AUTOPHAGY
2023, VOL. 19, NO. 1, 363–364
https://doi.org/10.1080/15548627.2022.2077552

Taylor & Francis
Taylor & Francis Group

AUTOPHAGIC PUNCTUM

SQSTM1 and its MAP1LC3B-binding domain induce forced mitophagy to degrade mitochondrial carryover during mitochondrial replacement therapy

Xiao-Yan Fan [a], Shen Yin [b], and Shi-Ming Luo [a]

[a]Fertility Preservation Lab, Guangdong-Hong Kong Metabolism & Reproduction Joint Laboratory, Reproductive Medicine Center, Guangdong Second Provincial General Hospital, Guangzhou, China; [b]College of Life Sciences, Key Laboratory of Animal Reproduction and Biotechnology in Universities of Shandong, Qingdao Agricultural University, Qingdao, China

ABSTRACT

Mitophagy is a process that selectively degrades mitochondria in cells, and it involves a series of signaling events. Our recent paper shows that the ectopic expression of SQSTM1 and its MAP1LC3B-binding domain (Binding) at the mitochondrial outer membrane, can directly cause mitophagy. To distinguish this mitophagy from others, we called it forced mitophagy. Further results show that the forced mitophagy can degrade half of the mitochondria and their DNA in HeLa cells and mouse embryos. Meanwhile, there are no apparent effects on mitochondrial membrane potential (MMP), reactive oxygen species (ROS), mitosis and embryo development. Thus, the forced mitophagy was examined to selectively degrade mitochondrial carryover in the nuclear donor embryos' mitochondria by pre-labeling with Binding before mitochondrial replacement therapy (MRT). The results show that the forced mitophagy can reduce mitochondrial carryover from an average of 4% to 0.09% compared to the controls in mouse embryos and tissues. In addition, the offspring from MRT mice show negligible effects on growth, reproduction, exercise and behavior. Furthermore, results from human tri-pronuclear embryos show that the forced mitophagy results in undetectable mitochondrial carryover in 77% of embryos following MRT. Therefore, forced mitophagy is efficient and safe for degrading mitochondrial carryover in MRT.

ARTICLE HISTORY
Received 3 May 2022
Revised 9 May 2022
Accepted 9 May 2022

KEYWORDS
Forced mitophagy; mitochondrial carryover; mitochondrial replacement therapy; NIX; SQSTM1

Mutations in mitochondrial DNA (mtDNA) can lead to mitochondrial diseases for which there is no effective treatment. However, considering that the mutated mtDNA is maternally transmitted, mitochondrial replacement therapy (MRT) is thought to be effective in blocking mitochondrial-related diseases. But MRT itself is a nuclear transfer in nature and thus inevitably causes mtDNA carryover, which may continue to transmit mitochondrial diseases due to enrichment and genetic drift. Thereby, how to eliminate the mtDNA carryover is a crucial issue for MRT.

Mitophagy is the process of selectively degrading mitochondria in cells. Therefore, if we pre-label the mitochondria of nuclear donor embryos with autophagy receptors, the targeted mitophagy will effectively degrade the prelabeled mtDNA carryover of MRT.

To label the mitochondria from nuclear donor embryos, we first constructed a plasmid that expresses a protein including a signal-anchored transmembrane peptide from CISD1 (CDGSH iron sulfur domain 1), a fluorescent protein and an autophagy receptor in this order starting at the N terminus [1]. The signal peptides can bring autophagy receptors to the mitochondrial outer membrane and leave them facing the cytoplasm; fluorescent proteins make mitochondria visible under a fluorescence microscope, and the autophagy receptors recruit MAP1LC3/LC3 (microtubule associated protein 1 light chain 3) proteins as part of phagophores to enwrap the mitochondria and form autophagosomes.

We initially tested the BNIP3L/NIX protein as an autophagy receptor (CISD1-RFP-BNIP3L/NIX). Although it localizes to mitochondria, the results showed that it fails to recruit LC3 proteins to form autophagosomes. Next, we tested the SQSTM1 protein as the autophagy receptor (CISD1-RFP-SQSTM1). The results indicated that the CISD1-RFP-SQSTM1 successfully recruits the LC3 proteins and induces a fusion between the mitochondria and lysosomes. Furthermore, by examining the mtDNA content of single cells with real-time PCR, we confirmed that CISD1-RFP-SQSTM1 can reduce mtDNA to half that of control cells. These results indicate that the CISD1-RFP-SQSTM1 can successfully induce mitophagy in HeLa cells. However, we found that SQSTM1 causes mitochondria to cluster around the nucleus, thus possibly bringing excess mitochondria to the nuclear recipient embryos in MRT.

To overcome SQSTM1-induced mitochondrial aggregation, we optimized the plasmid by replacing SQSTM1 with its MAP1LC3B-binding domain (CISD1-RFP-Binding). The results showed that CISD1-RFP-Binding can effectively induce forced mitophagy similar to the CISD1-RFP-SQSTM1 but with less mitochondrial aggregation. Therefore, CISD1-RFP-Binding is more suitable for MRT than CISD1-RFP-SQSTM1.

CONTACT Shen Yin syin@qau.edu.cn College of Life Sciences, Key Laboratory of Animal Reproduction and Biotechnology in Universities of Shandong, Qingdao Agricultural University, Qingdao 266109, Shandong, China; Shi-Ming Luo luoshm@gd2h.org.cn Fertility Preservation Lab, Guangdong-Hong Kong Metabolism & Reproduction Joint Laboratory, Reproductive Medicine Center, Guangdong Second Provincial General Hospital, Guangzhou 510317, Hong Kong

In addition to efficacy, the safety of forced mitophagy is another issue that needs to be confirmed. Therefore, we examined mitochondrial membrane potential (MMP), reactive oxygen species (ROS) production, and nuclear morphology in HeLa cells transfected with CISD1-RFP-SQSTM1 and CISD1-RFP-Binding. The results showed that neither CISD1-RFP-SQSTM1 nor CISD1-RFP-Binding have apparent effects on MMP, ROS and nuclear morphology. Interestingly, some positive CISD1-RFP-SQSTM1 and CISD1-RFP-Binding cells exhibit mitosis even 72 h after transfection. Furthermore, by microinjecting mRNA rather than transfecting plasmid, we shortened the expression time of CISD1-RFP-Binding and found some nascent tubular mitochondria similar to those in control cells. These results suggest that the reduction of MMPs and ROS increase are not involved in forced mitophagy. Thus, forced mitophagy is safe for cells to reduce mitochondrial numbers.

Due to the relative silencing of transcription in mature oocytes and zygotes, mRNAs are used instead of plasmids in all oocyte and embryo experiments. We microinjected mouse zygotes with mRNA encoding CISD1-RFP-Binding and found that 1.5 h after microinjection, CISD1-RFP-Binding localizes to mitochondria and recruits LC3 protein to enwrap nearly all mitochondria. Subsequently, the CISD1-RFP-Binding-labeled mitochondria begin to fuse with lysosomes in the 4-cell embryos and degrade half of the mitochondria at the morula stage. Furthermore, the developmental results indicated that the CISD1-RFP-Binding has little effect on blastocyst and offspring birth rates. Thus, the forced mitophagy mediated by CISD1-RFP-Binding is efficient and safe in mouse embryos.

To degrade the mtDNA carryover of MRT, we pre-labeled the mitochondria of nuclear donor embryos with CISD1-RFP-Binding before MRT. The results showed that MRT itself results in detectable mtDNA carryover in all samples, with an average rate of 3.89% and 4.56% in blastocysts and tissues. In contrast, when the MRT is combined with forced mitophagy, it results in undetectable mtDNA carryover in 70% of samples and reduces the mtDNA carryover to be 0.09% on average. Furthermore, the results from the safety examination indicated that the forced mitophagy used in MRT has little effect on the offspring's reproduction, growth and behavior.

The human tri-pronuclear embryo is clinically abandoned but useful for studying MRT. We used it to test the feasibility of forced mitophagy for MRT in humans. The results showed that CISD1-RFP-Binding can induce forced mitophagy and lead to mitochondria-lysosome fusion occurring at the morula stage. Utilizing forced mitophagy in MRT reduces the mtDNA detectable carryover from 100% to 23% while having no apparent effects on the embryo development.

In conclusion, we established a new method for reducing/eliminating mtDNA carryover from MRT using forced mitophagy and confirmed its efficacy and safety in mouse and human embryos. In addition to MRT, we suggest that by combining with other methods such as the tetracycline-inducible expression system, this method should be helpful for people to control the number of mitochondria in cells. Our study also found that forced mitophagy does not require or result in reduced MMPs and increased ROS, and LC3-wrapped mitochondria may have normal functions. All these combined results indicate that the forced mitophagy is distinct from non-forced mitophagy, and thus, future studies should focus on its mechanism, optimization and use.

Disclosure statement

No potential conflict of interest was reported by the author(s)

Funding

This study was supported by the National Natural Science Foundation of China (82071714) and the National Key Research and Development Program of China (2018YFC1004800).

ORCID

Xiao-Yan Fan http://orcid.org/0000-0003-4045-6136
Shen Yin http://orcid.org/0000-0003-4792-7885
Shi-Ming Luo http://orcid.org/0000-0002-8874-1510

Reference

[1] Fan XY, Guo L, Chen LN, et al. Reduction of mtDNA heteroplasmy in mitochondrial replacement therapy by inducing forced mitophagy. Nat Biomed Eng. 2022;6:339–350.