Instituto de Medicina Molecular

Master Project Proposal

Title: Dissecting the effect of Parkinson's Disease related PINK1 mutations on kinase activity

Synopsis:

Parkinson's disease (PD) is the second most common neurodegenerative movement disorder, affecting approximately 1% of the population over 65. This number is bound to increase, as lifespan of the populations increases as well. At present, there is only symptomatic but no causal cure for PD. PTEN-induced putative kinase 1 (PINK1) encodes a Serine/Threonine kinase with a mitochondrial targeting sequence, and mutations in this gene are linked to early-onset recessive PD. Cellular and animal loss-of-PINK1-function models show defects in mitochondrial homeostasis, impaired stress-response, decreased oxidative phosphorylation and defects in mitochondrial trafficking, dynamics and quality control. Recently, PINK1 has been reported to be autophosphorylated upon depolarization, hinting at a possible activation mechanism. Different residues have been identified as autophosphorylation sites after CCCP-induced mitochondrial membrane depolarization, namely S228, T257 and S402. Two residues, S228 and S402, appear to be involved in PINK1 dimerization and Parkin recruitment. A fourth putative phosphorylation site in PINK1 is T313, a residue that is regulated by the activity of Microtubule affinity regulating kinase 2 (MARK2). Although understanding the regulation of PINK1 activity is pivotal to interpret how PINK1 executes its different functions in both healthy and depolarized mitochondria, it remains rather unclear how PINK1 induced loss-of-function can affect the kinase activity and the overall (auto)phosphorylation status of PINK1. In this project we aim to understand how the PD-causing clinical mutations of PINK1 can affect its kinase activity and consequent substrate phosphorylation. For this we will use assays established in our previous work (Aerts et al., 2015 JBC) to generate PINK1 clinical mutant forms and access their activity in an in vitro phosphorylation assay where we will measure substrate phosphorylation (namely of Parkin, Ubiquitin and Complex I) and PINK1 (auto)phosphorylation. We will also further investigate the consequence of these clinical mutations on Parkin recruitment and mitochondrial clearance.

Supervisor: *Vanessa Morais, VMorais Lab, vmorais@medicina.ulisboa.pt*
Webpage of the group: https://imm.medicina.ulisboa.pt/en/investigacao/labs/morais-vanessa/