In recent years, leucine-rich repeat kinase 2 (LRRK2) mutations have been identified as having a central part in the development of Parkinson’s disease. Efforts have since been underway to better understand LRRK2 and develop therapeutic interventions that inhibit LRRK2 kinase activity. Earlier this year, an international public-private research consortium revealed that it has identified and validated a cellular role of LRRK2 kinase. Published in the open-access eLife journal, the finding illuminates a novel route of therapeutic development and intervention testing for Parkinson’s disease.

A team of investigators from the Max Planck Institute of Biochemistry, the University of Dundee, The Michael J. Fox Foundation for Parkinson’s Research, GlaxoSmithKline, and Merck collaborated on the systematic testing that determined the LRRK2 kinase regulates cellular trafficking by deactivating certain Rab proteins (3, 8, 10 and 12).

According to the Michael J. Fox Foundation, the newly discovered link between mutant LRRK2 and inappropriate deactivation of Rab function unlocks more than 20 years of accumulated knowledge of the roles of Rab proteins and may improve understanding of LRRK2 dysfunction in the Parkinson’s disease process. The research group now is working to further characterize the Rab proteins modified by LRRK2 and to understand how an imbalance in cellular trafficking leads to the degeneration of neurons seen in Parkinson’s disease.

Ahead, Marco Baptista, PhD, Associate Director of Research Programs for the Michael J. Fox Foundation, discusses the significance of the recent findings and shares insights on how LRRK2 may shape the future of therapy.

Can you talk a bit about LRRK2 and its significance within the spectrum of Parkinson’s disease?

“Parkinson’s disease traditionally was not considered a genetic disease, but over the last 20 years, this view has changed. Although rare, known genetic causes account for approximately 10 percent of Parkinson’s disease (PD) cases,” says Dr. Baptista. “Mutations in the LRRK2 gene are one of the most common known genetic causes of PD, accounting for about two percent of all cases. However, in certain ethnic groups such as Ashkenazi Jews and North African Arab Berbers a particular LRRK2 mutation (G2019S) accounts for 20 to 40 percent of cases.”

“The G2019S mutation increases LRRK2 kinase activity, which researchers believe to be pathological. This is the rationale for developing LRRK2 kinase inhibitors, and many drug companies are very good at making this drug class given their expertise in targeting kinases,” he states. “The clinical and pathological phenotypes of G2019S mutation carriers are not that different from idiopathic Parkinson’s (no known cause), which provides hope that a LRRK2 kinase inhibitor may be effective in all Parkinson’s cases.”

What does your recent finding about LRRK2 and Rab proteins illuminate about how Parkinson’s disease is understood and potentially treated?

“Kinases activate or inhibit signaling pathways (also referred to as substrates) via phosphorylation. It has been incredibly difficult to identify bona fide LRRK2 substrates because of LRRK2’s relatively low kinase activity. Although there have been published reports of potential LRRK2 substrates, none have stood up to rigorous validation,” Dr. Baptista observes. “Our identification of a subset of the enzyme family GTPases termed Rabs...
is the first time that a LRRK2 substrate has been fully validated by multiple labs, various tools and models, and molecular techniques. We are very confident that these Rab substrates—which play a role in cellular trafficking—will stand the test of time as bona fide proteins directly regulated by LRRK2. And this finding would not have happened without the open collaboration between academic laboratories and two major pharmaceutical companies, organized and facilitated by The Michael J. Fox Foundation.”

This discovery has three major implications, according to Dr. Baptista. “First, it allows us to make advances in understanding the biological role of normal and mutated LRRK2. The functional role of LRRK2 is still a bit of a mystery, but we now have a solid foundation for placing LRRK2 into the GTPase Rab pathway, which has been extensively characterized,” he says. “Second, to develop LRRK2 kinase inhibitors, it is important to have the ability to assess the effects of investigational compounds on LRRK2-mediated signaling. The Rab discovery presents a possible way to assess the pharmacological effects of the drugs at different doses and over time after administration. This would improve selection of doses and dosing regimen to be tested in PD patients to slow the progression of the disease. The ability to monitor phosphorylation of Rabs also provides the potential to select/enrich for patients who have hyperactivation of the LRRK2 pathway. Thus, as seen in the cancer field, personalized therapy may be approachable for LRRK2 kinase inhibitors,” notes Dr. Baptista. “Finally, the current strategy is to target LRRK2 via molecules that inhibit its kinase function. The identification of direct LRRK2 substrates offers the opportunity to target Rab signaling to correct an abnormal LRRK2 pathway in people with Parkinson’s.”

Can you talk a bit about LRRK2 kinase inhibitors and their potential impact on pulmonary and kidney function? How are those risks being addressed going forward?

“We have known for some years now that genetically altered rodents deficient in LRRK2 protein levels display an abnormal kidney and lung phenotype. The kidneys are enlarged and dark red, while the lung microscopy reveals abnormal accumulation of secretory lysosome-related organelles known as lamellar bodies in type II pneumocytes,” he explains.

“We began working with Genentech to determine if these histopathological abnormalities would be induced by LRRK2 kinase inhibitor. We tested two different compounds and demonstrated that neither compound affected lungs or kidneys in rodents but both induced abnormal lung pathology in monkeys. All other organs, including the brain and kidney, were unaffected in monkeys treated for 28 days with LRRK2 inhibitors,” says Dr. Baptista. “These findings were important because they strongly suggested that the lung phenotype was likely due to inhibition of LRRK2. It also demonstrated that the monkey was the more sensitive species for LRRK2 inhibitors.”

“However, two key concepts were not addressed by the study described above: (a) Since most drugs have the potential to hit alternative targets (the so-called off-target effects), we needed to further confirm that the long histopathology was directly attributable to LRRK2 inhibition. (b) We also needed to address whether the lung histopathology was reversible upon stopping the treatment, which is an important question for development of LRRK2 kinase inhibitors. To address these questions, we formed a consortium and solicited from industry partners structurally different LRRK2 kinase inhibitors with different off-target profiles and added a reversibility arm to the study design. To our delight, both Pfizer and Merck & Co. contributed potent and selective LRRK2 kinase inhibitors in a stunning display of pre-competitive collaboration. We conducted another monkey study testing two different doses of Pfizer’s and Merck’s compounds side by side with the Genentech molecule included as a positive control,” Dr. Baptista notes.

“This recently completed study showed that all compounds at higher doses induced the lung pathology while the low doses were without any adverse effects, confirming that the lung effects are mediated by LRRK2 inhibition. Importantly, the low doses of both compounds inhibited LRRK2 kinase activity by more than 80 percent in the brain and peripheral tissues. Thus there appears to be a margin of safety where LRRK2 may be inhibited significantly without inducing lung pathology. Secondly, we demonstrated that upon stopping the treatment with Genentech’s compound, the lung pathology was completely reversed in all monkeys, demonstrating the lack of per-
manent damage to lungs within the timeframe of dosing. We also assessed several lung functions at doses that produced the pathology and found no biologically significant effects. Importantly, drug withdrawal completely reversed the effect, and there were no functional pulmonary deficits. Taken together, these data significantly allay concerns around therapeutic targeting of LRRK2.”

Given the interest in LRRK2’s role in the disease process of PD, how do you see these recent findings impacting future development and care?

“From our discussions with several drug companies, there is none that sees the lung findings in the monkeys as an insurmountable obstacle to go into the clinic,” Dr. Baptista notes. “The key now is to clearly define the margin of safety for each individual molecule in development and to integrate the monitoring of lung and kidney functions and biomarkers of damage in clinical trials.”

What are the next steps in terms of research, and what is the current status of the LRRK2 kinase inhibitor pipeline?

Companies with LRRK2 kinase inhibitor programs have made significant advances in discovery and development of potent and selective LRRK2 kinase inhibitors with drug-like properties, according to Dr. Baptista. “These companies are starting to plan their long-term clinical development strategies. Although we are making good progress in understanding the potential safety issues of LRRK2 kinase inhibitors, three areas need to be focused on to facilitate drug development: (a) development and validation of assays to measure effects of investigational compounds on clinical samples, (b) an understanding of the degree of LRRK2 kinase inhibition required to produce disease modifying activity in PD patients, and (c) identification of a patient population that is most likely to respond to LRRK2 kinase inhibitors,” he explains.

“First, we need to develop assays that indicate LRRK2 kinase activity to be able to demonstrate that the compounds are adequately engaging the target. To this end, we are aggressively investing in reagents to assess Rab and LRRK2 phosphorylation in a variety of biological tissues. Secondly, to establish a relationship between LRRK2 kinase inhibition and anti-parkinsonian effects, we are continuing our industry collaborations with Merck and Pfizer to conduct animal model studies and are making good progress. For the patient enrichment strategy, we are aggressively recruiting and studying LRRK2 mutation-carrying PD patients within our landmark Parkinson’s Progression Markers Initiative study and other LRRK2 registries. These would facilitate access to patients and inform clinical end-points. In parallel, we are also funding studies to strengthen the role of LRRK2 in idiopathic PD patients to be able to target a wider PD population that is not hampered by limited numbers of patients.”

Do you have any take-home points about LRRK2 and the future of Parkinson’s therapy?

“LRRK2 is one of the most attractive drug targets for Parkinson’s disease, and we may be only a couple of years away from a first-in-human clinical trial,” says Dr. Baptista. However, targeting LRRK2 does not come without its challenges, he notes. “We need to realize that regardless of the drug target, drug discovery is a very challenging endeavor, and it takes more than throwing money at the issue to come to solutions. From our experience, it requires the determination and passion of many stakeholders who are willing to collaborate on projects that may not have been viewed as pre-competitive but that are essential to move the field forward. Our collaborations with big Pharma and academia demonstrate that challenging questions can be answered when we all find ways to work together for a common goal,” he observes. It also requires the active participation of Parkinson’s patients who play a crucial role in the success of clinical trials, according to Dr. Baptista. “We owe it to them to find new ways to collaborate so that we can deliver a safe and effective cure for Parkinson’s disease.”

To learn more about LRRK2 and the group’s research, visit www.michaeljfox.org.

1. http://dx.doi.org/10.7554/eLife.12813