Mitophagy is increased in the OPA1^{Q285STOP}/RedMIT/GFP-LC3 mouse: a model of Dominant Optic Atrophy for identifying drug modulators of mitophagy

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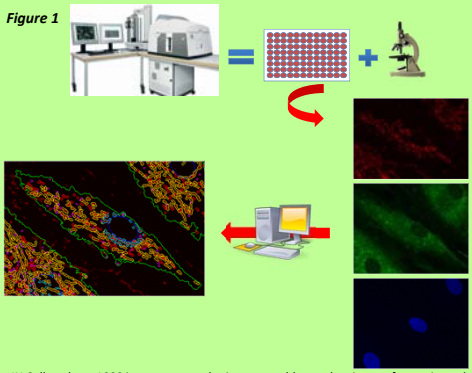
Mitophagy (recycling of damaged mitochondria) is central to the pathogenesis of Parkinson's disease and is likely to be an important determinant of mitochondrial disease severity. It occurs when a LC3-positive membrane structure, the phagophore, encloses mitochondrial fragments, resulting in a double-membrane autophagosome that fuses with a lysosome to become an autolysosome able to degrade its content.
Dominant optic atrophy (DOA) is the most common inherited optic neuropathy (prevalence 1:12 000), largely caused by mutations in OPA1. This gene encodes a ubiquitous protein involved in mitochondrial dynamics. Some of these patients develop a severe form of this disorder, known as DOA plus, with additional symptoms including deafness, peripheral neuropathies and occasionally Parkinson's disease. We previously showed that mitophagy is increased in patients with severe DOA plus.
In the *OPA1^{Q285STOP}* mouse model of DOA, loss of vision is accompanied by dendronopathy in retinal ganglion cells (RGCs), electron microscopy shows signs of autophagy and aberrant cristae that are consistent with, but not definitive evidence of, increased mitophagy. We crossed these mice with our RedMIT/GFP-LC3 mouse and then used high throughput imaging to quantitate mitophagy in mouse embryonic fibroblasts (MEFs) and splenocytes from this RedMIT/GFP-LC3/OPA1^{Q285STOP} mouse.

IN Cell system to quantify autophagy/mitophagy

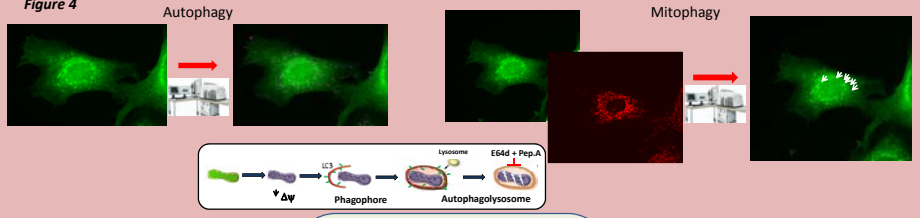
Figure 1

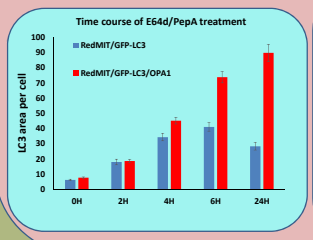


The IN Cell analyzer 1000 is an automated microscope able to take pictures from microculture plates (up to 384-well plate) in 3 different fluorescent channels. Using the developer toolbox software we are able to segment the cells, mitochondria, autophagosomes and then investigate the co-localisation between mitochondria and autophagosome and various parameters for each segmented target (length, area, number).

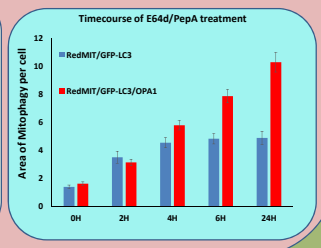
Increased mitophagy in RedMIT/GFP-LC3/OPA1^{Q285STOP}

Figure 4



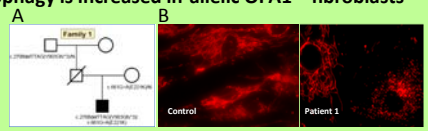


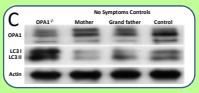
To investigate mitophagy in a DOA model, we crossed the OPA1^{Q285STOP} mouse with our RedMIT/GFP-LC3 mouse harbouring red fluorescent mitochondria and green fluorescent autophagosomes. Using IN Cell 1000 we showed increased co-localisation between mitochondria and autophagosomes and the OPA1Q285STOP/RedMIT/GFP-LC3 MEFs. Using lysosomal inhibitors E64D and pepstatin A, we showed that the increased number of autophagosomes was due to increased accumulation in the OPA1^{Q285STOP}/RedMIT/GFP-LC3 mice compared to the control (OPA1wt/RedMIT/GFP-LC3).

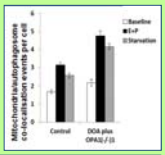


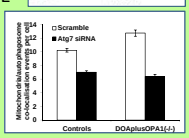
Mitophagy is increased in-allelic OPA1$^{-/-}$ fibroblasts

Figure 2







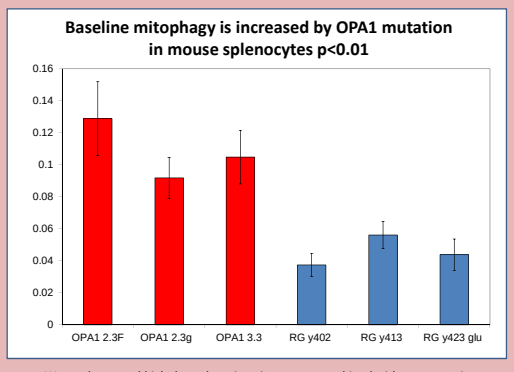


A) We identified two OPA1 mutations in a proband with a severe phenotype; bi-allelic "dominant" inherited optic atrophy plus ("DOA plus", phenotype). B) The OPA1 protein is essential for normal mitochondrial fusion, hence mitochondria were more fragmented in patient fibroblasts than controls. C) OPA1 protein was substantially less abundant in fibroblasts from the proband than controls or his transmitting relatives (right panel). These cells had abundant LC3-II compared to control. D) Mitophagy is characterised by co-localisation of mitochondria with the autophagosomes that engulf damaged mitochondrial fragments. We quantified autophagosomes co-localising with mitochondria in fibroblasts, using IN Cell 1000. This high through-put microscopy system showed increased autophagosomes and co-localisation of mitochondria with autophagosomes at baseline. Co-localising puncta were further increased by exposure to known modulators of mitophagy, (lysosomal inhibitors E64D and pepstatin A (E+P) and after autophagy was induced by starvation, suggesting increased mitophagic flux in fibroblasts from bi-allelic DOA plus patients compared to controls. E) Genetic knock-down of mitophagy protein ATG7 eliminated the differences between bi-allelic OPA1(-/-) fibroblasts and controls, that we attributed to mitophagy.

Increased mitophagy in RedMIT/GFP-LC3/OPA1^{Q285STOP}

Figure 5

Splenocytes analysed with Imagestream



We used a second high throughput imaging system combined with cytometry in a single platform, Imagestream (Amnis) to analyse splenocytes from this cross. The bar chart shows that there was significantly increased co-localisation between mitochondria and autophagosomes in three OPA1^{Q285STOP}/RedMIT/GFP-LC3 mice (red bars) compared to three control (blue bars) p<0.01.

Conclusion and Future Directions

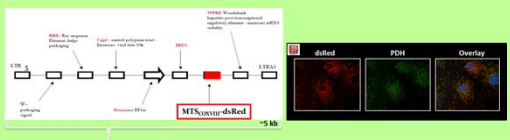
Mitophagy is rapid and occurs infrequently, hence is hard to document in either cells or whole animals. To overcome these difficulties we developed two high through-put methods for imaging co-localisation of mitochondria with autophagosomes, IN Cell 1000 (GE Healthcare, figure 1) and ImageStream (Amnis, figure 5).

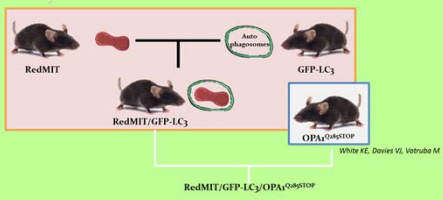
The RedMIT/GFP-LC3 mouse (figure 3) enables us to visualise fluorescently labelled mitochondria. We validated the utility of this model by crossing with a model of OPA1 haploinsufficiency and hence activated mitophagy, to generate OPA1^{Q285STOP}/RedMIT/GFP-LC3 mice. We used IN Cell 1000 and ImageStream to demonstrate increased mitophagy in MEFS and splenocytes (figures 4 and 5 respectively). We are now quantifying mitophagy in retinal ganglion cells, the cell type targeted in this disease.

Future work will determine whether increased mitophagy is causally related to neurodegeneration in DOA plus due to OPA1. We anticipate that the RedMIT/GFP-LC3 mouse will be equally useful for quantifying mitophagy in dopaminergic neurons, which will address important questions in Parkinsons research. For instance this model will be useful for screening drugs to identify modulators of mitophagy.

The RedMIT/GFP-LC3 mouse

Figure 3

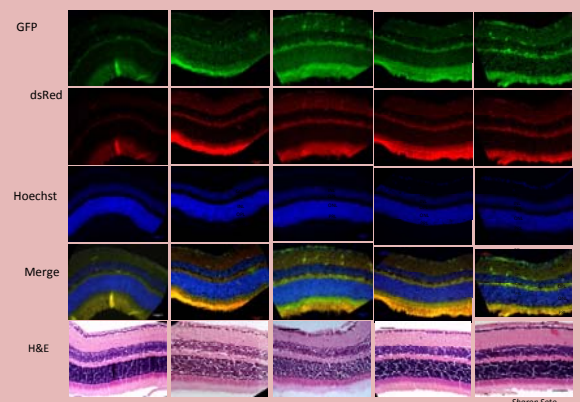




Top panel: The 5kb insert used to transform our RedMIT mouse. Ds-red was targeted to mitochondria using a COX VIII leader sequence, histochemistry using a co-localising antibody to mitochondrial matrix enzyme, pyruvate dehydrogenase (PDH), confirms correct targeting in mouse embryonic fibroblasts (MEFs).
Bottom Panel: Crossing the RedMIT mouse with the GFP-LC3 mouse enables us to visualise mitophagy, i.e. red mitochondria in green autophagic vesicles.

Investigating mitophagy in the retina

Figure 6



In the OPA1^{Q285STOP} mouse model, autophagy is dysregulated in retinal ganglion cells (RGCs). RGCs from these mice with fluorescently labelled organelles are under investigation to confirm that increased mitophagy accompanies the development of the disease.

Methods: Whole globe was fixed in 4% PFA , sucrose protected and embedded in OCT (Leica) embedding medium. 8um thick sections were placed on electrostatic charged microscope slides (Fisher), and air dried in the dark for 4 hours at room temperature. Sections were rehydrated in PBS pH 7.4 for 4 minutes, and Hoechst nuclear stain applied for 10 minutes at 1:1000 dilution. Sections were rinsed in PBS and washed for 3x 5 minutes with gentle agitation in a darkened slide chamber. Sections mounted in prolong gold mounting medium and stored at 4°C.