Loss of *Usp15* leads to mitochondrial abnormalities

Kseniia Sevostianova（筑波大学 生物学類）　　指導教員：Fuminori Tsuruta（筑波大学 生命環境系）

Introduction

The cerebral cortex is organised into 6 horizontal layers that are characterised by varying cell populations and density. Layer V (L5) consists of large pyramidal neurons. The long-projecting axons of these neurons travel to the spinal cord forming the corticospinal tract, which is the main pathway for voluntary motor control. In turn, axon degeneration is one of the features of motor and neurodegenerative disorders. Recent evidence suggests that the degeneration can be triggered by mitochondrial dysfunction.

In this study, I assess the role of ubiquitin-specific protease 15 (USP15), a deubiquinating enzyme (DUB) from the USP subfamily. *USP15* is a relevant gene in several motor and neurodegenerative disorders. Prior research demonstrates that the knock-out (KO) of *USP15* in mice leads to behavioural (impaired sociability and instincts) and physiological abnormalities (thinning of L5 in the cerebral cortex, hippocampus shrinkage). However, the molecular mechanisms of these changes are unknown.

USP15 is a known antagonist of Parkin and a mitophagy regulator, which makes USP15-mediated mitochondrial regulation a potential factor in the progression of neurodegeneration. I investigated the influence of USP15 on mitochondria under basal and stressful conditions. I found that the mitochondria in *USP15* KO MEFs have a distinct phenotype and conduct clearance of damaged mitochondria more efficiently.

Material & Methods

Cell culture and transfection: Wild type (WT) and *USP15* KO mouse embryonic fibroblasts (MEFs) were cultured in Dulbecco's modified Eagle's medium (high glucose) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin-glutamine in a 37°C incubator with 5% CO_2. Cells were plated at the density of 3.0 x 10^4 in 3 ml per well and incubated for 1 day. Cells were transfected with the pEYFP-Mito and/or Myc-USP15 plasmids using appropriate amounts of polyethyleneimine MAX and Opti-MEM according to the manufacturer's instructions.

Immunocytochemistry: Cells were fixed 24 hours after transfection with 4% paraformaldehyde in PBS, blocked in 0.4% Triton X-100 in blocking solution (5% BSA in PBS), and incubated with primary antibodies overnight at 4°C. After washing with PBS, cells were incubated with secondary antibodies for 30 min at room temperature. The coverslips were mounted onto slides, and fluorescence images were obtained using a confocal microscope and analysed by FIJI ImageJ software.

Rotenone treatment: Cells were incubated for 1 day after transfection and treated with rotenone at the final concentrations of 100, 250, 500, and 1000 nm for 4 or 8 hours before fixation.

Results

1. Loss of *USP15* changes mitochondrial morphology in MEFs

To assess the morphology of mitochondria in WT and *USP15* KO MEFs, I transfected pEYFP-Mito into MEFs and quantified relevant mitochondrial parameters. The mean area of all mitochondria in a cell was 19% lower, and the mean perimeter was 15% lower in KO cells on average (p = 0.003). The mitochondria in KO cells exhibited less branching. The mean form factor and aspect ratio indicated more elongated and fibrous organelles in WT cells as opposed to more circular ones in KO cells. Overall, mitochondria in *USP15* KO MEFs exhibited predominantly round morphology.

2. Loss of *USP15* promotes a more effective clearance of damaged mitochondria under stressful conditions

To investigate the influence of USP15 on mitophagy under stressful conditions, I treated pEYFP-Mito transfected cells with rotenone, a respiration inhibitor. In both 4- and 8-hour treatment groups, the mitochondria in KO cells displayed significantly fewer fluorescent signals than in WT cells at rotenone concentrations of 250 nm and above. To confirm the role of USP15 in regulating mitophagy, I conducted a rescue experiment cotransfecting KO cells with pEYFP-Mito and USP-Myc. After 4 hours of rotenone treatment, the mitochondrial area was larger in cotransfected cells even at the 500 nm rotenone concentration. These data suggest that loss of *USP15* can enhance mitophagy in response to stressful conditions.

Discussion

USP15 KO MEFs have a distinct mitochondrial phenotype, possibly implying a shift in the fission/fusion balance towards fission with mitochondria taking a shorter, more circular shape. Enhanced mitophagy in KO cells induced by mitochondrial stressors was also observed for other DUBs of the same family, such as USP30. Emerging evidence of functional differences of mitochondria in axons gives more grounds for a claim of USP15 - mitochondria interactions playing a substantial role in related disorders. Taken together, my results suggest that USP15-mediated changes in mitochondria regulation make USP15 a potential therapeutical target.