Published in final edited form as:

J Neurochem. 2014 June ; 129(6): 895–897. doi:10.1111/jnc.12691.

LRRK2: Dropping (kinase) inhibitions and seeking an (immune) response

Adamantios Mamais and Mark R. Cookson

Cell Biology and Gene Expression Section, Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, Maryland, USA

Since the discovery in 2004 of mutations in *LRRK2* that cause Parkinson's disease (PD), extensive effort has been made to understand the cellular roles of the encoded protein. *LRRK2* is a large (286kDa) multi-domain protein that harbours a kinase and GTPase domain. Most PD linked mutations are found in these two enzymatic domains while the most prevalent mutation, G2019S, enhances the kinase activity of the protein and induces neuronal cell death in cell and animal models. Because of the potential role of kinase activity in *LRRK2* pathogenesis, a number of small pharmacological inhibitors against the kinase activity of *LRRK2* have been developed that are now routine research tools.

Recently, *LRRK2* has been linked to inflammatory response signalling. Genome-wide association studies (GWAS) have identified common genetic variants around *LRRK2* as risk factors for Crohn's disease (Barrett *et al.*, 2008), leprosy (Zhang *et al.*, 2009) as well as PD (IPDGC and WTCCC2, 2011). *LRRK2* is highly expressed in immune cells and upregulated in response to microbial and viral particles (Hakimi *et al.*, 2011). Furthermore, Toll-Like Receptor (TLR) signalling has been shown to modulate *LRRK2* function via changes in constitutive phosphorylation in macrophages (Dzamko *et al.*, 2012). *LRRK2* is also upregulated in peripheral blood mononuclear cells (PBMCs) upon INF- γ stimulation (Gardet *et al.*, 2010), but the signalling events mediating this are largely unexplored. In this issue, Kuss *et al.* (2014) identify extracellular-signal-regulated kinase 5 (ERK5) as a modulator of *LRRK2* expression. The authors demonstrate that induction of *LRRK2* expression by INF- γ is attenuated by knock down or molecular inhibition of ERK5 in human monocytic leukemia THP-1 cells or human peripheral blood monocytes differentiated to macrophages. ERK5 belongs to the mitogen-activated protein kinase (MAPK) family and is involved in regulation of cell proliferation and differentiation. Inhibition of ERK5 blocks expression of TLR2 induced by a TLR1/2 agonist and other cytokines in monocytes (Wilhelmsen *et al.*, 2012), suggesting that ERK5 may play a role in INF- γ and TLR2 signaling.

The data from Kuss *et al.* (2014) provide an interesting concept for how *LRRK2* might have different functions in different cell types. ERK5 and *LRRK2* are both ubiquitously expressed in the human body but INF- γ signalling is predominantly restricted to immune

Corresponding author: cookson@mail.nih.gov.

Conflicts of interest: none

=> if 'none', insert "The authors have no conflict of interest to declare."

=> otherwise insert info unless it is already included

cells. This suggests that control of LRRK2 may vary between different cell types. Downstream of IFN- γ ERK5 may thus serve as a modular control for LRRK2 in different cells. ERK5 is activated by oxidative stress and plays a neuroprotective role in dopaminergic cells (Cavanaugh *et al.*, 2006). Accumulation of reactive oxygen species and oxidative damage in the aging brain could therefore impair physiological ERK5 signalling and dysregulate LRRK2. Therefore, common signalling events in both microglia and neurons might be critical for pathogenesis.

ERK5 is believed to mediate activation of Akt in response to growth factors to regulate cell survival but the signalling events involved are not fully defined. Kuss *et al.* (2014) suggest that LRRK2 is a downstream ERK5 effector whose levels are regulated in response to stimuli. As previous data has implicated LRRK2 in Akt phosphorylation (Ohta *et al.*, 2011) a testable hypothesis is that activated ERK5 promotes upregulation of LRRK2 that in turn can drive downstream dysregulation of Akt signalling, thought to occur in degenerating neurons (Timmons *et al.*, 2009). Again, these considerations support the idea that LRRK2 pathogenesis might include effects both on neuronal and non-neuronal cells, although this has yet to be tested *in vivo*.

Part of the way in which Kuss *et al.* (2014) identified ERK5 was by considering some apparently difficult data. Although one kinase inhibitor, LRRK2-IN-1, could attenuate IFN- γ -induced LRRK2 upregulation, this effect was not reproduced with four other chemically distinct LRRK2 kinase inhibitors. This therefore suggested that LRRK2 kinase inhibition is not the event mediating LRRK2 upregulation. A recent study by Luerman *et al.* (2013) used proteomics to show that LRRK2-IN-1 can inhibit ERK5, while also exhibiting a measurable effect on TNF α activation signalling. Kuss *et al.* (2014) therefore used two ERK5 selective inhibitors and knock down and observed similar effects to LRRK2-IN-1, indicating that ERK5 is involved in LRRK2 upregulation after IFN- γ stimulation. Although this underlines the limitations of any pharmacological approach, at the same time it presents a good example of how these non-specific cellular effects can be exploited to advance our understanding of LRRK2 biology.

In summary, the study by Kuss *et al.* (2014) has two novel observations: a) ERK5 is involved in LRRK2 signalling after IFN- γ activation, and b) the exploitation of off-target effects of pharmacological inhibitors in deciphering LRRK2 function. It is important that all the experiments looked at endogenous proteins, suggesting relevance to physiological signalling events. Future directions might be to consider whether genetic variants found in GWAS, either for PD or Crohn's disease, or familial mutations may further modulate the effects of ERK5 on LRRK2. It would also be important to investigate the nature of the apparent accumulation of mRNA; perhaps these are translational events which again would be important in understanding along with the effects of genetic variation around transcription factor binding sites. At the protein level, Kuss *et al.* (2014) propose that endogenous LRRK2 appears to accumulate in the cytoplasm of IFN- γ treated THP-1 cells. Following on from reports implicating ubiquitinating-proteasome pathway in degradation of LRRK2 (Ko *et al.*, 2009) it is possible that association with chaperone proteins mediates accumulation of the protein in the cytoplasm, which would be an additional mechanism for controlling activity of this protein.

Some additional questions are whether LRRK2 is involved in ERK5/IFN- γ signalling in microglia, whether LRRK2 expression levels are important in inflammation response in the brain, and how could altered microglia function, caused by aberrant LRRK2, influence neurodegeneration in PD? As discussed above, LRRK2 can be upregulated by IFN- γ (Kuss *et al.*, 2014) and TLR-mediated signalling can modulate LRRK2 phosphorylation. These events may be related and, potentially, co-ordinated during specific signalling situations (Figure 1). What is not known is how these events can modulate LRRK2 activity, which has been proposed to be important in autophagy and/or inflammation. Both these systems are believed to contribute to PD progression: dysregulation of macroautophagy signalling may partly mediate α -synuclein deposition, a key pathological marker of PD, while neuroinflammation can induce dopaminergic neuron loss (Qin *et al.*, 2007). Autophagy is an integral part of immune response mediating antigen presentation, pathogen clearance and intracellular antigen sensing. What is becoming apparent is that PD progression may be a multifactorial sequence of signalling events that take place in both microglia and neurons, and data suggest that LRRK2 may function in both systems.

Acknowledgments

This research was supported entirely by the Intramural Research Program of the NIH, National Institute on Aging.

This work was funded by Intramural Research Program of the NIH, National Institute on Aging (grant number): This information is usually included already, but please add to the Acknowledgments if not.

ARRIVE guidelines have been followed:

No

=> if No, skip complete sentence

=> if Yes, insert "All experiments were conducted in compliance with the ARRIVE guidelines."

Abbreviations

PD	Parkinson's disease
LRRK2	leucine-rich repeat kinase 2
GWAS	genome-wide association studies
IFN-γ	interferon- γ
TLR	toll-like receptor
ERK5	extracellular-signal-regulated kinase 5
MAPK	mitogen-activated protein kinase
PBMCs	peripheral blood mononuclear cells

References

Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat. Genet.* 2008; 40:955–962. [PubMed: 18587394]

- Cavanaugh JE, Jaumotte JD, Lakoski JM, Zigmond MJ. Neuroprotective role of ERK1/2 and ERK5 in a dopaminergic cell line under basal conditions and in response to oxidative stress. *J. Neurosci. Res.* 2006; 84:1367–1375. [PubMed: 16941494]
- Dzamko N, Inesta-Vaquera F, Zhang J, Xie C, Cai H, Arthur S, Tan L, Choi H, Gray N, Cohen P, et al. The IkappaB kinase family phosphorylates the Parkinson's disease kinase LRRK2 at Ser935 and Ser910 during Toll-like receptor signaling. *PLoS ONE.* 2012; 7:e39132. [PubMed: 22723946]
- Gardet A, Benita Y, Li C, Sands BE, Ballester I, Stevens C, Korzenik JR, Rioux JD, Daly MJ, Xavier RJ, et al. LRRK2 is involved in the IFN-gamma response and host response to pathogens. *J. Immunol.* 2010; 185:5577–5585. [PubMed: 20921534]
- Hakimi M, Selvanantham T, Swinton E, Padmore RF, Tong Y, Kabbach G, Venderova K, Girardin SE, Bulman DE, Scherzer CR, et al. Parkinson's disease-linked LRRK2 is expressed in circulating and tissue immune cells and upregulated following recognition of microbial structures. *J Neural Transm.* 2011; 118:795–808. [PubMed: 21552986]
- International Parkinson's Disease Genomics Consortium (IPDGC), and Wellcome Trust Case Control Consortium 2 (WTCCC2). A two-stage meta-analysis identifies several new loci for Parkinson's disease. *PLoS Genet.* 2011; 7:e1002142. [PubMed: 21738488]
- Ko HS, Bailey R, Smith WW, Liu Z, Shin J-H, Lee Y-I, Zhang Y-J, Jiang H, Ross CA, Moore DJ, et al. CHIP regulates leucine-rich repeat kinase-2 ubiquitination, degradation, and toxicity. *PNAS.* 2009; 106:2897–2902. [PubMed: 19196961]
- Kuss M, Adamopoulou E, Kahle PJ. Interferon- γ induces leucine-rich repeat kinase LRRK2 via extracellular signal-regulated kinase ERK5 in macrophages. *J. Neurochem.* 2014
- Luerman GC, Nguyen C, Samaroo H, Loos P, Xi H, Hurtado-Lorenzo A, Needle E, Stephen Noell G, Galatsis P, Dunlop J, et al. Phosphoproteomic evaluation of pharmacological inhibition of leucine-rich repeat kinase 2 reveals significant off-target effects of LRRK-2-IN-1. *J. Neurochem.* 2013
- Ohta E, Kawakami F, Kubo M, Obata F. LRRK2 directly phosphorylates Akt1 as a possible physiological substrate: impairment of the kinase activity by Parkinson's disease-associated mutations. *FEBS Lett.* 2011; 585:2165–2170. [PubMed: 21658387]
- Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong J-S, Knapp DJ, Crews FT. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia.* 2007; 55:453–462. [PubMed: 17203472]
- Timmons S, Coakley MF, Moloney AM, O' Neill C. Akt signal transduction dysfunction in Parkinson's disease. *Neuroscience Letters.* 2009; 467:30–35. [PubMed: 19800394]
- Wilhelmsen K, Mesa KR, Lucero J, Xu F, Hellman J. ERK5 protein promotes, whereas MEK1 protein differentially regulates, the Toll-like receptor 2 protein-dependent activation of human endothelial cells and monocytes. *J. Biol. Chem.* 2012; 287:26478–26494. [PubMed: 22707717]
- Zhang F-R, Huang W, Chen S-M, Sun L-D, Liu H, Li Y, Cui Y, Yan X-X, Yang H-T, Yang R-D, et al. Genomewide association study of leprosy. *N. Engl. J. Med.* 2009; 361:2609–2618. [PubMed: 20018961]

Figure 1.

LRRK2 is involved in inflammatory signalling. In response to IFN- γ and via ERK5 LRRK2 is upregulated in macrophage-like cells, while TLR activation mediates LRRK2 phosphorylation via IKK. Apparent involvement of LRRK2 in signalling events of inflammation and autophagy raise the possibility that the role of LRRK2 in PD may be non-cell autonomous, perhaps mediated through diverse functions in both microglia and neuronal cells.