Final Report

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Title of Project: *Mechanisms to augment mitophagy as treatment for Parkinson's disease*

Summary: (approximately 1,000 words)

Parkinson's disease (PD) is an incurable disease characterised by progressive motor system dysfunction. PD affects 1% of the population over the age of 60 (~110,000 patients in Australia and more than 10 million patients world-wide). Currently, there are no treatments that cure or slow disease progression. The total economic cost of PD is enormous, and it is expected to result in an increasing burden on society as the population ages.

The cause of Parkinson's disease is not fully understood. Rare, inherited Parkinson's disease can be caused by mutations in proteins, Pink1 and Parkin, that regulate the removal of mitochondria from cells in a process known as mitophagy. There has been a great deal of research into the development of Parkinson's disease therapies designed to reactivate mutant Parkin through the use of small molecules.

The aim of this project is to set the stage for the development of strategies to re-activate the mitophagy pathway in cells. We have discovered a regulatory pathway that keeps mitophagy levels low in cells. This could lead to the development of activators of mitophagy which could serve as treatments for Parkinson's disease.

Hypothesis vs Findings

The hypothesis we tested was: **Mitophagy is induced by preventing the degradation of mitophagy receptors located on the mitochondrial outer membrane.** If proven correct, the mechanisms leading to the stabilisation of mitophagy receptors represent potential interventions to upregulate mitophagy in diseases, such as Parkinson's, in which mitophagy is deficient.

We tested this hypothesis by testing cellular mitophagy in three conditions:

- Chemical inhibition of mitophagy receptor degradation, using an inhibitor that prevents E3 ubiquitin ligase function.
- Inhibition of mitophagy receptor degradation by siRNA mediated depletion of SCF ubiquitin ligase function
- Generation of mutant mitophagy receptors that are constitutively stabilised in cells (i.e. expressing non-degradable mutants in cells).

As hypothesised, we found that mitophagy was indeed induced by inhibition of Cullin-RING ligase function, and by expressing mutations in the degron region of the mitophagy receptors that prevent their degradation. Thus, stabilisation of mitophagy receptors can induce mitophagy in cells.

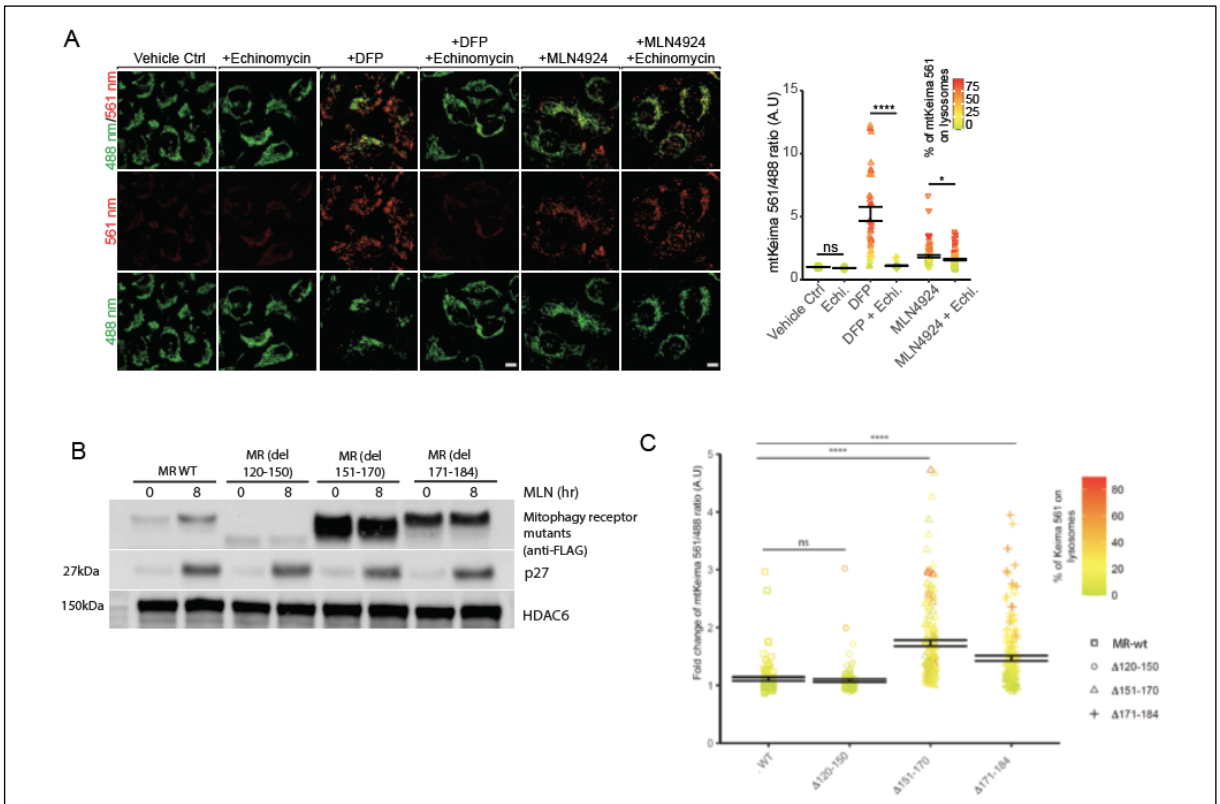

Figure 1. A) Cullin-RING ligase(s) oppose mitophagy in cells. Establishment of mitophagy assay using mtKeima pH sensitive reporter. Cells treated with DFP (a HIF activator) display robust mitophagy which is eliminated after inhibition of HIF1 transcription factor using Echinomycin (a HIF1 inhibitor). MLN4924 (a Cullin-RING E3 ligase inhibitor) also induces mitophagy but in a HIF1-independent manner. Mitophagy is represented as the ratio of red 561 nm pixels divided by the green 488 pixels in each cell. B) Generation of non-degradable mitophagy receptor cell lines. To identify amino acid sequences conferring instability to the mitophagy receptor (MR), cells inducibly-expressing FLAG-tagged deletion mutants of MR were treated with MLN4924. MR(del151-184) was significantly increased compared with wild-type and no longer responsive to MLN4924, suggesting that the degron region in MR resides between amino acid 151-184 of MR. C) mtKeima assay in cells induced to express non-degradable MR. The levels of mitophagy correlate with the level of MR within the cell.

Unanswered Questions

We are interested in identifying any upstream signalling pathways that control mitophagy receptor degradation (e.g. kinases and phosphatases). We are also interested in understanding more about the long-term effects of stabilisation of mitophagy receptors.

What these research outcomes mean

Our research sets the stage for development of activators of mitophagy which could serve as treatments for Parkinson's disease.

Please include any appropriate photos or diagrams.

Please submit this report as a PDF using the following naming convention:

Lastname Firstname – Simplified Project Title

For example: Smith Jane – The anatomy of the Brain.PDF