

# LRRK2-NFATc2 Pathway Associated with Neuroinflammation May Be a Potential Therapeutic Target for Parkinson's Disease

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**Abstract:** Neuroinflammation plays an important role in the pathogenesis of Parkinson's disease (PD). However, the molecular mechanisms involved in extracellular  $\alpha$ -synuclein-induced proinflammatory microglial responses through Toll-like receptor 2 (TLR2) are unclear. Leucine-rich repeat kinase 2 (LRRK2) is a serine/threonine kinase, and its mutations are closely related to autosomal dominant PD. Recently, Masliah et al characterized a novel-specific neuroinflammation cascade dependent on LRRK2-NFATc2 in microglia activated by neuron-released  $\alpha$ -synuclein. LRRK2 selectively phosphorylated and induced nuclear translocation of NFATc2 to activate a neuroinflammation cascade. In this cascade, LRRK2 kinase was activated by neuron-released  $\alpha$ -synuclein in microglia via TLR2. Further, NFATc2, as a kinase substrate for LRRK2, was directly phosphorylated, which accelerated nuclear translocation of NFATc2, where cytokine/chemokine gene expression including TNF- $\alpha$  and IL-6 is regulated by NFATc2 transcriptional activity, resulting in a neurotoxic inflammatory environment. Moreover, an abnormal increase of NFATc2 in nuclear was observed in the brains of patients and a mouse model of PD. Additionally, the administration of an LRRK2 inhibitor could ameliorate neuroinflammation, prevent neuronal loss, and improve motor function. Therefore, modulation of LRRK2-NFATc2 signaling cascade might be a potential therapeutic target for the treatment of PD.

**Keywords:** Parkinson's disease, neuroinflammation, LRRK2, NFATc2,  $\alpha$ -synuclein

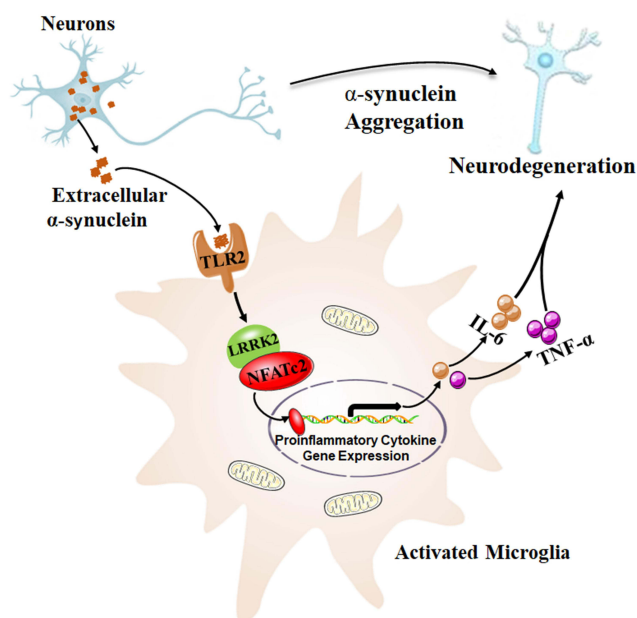
Neuroinflammation plays an important role in the pathogenesis of Parkinson's disease (PD),<sup>1</sup> therefore, targeting crucial factors of different neuroinflammatory signaling pathways is a potential therapeutic strategy for PD. It is well known that PD is a chronic neurodegenerative disorder characterized by abnormal accumulation of  $\alpha$ -synuclein in the degenerative dopaminergic neurons of substantia nigra, which can induce cell-autonomous neurotoxicity.<sup>2,3</sup> Notably, a large number of studies have shown that  $\alpha$ -synuclein can be secreted by neurons. This extracellular  $\alpha$ -synuclein can be directly transferred to neighboring neurons resulting in neurotoxic  $\alpha$ -synuclein deposition, and it can further lead to neuroinflammatory responses in microglia that can produce and release pro-inflammatory cytokines, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1beta (IL-1 $\beta$ ), interleukin-6 (IL-6) and so on, which in turn aggravate neuronal degeneration and neuroinflammation.<sup>3</sup> Previous studies have shown that extracellular  $\alpha$ -synuclein activates microglia through Toll-like receptor 2 (TLR2), which is a  $\alpha$ -synuclein receptor expressed in neurons and microglia, leading to non-cell-autonomous

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neurotoxicity.<sup>4,5</sup> The mechanism probably involves the production and release neurotoxic pro-inflammatory cytokines by activated microglia via nuclear factor B (NF- $\kappa$ B) and p38 mitogen-activated protein kinase (MAPK) downstream signaling pathways.<sup>4</sup> Furthermore, increased expression of TLR2 was observed in the brains of patients and a mouse model of PD.<sup>6,7</sup> However, the molecular mechanisms involved in extracellular  $\alpha$ -synuclein-induced neurotoxic proinflammatory microglial responses through TLR2 are unclear.

Leucine-rich repeat kinase 2 (LRRK2) is a serine/threonine kinase,<sup>8</sup> and its mutations are closely related to autosomal dominant PD.<sup>9,10</sup> Mounting evidence indicates that LRRK2 is highly expressed in immune cells and is frequently associated with neuroinflammation in PD.<sup>11,14</sup> It has been reported that the production of pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  decreased in primary microglia with LRRK2 knockdown or kinase inhibition.<sup>12,13</sup> Therefore, an understanding of the interplay between LRRK2-mediated neuroinflammatory responses and PD is essential to clearly define the upstream and downstream factors of this pathway. Although knockdown of LRRK2 can reduce the transcriptional activity of NF- $\kappa$ B in activated microglia,<sup>14</sup> it might raise the question whether there are other LRRK2-mediated neuroinflammatory signaling pathways in activated microglia via extracellular  $\alpha$ -synuclein.

Recently, Masliah et al characterized a novel neuroinflammation cascade dependent on LRRK2-nuclear factor of activated T cells, cytoplasmic 2 (LRRK2-NFATc2) in microglia activated by neuron-released  $\alpha$ -synuclein<sup>15</sup> (Figure 1). In this study, the level of LRRK2 phosphorylation and activity increased in mouse primary microglia with extracellular  $\alpha$ -synuclein treatment. However, the increase disappeared when the microglia were pretreated with TLR2 function blocking antibody T2.5, suggesting that extracellular  $\alpha$ -synuclein activated LRRK2 by TLR2 in microglial cultures. Furthermore, *Lrrk2* knockout significantly suppressed  $\alpha$ -synuclein-mediated microglial neurotoxicity by decreasing of TNF- $\alpha$  and IL-6 expression. It has been reported that TLR2 is a pattern recognition receptor that plays a key role in the innate immune response to micro-organisms.<sup>16,17</sup> Single-nucleotide polymorphisms of *Lrrk2* or its adjacent genes are susceptible to leprosy infection in humans.<sup>18</sup> Although evidence suggests that TLR2 and LRRK2 are involved in the innate immune response to micro-organisms, the underlying interaction between them is not completely understood. It has been



**Figure 1** Neuron-released  $\alpha$ -synuclein activated microglia through TLR2, and subsequently activated LRRK2 kinase. NFATc2, as a kinase substrate of LRRK2, was directly phosphorylated, which accelerated nuclear translocation of NFATc2 where cytokine/chemokine gene expression such as IL-6 and TNF- $\alpha$ , were upregulated by NFATc2 transcriptional activity. This resulted in a neurotoxic inflammatory environment, which in return aggravated the neuronal degeneration in a mouse model of PD. Data from Kim et al.<sup>15</sup>

shown that marked phosphorylation of LRRK2 at Ser910 and Ser935 is induced by the canonical I $\kappa$ B kinase family during TLR signaling.<sup>19</sup> While Masliah et al did not provide additional information regarding the interactions between TLR2 and LRRK2, further studies are needed to elucidate this mechanism.<sup>15</sup>

To address the mechanisms underlying the role of LRRK2 in microglial activation via extracellular  $\alpha$ -synuclein, Masliah et al analyzed transcriptome data (GSE26532) obtained from primary rat microglia exposed to extracellular  $\alpha$ -synuclein. Forty-three up-regulated genes, which involved in TLR signaling and other immune signaling pathways, were selected and a signaling network was reconstructed to describe their interactions using functional gene enrichment analysis and information on protein-protein interactions.<sup>15</sup> According to the analysis results, Masliah et al hypothesized that LRRK2 and NFATc2 selectively modulate cytokine and chemokine expression in extracellular  $\alpha$ -synuclein-mediated microglia via TLR2 signaling pathway.<sup>15</sup> It has been shown that NFATc2, as an immune transcription factor, can translocate to the nucleus, and NFAT responsive elements are located in the promoter region of multiple immune-related genes such as TNF- $\alpha$ , IL-6 and so on.<sup>20,21</sup> Besides,

calcineurin/NFAT signaling cascade is frequently associated with the neuroinflammation in mouse models of Alzheimer's disease (AD)<sup>22</sup> and PD.<sup>23</sup> Additionally, phosphorylation of NFATc1 is closely related to the transcription of genes associated with neuroinflammation in AD mice.<sup>24</sup> Notably, the NFATc2 isoform was the most highly expressed in murine microglia cultures, and the ability of the microglia to secrete cytokines was attenuated by deletion of NFATc2 in mouse models of AD.<sup>25</sup> The above findings are strong evidence to support the hypothesis that LRRK2 and NFATc2 selectively modulate pro-inflammatory cytokine expression in extracellular  $\alpha$ -synuclein-mediated microglia via TLR2 signaling pathway.

Interestingly, the direct interaction between NFATc2 and LRRK2 was first identified by automated image analysis in this study. Specifically, LRRK2 selectively phosphorylated and induced the nuclear translocation of NFATc2 to activate a neuroinflammation cascade. In this cascade, LRRK2 kinase was activated by neuron-released  $\alpha$ -synuclein in microglia via TLR2. Further, NFATc2, as a kinase substrate for LRRK2, was directly phosphorylated, which accelerated the nuclear translocation of NFATc2 where the cytokine/chemokine gene expression including IL-6 and TNF- $\alpha$  is regulated by NFATc2 transcriptional activity, resulting in a neurotoxic inflammatory environment<sup>15</sup> (Figure 1). Moreover, an abnormal increase of NFATc2 in the nucleus was observed in the brains of patients and mouse models of PD.<sup>15</sup> Additionally, the administration of an LRRK2 inhibitor (HG-10-102-01) could ameliorate neuroinflammation, prevent neuronal loss, and improve motor function in a mouse model of PD.<sup>15</sup> Although LRRK2 inhibitor clinical trials provide opportunities to refine our understanding of LRRK2 in human immune function, it is unknown whether LRRK2 inhibition increases the risk of opportunistic infections.<sup>11</sup> In addition, it is unclear whether LRRK2 inhibitors have disease modifier role in PD patients with LRRK2 mutations.<sup>11</sup> Although it is difficult to develop specific antibodies targeting NFATc2 because of multiple phosphorylation residues, modulation of the LRRK2-NFATc2 signaling cascade might be a potential therapeutic target for the treatment of PD.

Altogether, this study is the first to report a novel LRRK2-NFATc2 signaling cascade that plays an important role in neuroinflammation in PD, and provides new clues for the clinical treatment of PD.

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## Disclosure

The authors report no conflicts of interest in this work.

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