A whole genome CRISPRi mitophagy screen for regulators of mitochondrial protein import quality control

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Project description:
Mitophagy is a highly selective form of autophagy (cellular self-eating) for the removal of damaged or redundant mitochondria via lysosomal degradation. It is an essential facet of the cellular response to physiological and environmental stress, and consequently, failures in mitophagy regulation are implicated in numerous human diseases (including neurodegenerative diseases) and contribute to the ageing process. Over recent years, our understanding of the molecules and pathways that coordinate mitophagy in mammalian cells has advanced through cell-based studies using drugs and overexpressed proteins; however, we still lack essential knowledge of the regulatory pathways that initiate and control mitophagy during (patho)physiological mitochondrial stress, such as mitochondrial protein import arrest. Understanding these pathways could lead to treatments that boost mitochondrial quality control, and thus mitochondrial population fitness, for sustained cell and tissue health across the lifespan.

This project will bridge this crucial knowledge gap by screening for individual proteins and associated pathways that coordinate the mitophagy response specifically during mitochondrial protein import arrest. Mitophagy reporter cell-lines will be adapted as platforms for CRISPRi (CRISPR interference)-based screening. These cells will be treated with chemical and/or genetic mitochondrial protein import blockers, and cells with impaired or elevated mitophagy responses will be isolated by fluorescence-activated cell sorting (FACs). Guide RNAs targeting influential genes will then be identified and categorised by next generation sequencing and bioinformatics. Mechanistic studies will then be carried out in whole cells and using isolated mitochondria to determine the roles of important individual proteins and their pathways acting in the cellular response to mitochondrial protein import arrest.

Multidisciplinary training will be provided in key areas, including: state-of-the-art widefield and confocal microscopy, including automated high content analysis; mitochondrial isolation from mammalian cells for in vitro protein import assays; CRISPRi assay design, including molecular cloning and lentivirus production; bioinformatics and large data set analytical methods; mechanistic cell biology.