AUTOPHAGY
2022, VOL. 18, NO. 12, 3043–3045
https://doi.org/10.1080/15548627.2022.2069956

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AUTOPHAGIC PUNCTUM

Essential role of lysosomal Ca2+-mediated TFEB activation in mitophagy and functional adaptation of pancreatic β-cells to metabolic stress

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ABSTRACT

Although the role of pancreatic β-cell macroautophagy/autophagy is well known, that of β-cell mitophagy is unclear. We investigated the changes of lysosomal Ca^{2+} by mitochondrial or metabolic stress that can modulate TFEB activation and, additionally, the role of TFEB-induced mitophagy in β-cell function. Mitochondrial or metabolic stress induces mitophagy, which is mediated by lysosomal Ca^{2+} release, increased cytosolic [Ca^{2+}] and subsequent TFEB activation. Lysosomal Ca^{2+} release is replenished by ER→lysosome Ca^{2+} refilling through ER Ca^{2+} exit channels, which is important for the increase of cytosolic [Ca^{2+}] and mitophagy by mitochondria stressors. High-fat diet (HFD) feeding augments pancreatic β-cell mitophagy, probably as an adaptation to metabolic stress. HFD-induced increase ofβ-cell mitophagy is reduced by *tfeb* KO, leading to increased ROS and decreased mitochondrial complex activity or oxygen consumption in *tfeb*-KO islets. In *tfeb* Δβ-cell mice, HFD-induced glucose intolerance and β-cell dysfunction are aggravated. Expression of mitophagy receptor genes including *Optn* or *Calcoco2* is increased by mitochondrial or metabolic stressors in a TFEB-dependent manner, likely contributing to increased mitophagy. These results suggest that lysosomal Ca^{2+} release in conjunction with ER→lysosome Ca^{2+} refilling is important for TFEB activation and mitophagy induction, which contributes to pancreatic β-cell adaptation to metabolic stress.

ARTICLE HISTORY
Received 3 April 2022
Revised 21 April 2022
Accepted 21 April 2022

KEYWORDS
Ca^{2+}; lysosome; mitophagy; pancreatic β-cells; TFEB

Mitochondrial function is essential for insulin release and survival of pancreatic β-cells. It is also well known that autophagy is important in the maintenance of β-cell function and mitochondrial integrity because *atg7*-knockout (KO) pancreatic β-cells show reduced glucose-stimulated insulin release and mitochondrial abnormalities. However, the role and mechanism of mitophagy in pancreatic β-cells have been unclear. TFEB (transcription factor EB) acts as a master regulator of lysosome biogenesis or autophagy gene expression, and also play a role in mitophagy. In TFEB activation and mitophagy by mitochondrial stressors, lysosomal Ca^{2+} is likely to play a part, although the detailed mechanism is unclear.

We investigated the mechanism and functional role of mitophagy in pancreatic β-cells, focusing on TFEB and lysosomal Ca^{2+} release as a regulator of TFEB [1]. We found that mitochondrial stressors such as rotenone or oligomycin plus antimycin A (O/A) combination induce mitophagy detected as mito-Keima red puncta or colocalization between mRFP-LC3 puncta with TOMM20, a mitochondrial outer membrane protein. Mitochondrial stressors also induce TFEB nuclear translocation which is important for mitophagy, as *tfeb*-KO INS-1 insulinoma cells show significantly reduced mitophagy after mitochondrial stressor treatment. TFEB nuclear translocation indicating TFEB activation after mitochondrial stressor treatment is mediated by PPP3/calcineurin, the single most important phosphatase inducing TFEB dephosphorylation; a dominant-negative PPP3/calcineurin mutant inhibits TFEB nuclear translocation and mitophagy induction by mitochondrial stressors.

We observed that release of lysosomal Ca^{2+} occurs after mitochondrial stressor treatment, leading to increased cytosolic [Ca^{2+}], PPP3/calcineurin activation and TFEB activation (Figure 1). An important role of increased cytosolic [Ca^{2+}] in TFEB nuclear translocation is supported by abrogation of mitochondrial stress-induced TFEB activation and mitophagy by BAPTA-AM, a cell-permeable Ca^{2+} chelator. Lysosomal Ca^{2+} release is mediated by mitochondrial ROS-induced lysosomal Ca^{2+} channel activation (Figure 1), because MitoTempo scavenging mitochondrial ROS inhibits the increase of cytosolic [Ca^{2+}] and the decrease of lysosomal [Ca^{2+}] by mitochondria stressors. The lysosomal Ca^{2+} channel responsible for lysosomal Ca^{2+} release after mitochondrial stressors appears to be MCOLN1 because ML-SI3, an inhibitor of the MCOLN1 channel or *Mcoln1* knockdown downregulates stress-induced mitophagy.

We next studied ER Ca^{2+} because the lysosomal Ca^{2+} reservoir might not be enough to accomplish cellular events requiring lysosomal Ca^{2+} due to its small volume. To support full progression of these processes, ER Ca^{2+} might be necessary, as the ER is the largest intracellular Ca^{2+} reservoir. Indeed, employing the technique of simultaneous measurement of lysosomal [Ca^{2+}] and ER [Ca^{2+}], we observed that ER→lysosome Ca^{2+} refilling occurs after treatment with mitochondrial stressors (Figure 1). Furthermore, blockade

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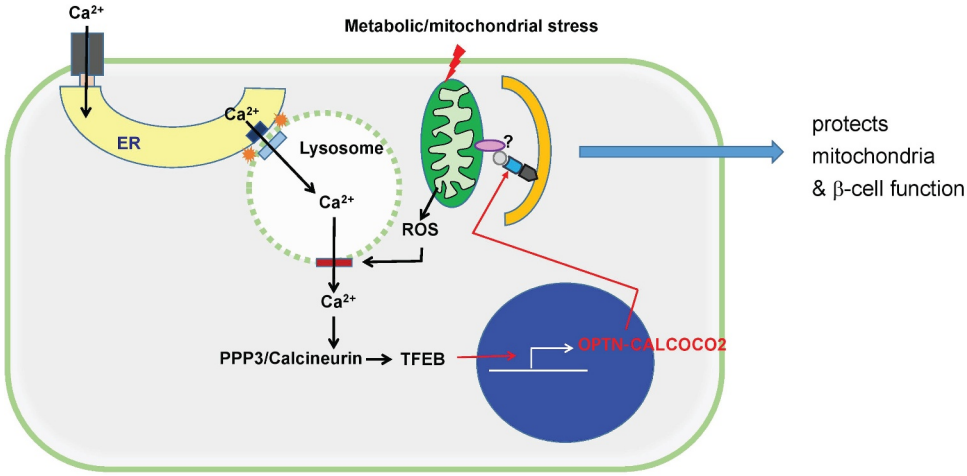

Figure 1. A schematic illustration of the mechanism of mitophagy induction in pancreatic β-cells by mitochondrial or metabolic stress. Mitochondrial or metabolic stress induces the generation of mitochondrial reactive oxygen species (ROS) that activate lysosomal Ca^{2+} exit channels such as MCOLN1 and consequently increases cytosolic [Ca^{2+}]. TFEB is activated through PPP3/calcineurin-mediated dephosphorylation, and then moves to the nuclei to induce expression of mitophagy receptor genes such as *Optn* or *Calcoco2*. Induction of mitophagy receptors could facilitate mitophagy through interaction with LC3 and mitophagy cargo, while the nature of the cargo is unclear. Lysosomal Ca^{2+} release is replenished by Ca^{2+} from the ER, the largest intracellular Ca^{2+} reservoir, which is facilitated by ER-lysosome contact (ER→lysosome Ca^{2+} refilling). ER Ca^{2+} depletion, in turn, activates SOCE of extracellular Ca^{2+}. Mitophagy induction by mitochondrial or metabolic stress helps maintain mitochondrial function and insulin release of pancreatic β-cells. (, OPTN/CALCOCO2; , LC3; , cargo; , ubiquitin; , ER-lysosome contact).

of ER→lysosome Ca^{2+} refilling using ER Ca^{2+} exit blockers such as xestospogin C, an ITPR/IP3R (inositol-1,4,5-phosphate receptor) antagonist or dantrolene, an RYR (ryanodine receptor) channel antagonist, reduces mitophagy by inhibiting mitochondrial stressor-induced increase of cytosolic [Ca^{2+}]. TPEN, a low-affinity Ca^{2+} chelator that can chelate ER Ca^{2+} but not cytosolic Ca^{2+}, also inhibits mitochondrial stressor-induced increase of cytosolic [Ca^{2+}], supporting the role of ER Ca^{2+} in this process. ER→lysosome Ca^{2+} refilling might be facilitated by ER-lysosomal contact established after treatment with mitochondrial stressors, which is illustrated by a proximity ligation assay and pBIR biotin ligase complementation assay. In turn, ER→lysosome Ca^{2+} refilling is maintained by store-operated Ca^{2+} entry (SOCE) that is activated by ER Ca^{2+} depletion, as demonstrated by inhibition of mitophagy by SOCE inhibitors such as BTP2 or chelation of extracellular Ca^{2+} by EGTA (Figure 1). SOCE activation after mitochondrial stressor treatment is further supported by STIM1 oligomerization and colocalization with ORAI1, a SOCE channel, which occurs when ER Ca^{2+} is depleted.

Most of the changes observed after mitochondrial stressor treatment such as mitochondrial ROS generation, Ca^{2+} flux, TFEB activation and occurrence of mitophagy are observed after treatment with palmitic acid, an effector molecule of metabolic stress in vitro. To study the in vivo role of TFEB activation and mitophagy in response to metabolic stress in vivo, we generated β-cell-specific *tfeb*-KO (*tfeb* Δβ-cell) mice and fed them a high-fat diet (HFD) to impose metabolic stress. By studying colocalization between LC3 and TOMM20 or that between LAMP2 and TOMM20, we found that mitophagy is significantly increased in pancreatic islets of HFD-fed mice, likely as an adaptation to metabolic stress in vivo. The increase of mitophagy after HFD feeding is significantly reduced in pancreatic islets of *tfeb* Δβ-cell mice, supporting the role of TFEB in metabolic stress-induced mitophagy. ROS accumulation observed in pancreatic islets of HFD-fed mice is aggravated in those of *tfeb* Δβ-cell mice, probably due to insufficient mitophagy. Conversely, mitochondrial complex IV activity is increased in pancreatic islets of HFD-fed wild-type mice, likely as an adaptation to metabolic stress. This adaptive increase of mitochondrial complex IV activity is abrogated in pancreatic islets of *tfeb* Δβ-cell mice, again probably due to insufficient mitophagy. In a similar vein, mitochondrial oxygen consumption of pancreatic islets is increased after HFD feeding probably as an adaptation to metabolic stress, which is also abrogated by β-cell-specific *tfeb* KO.

In an attempt to elucidate target genes related to TFEB-mediated mitophagy and protection of β-cells against metabolic stress, we conducted real-time RT-PCR using mRNA from pancreatic islets of HFD-fed mice. We observed that expression of *Optn* and *Calcoco2* is significantly increased by HFD feeding, which is suppressed in islets of *tfeb* Δβ-cell mice, suggesting that these two important mitophagy receptor genes could be TFEB targets associated with metabolic stress. Expression of other putative mitophagy receptors such as *Nbr1, Tbk1* or *Taxbp1* is also increased in pancreatic islets of HFD-fed mice, although statistical significance is not achieved. We confirm that *Optn* and *Calcoco2* are TFEB targets by employing a reporter assay and chromatin immunoprecipitation.

These results suggest that TFEB is activated by lysosomal Ca^{2+} release coupled with ER→lysosome Ca^{2+} refilling during mitochondrial or metabolic stress, and that TFEB-dependent mitophagy induction, facilitated by transactivation of mitophagy receptors such as *Optn* or *Calcoco2*, plays an important role in β-cell adaptation to metabolic stress through improved mitochondrial function (Figure 1).

Disclosure Statement

M.-S. L. is the CEO of LysoTech, Inc.

Funding

This study was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2019R1A2C3002924) and by the Bio&Medical Technology Development Program (2017M3A9G7073521).

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