Molecular Regulations of FUNDC1 at ER-Mitochondria Contacts Under Hypoxic Stress

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Abstract
A recent research paper published in Journal of Cell Biology by Chen and colleagues describes a novel mechanism by which the MAM (Mitochondrial-associated endoplasmic reticulum membrane) protein FUNDC1 (FUN14 domain-containing protein 1) regulates mitochondrial division through altered protein post-translational modifications under hypoxic stress. The authors found that in a hypoxic environment, the endoplasmic reticulum-localized deubiquitinating enzyme USP19 accumulates at the MAM and interacts with the enriched mitochondrial outer membrane protein FUNDC1, which subsequently induces its deubiquitination and promotes the oligomerization and activity of DRP1, and mitochondria eventually divide in the presence of DRP1. This article provides new insights into the regulation of mitochondrial dynamics by FUNDC1 under hypoxic condition.

Keywords
MAM, FUNDC1, DRP1, USP19, Mitochondria

In eukaryotic cells, the physical contact interface between mitochondria and the ER, called the mitochondria-associated endoplasmic reticulum membrane (MAM), serves as a key hub involved in numerous intracellular activities, including lipid synthesis and transport, calcium ion homeostasis, reactive oxygen species signaling, and autophagosome formation (Rowland & Voeltz, 2012; Lin et al., 2021). Previous studies have shown that under stressful conditions, the contact between mitochondria and the ER increases (Sood et al., 2014), followed by mitochondrial division and autophagosome formation, and the autophagosome is then used for two main types of autophagy: non-selective autophagy or selective autophagy (Nakatogawa, 2020), allowing cells to cope with various external stresses. Ten years ago, Dr. Quan Chen’s lab discovered that mitophagy induced by hypoxic stress is mediated by the mitochondrial membrane protein FUNDC1 (Liu et al., 2012). It is anchored to the mitochondrial outer membrane by three transmembrane domains at its C-terminus and contains a LIR domain that interacts with LC3 at the N-terminal side which is exposed to the cytoplasm, and is capable of recruiting isolated membranes to mitochondria under hypoxic stress. Subsequently, our group further found that hypoxic stress promotes MAM formation and the hydrophilic loop of FUNDC1 exposed on the cytoplasmic side interacts with CANX and DNM1L (DRP1), which induces mitochondrial division and ultimately leads to mitophagy (Wu et al., 2016). The activation of FUNDC1 involves several key post-translational modifications that synergistically regulate the action of FUNDC1 including successive PGAM5-mediated dephosphorylation at Ser 13

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(Chen et al., 2014), ULK1-mediated phosphorylation at Ser 17 (Wu et al., 2014), and, notably, MARCH5-mediated ubiquitination at Lys 119, which is closely related to its regulation of mitochondrial dynamics and mitophagy. Since FUNDC1 is degraded by proteasomes following ubiquitination modifications to alleviate mitophagy, can FUNDC1 be deubiquitinated to resist the degradation tendency and thus induce mitophagy to proceed? Recently, Chai et al. found that the deubiquitinating enzyme USP19 catalyzing deubiquitination of FUNDC1 under hypoxic stress allows FUNDC1 to be stably enriched on the MAM, which ensures mitochondrial division. (Chai et al., 2021). USP19 is an ER-resident deubiquitinating enzyme that stabilizes the hypoxia-inducible factor HIF1α under hypoxic stress. To verify its function in hypoxic stress, the authors constructed a USP19 stable knockdown cell line. After cells were treated with hypoxic stress, the authors found that only 26% of cells containing fragmented mitochondria, meanwhile USP19 deficiency did not change the mitochondrial morphology under normoxia. The rescue experiments showed that this process is dependent on the ER localization of USP19 as well as its deubiquitinating enzyme activity. Observation of mitochondrial fusion and fission using Mito-PAGFP as well as MitoTracker revealed that UPS19 affects mitochondrial division.

The MAM is known to be important for mitochondrial division, so the authors further examined the subcellular localization of USP19. Through subcellular fractionation experiments, they found that USP19 is localized to the MAM and ER. Although hypoxic stress increases the accumulation of USP19 to the MAM, the level of ER-localized USP19 remains unchanged, which was subsequently confirmed by immune-fluorescence, proximity labeling experiments, and immune-electron microscopy. Therefore, how does more USP19 translocate to MAMs requires further exploration. Because previous studies have demonstrated that FUNDC1 translocates to the MAM in response to hypoxic stress and regulates mitochondrial dynamics similarly to USP19, they examined the interaction of USP19 with FUNDC1. Interestingly, the authors found a direct interaction between USP19 and FUNDC1, and the interaction is dependent on the formation of the MAM structure, as their interactions were reduced in MFN2 KO cells. In contrast, USP19 KO inhibits the MAM translocation of FUNDC1, suggesting that the MAM translocation of FUNDC1 may require the assistance of USP19. Because mitochondrial division under hypoxic stress is dependent on the deubiquitinating enzyme activity of USP19, and in normoxia the degradation of FUNDC1 requires ubiquitination modification of its Lys119 by MARCH5 (Chen et al., 2017), does USP19 ensure the stability of FUNDC1 through deubiquitination of Lys119 so that mitochondria can divide normally and subsequently undergo mitophagy?

The answer is yes, USP19 stabilizes FUNDC1 at the MAM by deubiquitinating its Lys119 during hypoxic stress. Finally, the authors further explored the effect of USP19 on the MAM translocation of DRP1 and analyzed the changes in its GTP-binding activity and hydrolase activity, demonstrating that USP19 contributes to the oligomerization of DRP1 by stabilizing FUNDC1 at the MAM, which ultimately leads to mitochondrial division.

Overall, the recent findings of Chai et al. identified a novel deubiquitinating enzyme USP19 that regulates FUNDC1 activity and stabilizes FUNDC1 at the MAM under hypoxic stress, which counteracts the MARCH5-FUNDC1 signaling axis. The presence of MARCH5 allows redundant FUNDC1 to be smoothly degraded and prevents excessive mitochondrial division as well as degradation in normoxia. The stabilizing effect of USP19 on FUNDC1 under hypoxia allows mitochondria to divide and degrade smoothly, allowing the cell to resist stress (Figure 1). The findings in this paper suggest that searching for new regulatory proteins of mitochondrial dynamics can also start from studying the post-translational modifications of these key regulatory proteins in the future. However, the pathophysiological significance of USP19 regulation of FUNDC1 is unclear and needs to be further explored. As a key receptor of mitophagy, FUNDC1 also plays an important role in many kinds of human diseases, especially in cardiovascular diseases. In obese mice induced by High Fat Diet, FUNDC1 interacts with FBXL2 to preserve cardiac homeostasis (Ren et al., 2020). During reperfusion injury, the elevated expression of Ripk3 phosphorylating FUNDC1, contributing to dysfunctional mitophagy and increasing the likelihood of apoptosis (Zhou et al., 2017). After reperfusion injury, CK2α serves as a negative regulator of mitochondrial division.
homeostasis via suppression of FUNDC1-mediated mitophagy (Zhou et al., 2018). FUNDC1 also triggers UPR\textsuperscript{mit} to preserve mitochondrial quality control in reperfusion injury (Ji et al., 2022). In cardiomyocytes from mice subjected to CRS-3, BI-1 expression enhances FUNDC1-related mitophagy to ameliorate myocardial injury (Wang et al., 2022). All of these findings showed that FUNDC1 in the regulation of mitochondrial dynamics and mitophagy is closely related to cardiovascular diseases. It is clear that further studies involving FUNDC1 are still needed to help reveal the mysteries of mitophagy and mitochondrial dynamics.

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