; Zarranz et al., 2004). Furthermore, the triplication and duplication of the α-syn locus (Chartier-Harlin et al., 2004; Singleton et al., 2003), overproducing the wild-type (*WT*) protein, can also cause the disease. In addition, α-syn aggregates can be found in Lewy bodies (LBs), the pathological hallmark of PD (Spillantini et al., 1998), being involved in sporadic cases as well (Lee et al., 2006). Therefore, it is of great necessity to explore the pathological changes induced by α-syn in PD.

Several studies indicated that the overexpression of α-syn either directly caused neuronal impairments or made the cells more susceptible to neurotoxins (Lee et al., 2006). Although the exact mechanisms remain elusive, the abnormal aggregation and deposition of α-syn have been pivotally implicated. The attenuation of macroautophagy and ubiquitin proteasome system (UPS) function were recognized as some important initiators of PD (Dehay et al., 2010). Furthermore, the abnormal accumulation of α-syn also impaired normal degradation of α-syn, causing proteasomal dysfunction (Lindersson et al., 2004), and lysosomal defects (Cuervo et al., 2004), and finally promoted α-syn aggregation and deposition. Taken together, the activation of the protein degradation seems to be a good strategy to fight the neurodegeneration caused by α-syn in PD (Ghavami et al., 2014).

In this study, we focused mainly on *A53T* α-syn, which was reported to be more potent than *WT* α-syn with regard to neurotoxicity. Several lines of evidence implied that *WT* α-syn could be degraded by both UPS and macroautophagy, while *A53T* α-syn gained a toxic function, and blocked the chaperone-mediated autophagy (CMA) (Cuervo et al., 2004; Lan et al., 2012). However, the exact manner of *A53T* α-syn degradation in neurons remains contentious. Our previous studies indicated that *A53T* α-syn accumulation could be degraded by the activation of macroautophagy (Lan et al., 2012), supporting autophagy as a potential drug target for the elimination of *A53T* α-syn.

Fasudil, a ROCK inhibitor, has been successfully implemented into clinical practice for the treatment of subarachnoid hemorrhage in Japan (Chen et al., 2013). Increasing bodies of evidence suggested that fasudil could exhibit markedly therapeutic effect on the disorders of central nervous system, including PD. In the $MPP^+$/MPTP induced models of PD, the treatment of fasudil not only increased dopaminergic cell survival, but also protected the neuritic network in vitro and the striatal axonal innervation in vivo (Tonges et al., 2012). In ovariectomized mice treated with MPTP, the inhibition of Rho kinase mediates the neuroprotective effects of estrogen in the MPTP induced model of PD (Rodriguez-Perez et al., 2013). In the C57Bl/6 mice lesioned by striatal stereotactic injections of 6-OHDA, high therapeutic concentrations of fasudil were suggestive of a proregenerative potential for dopaminergic neurons (Tatenhorst et al., 2014). These studies implied the neuroprotective role of fasudil in PD, but the exact mechanisms remained elusive. In a study of Huntington Disease, the inhibition of Rho kinase was found to activate the main cellular degradation pathways, including macroautophagy (Bauer et al., 2009). Furthermore, a pathway study proposed a novel role of Rho kinase in the regulation of autophagosome formation (Mleczak et al., 2013). Therefore, it is of great interest to detect the effect of fasudil, the Rho kinase inhibitor, on the PD model caused by *A53T* α-syn overexpression.

Herein, we evaluated whether fasudil could ameliorate the neurotoxicity induced by *A53T* α-syn overexpression in SH-SY5Y cells, and detected the autophagy activity and possible molecular pathways involved. Our study might be able to deepen our understanding of the pathogenesis of PD, and offer a new insight into developing novel treatment strategies.

## 2. Results

### 2.1. *A53T α-syn overexpression could decrease the neurite outgrowth with the Rho kinase activity being enhanced*

To study the effects of *A53T* α-syn overexpression on neurons, we measured the total length of neurites in cultured SH-SY5Y cells, since the loss of neurites can result in synaptic dysfunction and cause neuronal degeneration. In cultured SH-SY5Y cells, *A53T* α-syn overexpression resulted in a significantly decreased neurite length per cell (Fig. 1, $21.83 \pm 2.70\%$ of control; $^{**}p < 0.01$). At the same time, the ROCK2 expression increased significantly (Fig. 2A, $331.60 \pm 23.56\%$ of control; $^{**}p < 0.01$), indicating that the Rho kinase activity increased in the condition of A53T α-syn overexpression.

### 2.2. *Fasudil could attenuate the injury of neurite outgrowth caused by overexpressed A53T α-syn*

In the current study, the application of fasudil could significantly decrease the activation of Rho kinase (Fig. 2A, $154.57 \pm 16.66\%$; $^{**}p < 0.01$, *A53T*+fasudil group vs. *A53T* group.) induced by *A53T* α-syn. Furthermore, the application of fasudil at the optimal dose of 15 μg/ml attenuated the axon injury (Fig. 1) caused by