

EDITORIAL COMMENT

# TRAF2 Mediates Physiological Mitophagy\*



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Mitochondria are the powerhouses of cardiomyocytes, but they are easily damaged by oxidative stress, and, once they are damaged, they become a major source of reactive oxygen species and promote programmed cell death, including apoptosis.

Mitophagy is the most prominent mechanism by which damaged or unnecessary mitochondria are removed in cardiomyocytes. Because of their continuous use of oxidative phosphorylation and the consequent production of reactive oxygen species as byproducts, cardiomyocytes are equipped with stringent mitochondrial quality control mechanisms, including mitophagy. A comparison of mitophagic activity among multiple organs in a mitophagy-indicator mouse showed that the heart and cardiomyocytes have high mitophagic activities even under baseline conditions. Previous studies have shown that cardiomyocytes activate mitophagy in response to pathological stimuli, including ischemia, pressure overload, and high fat diet consumption, through either conventional or unconventional mechanisms.<sup>1</sup> However, the molecular mechanism through which mitophagy is mediated in the heart at baseline and its functional significance remain obscure. PINK1-Parkin-mediated mitophagy is the most well-characterized form of mitophagy in many organs and cell types. Stabilization of PINK1 in

depolarized mitochondria induces mitochondrial translocation of Parkin, an E3 ligase. Damaged mitochondria are marked with K63-linked polyubiquitination for engulfment by autophagosomes and lysosomal degradation. Mice genetically deficient in PINK1 and Parkin exhibit a normal cardiac phenotype at young ages, and mitophagy appears to be maintained at baseline. Thus, how mitophagy at baseline is maintained in the heart has been unclear.

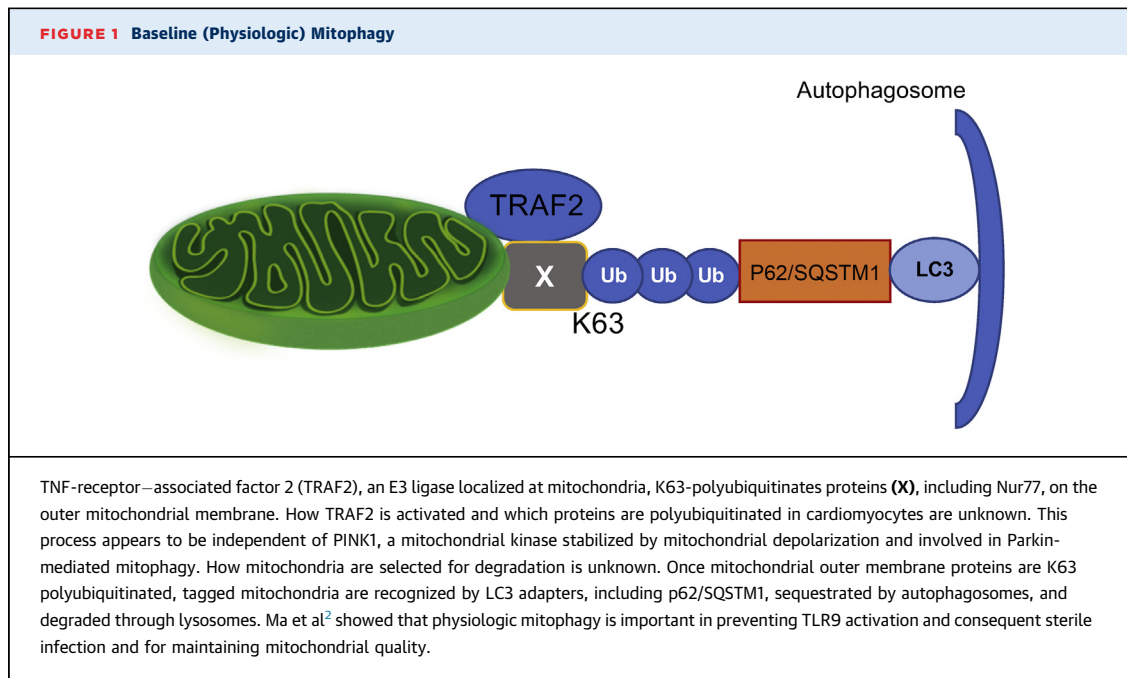
In this issue of *JACC: Basic to Translational Science*, using conditional cardiac-specific TRAF2 knock-out (KO) mice, Ma et al<sup>2</sup> showed that TNF-receptor-associated factor 2 (TRAF2), a mitochondrially localized E3 ubiquitin ligase, mediates mitophagy, and therefore plays a protective role in the adult heart at baseline. Previous studies showed that TRAF2 mediates cytoprotective TNF receptor signaling in the heart during stress, and, that TRAF2, in concert with Parkin, mediates mitophagy in cardiomyocytes in response to stress.<sup>3</sup> By extending these findings, Ma et al<sup>2</sup> showed that the loss of TRAF2 function attenuated mitophagy at baseline in the adult heart, accompanied by accumulation of mitochondrial proteins and mitochondrial dysfunction. Furthermore, the loss of TRAF2 function led to stimulation of TLR9 and macrophage infiltration, apoptosis, and cardiac dysfunction, which suggested that endogenous TRAF2 protects the heart against TLR9-induced sterile infection by facilitating safe disposal of mitochondrial DNA through activation of mitophagy, even without stress.<sup>2</sup>

The study by Ma et al<sup>2</sup> is highly significant for multiple reasons. To my knowledge, this is the first report that clearly shows the molecular mechanism that mediates mitophagy, namely, the involvement of TRAF2 in the adult heart at baseline. The study showed that mitophagy at baseline not only maintains mitochondrial function but also protects the heart against sterile infection caused by TLR9

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activation, which is caused by undigested mitochondrial DNA. Because TRAF2 is involved in innate immunity by transducing TNF receptor signaling, TRAF2 also mediates an additional cell-protective mechanism by preventing TLR9 activation through activation of mitophagy.

Although the investigators showed convincing evidence to support the role of TRAF2 in mediating physiological mitophagy, several questions remain. First, TRAF2 mediates multiple cardioprotective cellular mechanisms. For example, TRAF2 inhibits necroptosis and apoptosis in the heart. Although the investigators showed that conditional down-regulation of TRAF2 in the adult heart did not induce necroptosis or systemic inflammation, it was unclear why these mechanisms were unaffected in the adult TRAF2 KO heart. Because TRAF2 can polyubiquitinate multiple targets at K63, it would have been useful to conduct the specific rescue of mitochondrial E3 ligase targets in TRAF2 KO mice.

Although the investigators showed that the rescue of mitophagy through Parkin overexpression improved the function of TRAF2-deficient murine embryonic fibroblast cells, ideally, it would have been good to test whether the rescue of mitophagy could normalize cardiac function in TRAF2 KO mice *in vivo*.

Second, the investigators previously showed that TRAF2 and Parkin, both of which are E3 ligases, have well-coordinated roles in mediating mitophagy during stress. However, Parkin does not compensate for the loss of TRAF2 function in the adult heart at

baseline. Because Parkin KO mice show no obvious cardiac phenotype at baseline in the adult heart, it is possible that TRAF2 may compensate for the loss of Parkin. Parkin promotes proteasomal degradation of TRAF2, and down-regulation of Parkin extends the half-life of TRAF2 in 293 and SH-SY5Y cells.<sup>4</sup> Ma et al<sup>2</sup> showed that the level of Parkin was not altered in TRAF2 KO mice. Although overexpression of Parkin can rescue the loss of mitophagy in TRAF2-deficient murine embryonic fibroblast cells, the insufficient upregulation of Parkin in the presence of TRAF2 down-regulation appears to explain the distinct cardiac phenotype in TRAF2 and Parkin KO mice. Further investigation is needed to test this hypothesis.

Finally, the molecular mechanism through which TRAF2 mediates mitophagy at baseline requires further investigation. The investigators suggested that TRAF2-induced mitophagy at baseline was PINK1-independent. This raises a question as to how damaged mitochondria are selectively eliminated by mitophagy. Although mitophagy is often triggered by mitochondrial depolarization, it can be activated even in polarized mitochondria. For example, activation of the mitochondrial unfolded protein response can induce PINK1 accumulation.<sup>5</sup> Because TRAF2-induced mitophagy at baseline appears to be PINK1-independent, further investigation is required to determine how physiological mitophagy is executed. A previous study showed that TRAF2 K63 polyubiquitinates Nur77, thus promoting its

interaction with p62, an LC3 adapter protein, and mitophagy (Figure 1). TRAF2 also interacts with RIPK1, thereby mediating NF- $\kappa$ B activation in 293 cells. We have shown recently that RIPK1 plays an essential role in mediating alternative mitophagy, which does not rely on the conventional mechanism of mitophagy in cardiomyocytes.<sup>1</sup> It would be interesting to investigate whether K63 polyubiquitination of RIPK1 mediates TRAF2-induced mitophagy.

In summary, the study by Ma et al<sup>2</sup> clearly shows the importance of TRAF2 in mediating physiological mitophagy in the heart and the cardiomyocytes therein. Increasing lines of evidence suggest that preserving mitochondrial quality is the key to maintaining cardiac function. For example, mitophagy is downregulated during aging, and aging is an important risk factor for incident heart failure.<sup>1</sup> Thus, a better understanding of the molecular mechanisms involved in maintaining mitochondrial quality in the heart at baseline is essential, and pharmacological

modulation of TRAF2 is certainly an attractive approach to addressing this issue. However, caution should be exercised because the function of TRAF2 is dose-dependent and cell type-specific.

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