AUTOPHAGY
2020, VOL. 16, NO. 6, 1159–1161
https://doi.org/10.1080/15548627.2020.1753927

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AUTOPHAGIC PUNCTUM

Methylmalonyl acidemia: from mitochondrial metabolism to defective mitophagy and disease

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ABSTRACT

Methylmalonic acidemia (MMA) is an autosomal recessive inborn error of metabolism due to the deficiency of mitochondrial MMUT (methylmalonyl-CoA mutase) – an enzyme that mediates the cellular breakdown of certain amino acids and lipids. The loss of MMUT leads to the accumulation of toxic organic acids causing severe organ dysfunctions and life-threatening complications. The mechanisms linking MMUT deficiency, mitochondrial alterations and cell toxicity remain uncharacterized. Using cell and animal-based models, we recently unveiled that MMUT deficiency impedes the PINK1-induced translocation of PRKN/Parkin to MMA-damaged mitochondria, thereby halting their delivery and subsequent degradation by macroautophagy/autophagy-lysosome systems. In turn, this defective mitophagy process instigates the accumulation of dysfunctional mitochondria that spark epithelial distress and tissue damage. Correction of PINK1-directed mitophagy defects or mitochondrial dysfunctions rescues epithelial distress in MMA cells and alleviates disease-relevant phenotypes in *mmut*–deficient zebrafish. Our findings suggest a link between primary MMUT deficiency and diseased mitochondria, mitophagy dysfunction and cell distress, offering potential therapeutic perspectives for MMA and other metabolic diseases.

ARTICLE HISTORY
Received 1 April 2020
Revised 4 April 2020
Accepted 6 April 2020

KEYWORDS
Cell damage; inherited metabolic disorders; kidney tubule; metabolism; mitochondria; mitophagy; oxidative stress

Cellular organelles called mitochondria are key to maintaining energy metabolism and physiological homeostasis, and their dysregulation drives metabolic dysfunction and disease. Methylmalonic acidemia (MMA; MIM #251000) – the most common form of organic aciduria – is caused by recessive, inactivating mutations in the *MMUT* gene encoding the mitochondrial enzyme methylmalonyl-CoA mutase that mediates the terminal step of branched-chain amino acid and certain lipid metabolism. Complete ($MMUT^0$) or partial ($MMUT^-$) loss of the enzyme MMUT leads to the accumulation of toxic organic acids (*e.g.*, methylmalonic acid, propionic acid and 2-methylcitric acid) within the mitochondrial matrix, triggering structural and functional abnormalities in the mitochondrial network that drive severe organ dysfunction affecting primarily the brain, liver and kidney. The mechanisms linking MMUT deficiency, mitochondrial distress and cell toxicity remain unclear, restricting treatment for this life-threatening disorder to supportive care.

Studies using kidney tubular cells derived from the urine of patients with MMA have revealed that MMUT deficiency is causing accumulation of damaged and dysfunctional mitochondria, triggering the generation of a large amount of reactive oxygen species (ROS) and cell distress. These dysfunctions are associated with an exaggerated production of LCN2 (lipocalin 2), a small iron-transporting protein largely produced by kidney tubular cells following cellular damage. Such modifications were confirmed in mouse kidney tubule cells following conditional inactivation of *Mmut* and in the kidney and liver of a zebrafish mutant line lacking *mmut*. Likewise, morphologically abnormal mitochondria were described in the kidney tubules and explanted livers of patients with MMA, suggesting an involvement of mitochondrial quality control and surveillance systems in the disease.

Quality control of worn out organelles, including mitochondria, is regulated by autophagy, an evolutionarily conserved, homeostatic process that catabolizes aged and/or malfunctioning cellular constituents confined within a double-membrane structure called an autophagosome. As autophagy safeguards the mitochondrial quality control, we hypothesized that mitochondrial abnormalities in MMA-affected kidney cells might reflect changes in the autophagy pathway. Using control and MMA cells, we measured autophagy by detecting the conversion of the non-lipidated form of LC3-I to the lipidated, autophagosome-associated form LC3-II through immunoblotting and/or by quantifying the numbers of punctate $LC3^+$ vesicles through confocal microscopy and/or the abundance of electron microscopy (EM)-structures compatible with autophagic vacuoles. Compared to control cells, we detected in MMA cells an elevated conversion of LC3-I to LC3-II and higher numbers of punctate LC3-positive structures, and more EM structures compatible with autophagic vacuoles. Treatment with bafilomycin A_1 – a vacuolar-type H^+-translocating ATPase inhibitor that blocks the cellular degradation of autophagosomes – further heightens the already increased steady-levels of LC3-II and the number of punctate $LC3^+$ structures, indicating a *bona fide* increase in autophagy flux. Furthermore, upstream signaling cascades

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that regulate autophagy machinery, such as MTORC1 and ULK1 complexes, are significantly downregulated and upregulated, respectively, supporting the link between MMUT deficiency and induction of autophagy.

The question remained how MMUT deficiency might impair the function and homeostasis of the mitochondrial network. Prolonged or unrepairable damage can lead to elimination of mitochondria through a selective autophagic process termed mitophagy. In this pathway, mitochondrial damage activates the mitochondria-associated kinase PINK1, which recruits and activates PRKN's E3 ubiquitin ligase activity, driving signaling cascades that mark damaged mitochondria for their engulfment and degradation within lysosomes. Considering the accumulation of damaged mitochondrial and autophagic vesicles, we reasoned that MMUT deficiency might compromise the PINK1-PRKN-directed priming of MMA stressed mitochondria to autophagic-lysosomal degradation. To test this hypothesis, we treated both control and MMA cells with rotenone to damage mitochondria and selectively activate their mitophagy-mediated degradation. Using the translocation of PRKN to damaged mitochondria as a *bona fide* reporter of PINK1-PRKN-dependent priming mechanisms, we noted that MMA cells display a decrease in numbers of $PRKN^{+}$ clusters and translocation of PRKN to damaged mitochondria, in both normal and mitophagy-induced conditions. The faulty execution of PINK1-PRKN-mediated priming programs halts the delivery of MMA stressed mitochondria to autophagy-lysosome degradation

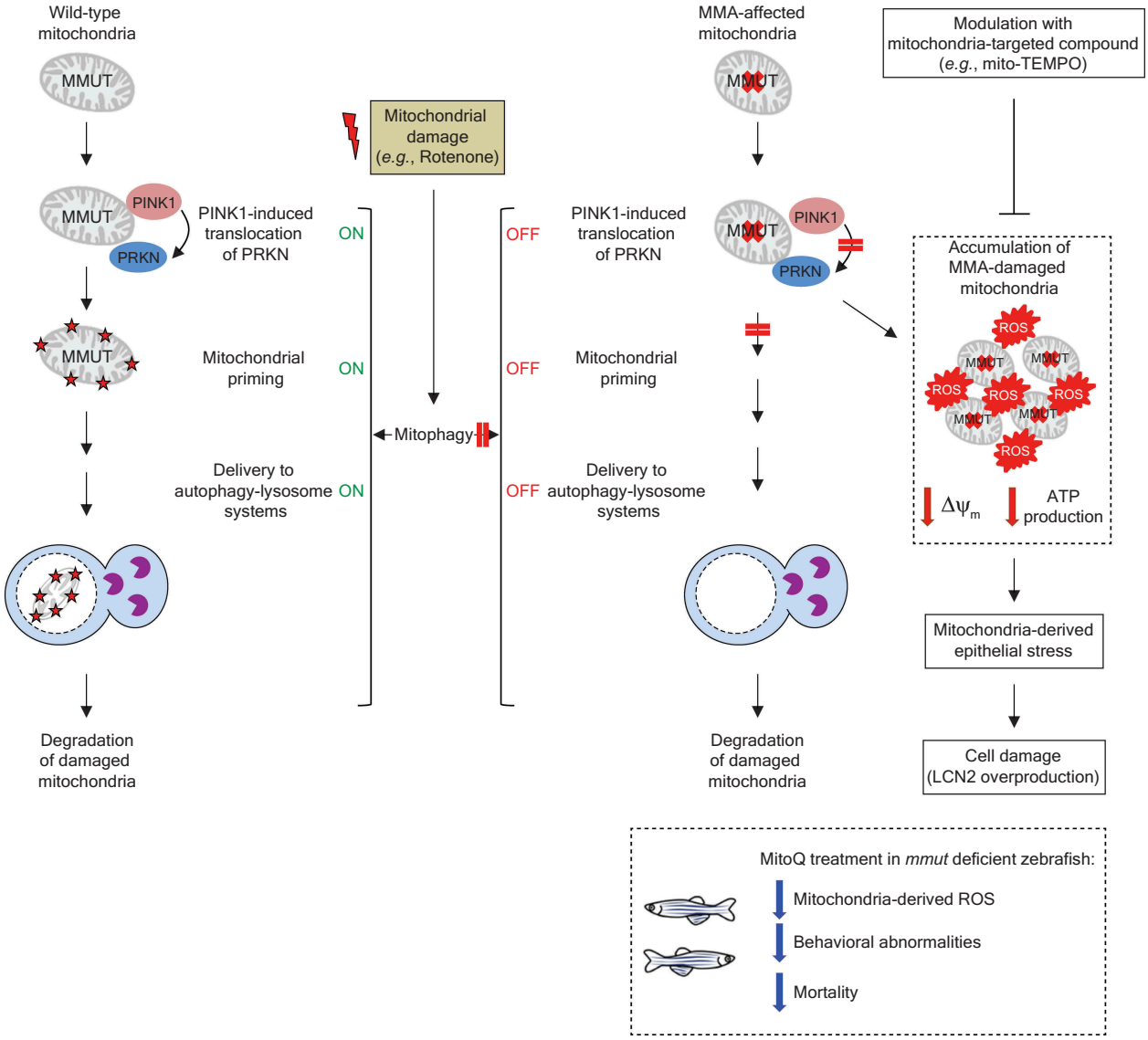

Figure 1. Model depicting the link between MMUT deficiency, mitochondrial alterations and epithelial stress in methylmalonyl acidemia. In wild-type kidney cells (left), mitochondrial stress (*e.g.*, treatment with rotenone) stimulates PINK1-induced translocation of PRKN to damaged mitochondria. This triggers mitophagy and the subsequent disposal of dysfunctional mitochondria through autophagy-lysosome degradation systems. By contrast, in MMA-affected kidney cells (right), the faulty execution of PINK-PRKN-mediated mitophagy prevents the delivery of diseased mitochondria to autophagy-lysosome pathways. This leads to the accumulation of MMA-damaged mitochondria characterized by collapsed mitochondrial membrane potential ($\Delta\psi_m$), abnormal bioenergetics profiling, and increased mitochondrial ROS. The resulting mitochondrial defects generate epithelial stress, ultimately causing cell damage (LCN2 overproduction). Treatment with mitochondria-targeted antioxidants (*e.g.*, mito-TEMPO or MitoQ) repairs mitochondrial dysfunctions, neutralizes epithelial stress and cell damage in MMA cells, and improves disease-relevant phenotypes in *mmut*-deficient zebrafish.

systems, as detected by the sensitive mt-Keima imaging-based assay and electron microscopy. These cellular defects compromise the clearance of MMA-diseased mitochondria, ultimately triggering a level of mitochondrial dysfunction that causes cellular distress and damage. Restoring mitophagy-mediated degradation through gain-of-function interventions targeting *PINK1* improves the function of the mitochondrial network and homeostasis in mutant kidney cells, averting mitochondria-derived epithelial stress and cell damage. Furthermore, restoring mitochondrial function and homeostasis with mitochondria-targeted antioxidants, which are clinically tested in a variety of diseases, hampers epithelial distress and damage in MMA cells, and ameliorates disease-relevant phenotypes in *mmut*-deficient zebrafish [1].

In conclusion, we identified a pathway that links a primary deficiency of a mitochondrial enzyme with dysfunction of mitophagy, accumulation of damaged mitochondria, and epithelial distress (Figure 1). These findings reveal a heretofore undescribed role of the enzyme MMUT – beyond its function in cellular metabolism – in maintaining mitochondrial quality control and hence the homeostasis of specialized epithelial cells. These insights offer promising therapeutic avenues for repairing mitochondria in MMA and in other mitochondrial diseases.

Disclosure statement

The authors declare no competing interest.

Funding

We are grateful to the European Reference Network for Rare Kidney Diseases (ERKNet) – project ID N° 739532; the Cystinosis Research Foundation (Irvine, CA, USA); the Swiss National Science Foundation (310030_189044); the clinical research priority programs (KFSP) radiz (Rare Disease Initiative Zurich) and Molecular Imaging Network Zurich (MINZ) at the University of Zurich; the Swiss National Centre of Competence in Research (NCCR) Kidney Control of Homeostasis (Kidney.CH) for support and Junior Grant (to A.L.) and the Wolfermann–Nägeli Shiftung.

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Reference

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