Mitochondrial ubiquitin landscape in neurons

Introduction

The identification of PINK1 and Parkin as genes mutated in heritable forms of Parkinson's disease have driven investigation into mechanistic studies aimed to elucidate their neurologic implications. In response to mitochondrial depolarisation, PINK1 kinase is activated and in turn activates Parkin ubiquitin E3 ligase and this has been shown collectively to function in the morphological maintenance of the mitochondrial network and also to critically regulate the removal of dysfunctional mitochondria through mitophagy. Parkin ubiquitylates a number of proteins of the outer mitochondrial membrane by forming different types of ubiquitin chains, which are further phosphorylated by PINK1. The question of whether this process is relevant to primary cells, particularly to neurons, under physiological conditions, has been highly debated.

The Mootha group in Harvard have aided the field by providing comprehensive lists of the mitochondrial proteome. This has been termed as MitoCarta 2.0. Both a human version (https://www.broadinstitute.org/files/shared/metabolism/mitocarta/human.mitocarta2.0.html) and a mouse version (https://www.broadinstitute.org/files/shared/metabolism/mitocarta/mouse.mitocarta.2.0.html) have been completed. The full file can be downloaded from these sites in excel format. The data is for 1,158 mouse genes with high confidence of mitochondrial localization (based on integrated proteomics, computation, and microscopy)

However, to date there has been few studies of ubiquitylated proteins that reside within the mitochondria and certainly none within primary cells.

Methods

In collaboration with the Wade Harper lab (Harvard, US), we performed a large-scale quantitative diGlycine (diGly) capture proteomics to identify Parkin-dependent ubiquitylation on lysine residues in proteins modified upon mitochondrial depolarization. Primary cortical neuron cultures derived from E16.5 C57BL/6J mouse embryos, cultured for 21 days *in vitro* and stimulated with 10uM Antimycin A and 1uM Oligomycin (A/O) for 5 hours to induce mitochondrial depolarisation and PINK1 activation. Membrane fractions were obtained that are *enriched for mitochondria* and analysed by mass spectrometry for both ubiquitylated peptides and the total proteome. A total of five biological replicates were carried out. ~8537 Kgg (Gly-Gly-modified lysine) peptides of which 5698 unique sites were identified in each of the unstimulated/basal condition and the stimulated/depolarized (A/O) condition. Of those 5698 unique sites, ~658 were statistically up-regulated and ~1253 sites were down-regulated upon A/O treatment.

Questions

1. Using our dataset from the primary neurons, we wish to analyse the landscape of ubiquitylated proteins that are mitochondrial **in the unstimulated basal conditions**. We are interested in understanding the following sub-questions

a. Based on the di-gly-gly data, the identity of ubiquitylated proteins in each compartment – matrix, inner membrane, intermembranous space and outer membrane
    i. Also are any E3 ligases (ie. Auto-ubiquitylated sites)
b. Based on the whole proteome the number and identity of ubiquitin E3 ligases in mitochondria.
c. Based on the whole proteome the number and identity of ubiquitin E3 ligases that are not mitochondrial.
d. Based on the whole proteome, the number and identity of ubiquitin E2 enzymes in mitochondria

2. The Harper lab have also studied mitochondrial ubiquitylation in HeLa cells, HCT116 cells and SH-SY5Y cells – would it be possible to compare the basal (unstimulated) mitochondrial ubiquitylome in neurons with that of these cell lines https://www.nature.com/articles/nature12043
3. The Harper lab have also studied mitochondrial ubiquitylation in human induced neurons – would it be possible to compare the basal (unstimulated) mitochondrial ubiquitylome in neurons with that of these cell lines https://www.sciencedirect.com/science/article/pii/S1097276519308421