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EDITORIAL COMMENT

Targeting Syntaxin 17 to Improve Mitophagy in Heart Failure*

Maurizio Forte, PhD,[a] Gianmarco Sarto, MD,[b] Sebastiano Sciarretta, MD, PhD[a,c]

Heart failure (HF) is a serious health concern worldwide and represents the final consequence of several cardiovascular diseases, such as ischemic heart disease and hypertrophic and dilated cardiomyopathy. New classes of drugs, such as angiotensin receptor-neprilysin inhibitors and sodium-glucose cotransporter 2 blockers, have emerged as effective therapies to improve heart function and survival in patients with HF with reduced ejection fraction. However, the overall mortality associated with HF remains high. Therefore, a better understanding of the molecular mechanisms underlying the development of HF still represents a major challenge. In this regard, basic research can help the discovery of novel and interesting molecular targets. Mitochondrial dysfunction is an important determinant of cardiac derangements in HF. Mitochondrial health is ensured by well-coordinated quality control mechanisms, which include mitochondrial biogenesis, dynamics (fusion and fission), and mitophagy.[1] Damaged and dysfunctional mitochondria that accumulate within cardiomyocytes in response to stress are digested and removed by mitophagy, a selective form of autophagy. Previous evidence demonstrated that mitophagy is an adaptive mechanism in the heart in response to pressure overload (PO). Mice undergoing PO by transverse aortic constriction (TAC) show transient mitophagy activation from day 3 to day 7 with a subsequent decline, which leads to mitochondrial dysfunction.[1] At a molecular level, the small GTPase dynamin-related protein 1 (Drp1) mediates the activation of mitophagy in response to PO in a stepwise manner, by ensuring mitochondrial fission first and then the elimination of damaged mitochondrial fragments by mitochondrial autophagy. In fact, the reduced translocation of Drp1 from the cytosol to the outer mitochondrial membrane (OMM) occurs in parallel with the decline in mitophagy observed after TAC. The progression of HF is also accelerated in Drp1 knockout mice. Another work also demonstrated that 2 forms of mitophagy exist: conventional and alterative mitophagy. The latter does not depend on conventional autophagy-related 7 conjugation system and LC3, but on a protein complex composed of Unc-51 Like Autophagy Activating Kinase 1, Rab9, and receptor-interacting serine/threonine protein kinase 1, which ultimately phosphorylates and induces Drp1 activation.[2] Both conventional and alternative mitophagy contribute at different time points to maintain mitochondrial quality and cardiac function in response to PO, with a very early activation of conventional mitophagy, which is then rapidly inhibited and replaced by alternative mitophagy. The latter peaks 3 days after TAC and then declines to basal levels within 14 days. Restoration of mitophagy in mice undergoing PO-induced HF through the synthetic peptide Tat-Beclin 1 reduces mitochondrial dysfunction and HF, suggesting that mitophagy may represent an interesting therapeutic target to reduce HF progression.

In this issue of *JACC: Basic to Translational Science*, Xu et al[3] provide further mechanistic insights into the role of mitophagy in HF, highlighting for the first time the role of Syntaxin 17 (STX17) in the regulation of mitochondrial fission, mitophagy, and cardiac function preservation in response to TAC-induced PO. STX17 is a SNARE scaffold protein localized in

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From the [a]IRCCS Neuromed, Pozzilli, Italy; [b]ICOT Istituto Marco Pasquali, Latina, Italy; and the [c]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy.

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mitochondria, endoplasmic reticulum (ER), and mitochondria-associated endoplasmic reticulum membranes (MAMs), the portions of mitochondrial membranes tightly adherent to ER membranes. Previous work clearly showed that MAMs play a major role in the regulation of mitochondrial quality control, mitochondrial dynamics, and mitophagy in a coordinated manner. The OMM protein FUNDC1 was previously found to localize at MAMs and favor mitochondrial fission and subsequently mitophagy in response to hypoxia in cell lines, by recruiting Drp1 and interacting with autophagosome protein LC3.[4] In this new study, Xu et al[3] extended this evidence by demonstrating that STX17 is required for Drp1 recruitment at MAMs during stress in cardiomyocytes. They found reduced levels of STX17 in the hearts of patients with HF and in mice after 2 and 4 weeks of TAC. Mice with cardiac-specific deletion STX17 develop cardiac dysfunction at baseline, along with impaired mitophagy and mitochondrial dysfunction. Notably, STX17 overexpression by an adenoviral vector improves cardiac performance in mice subjected to TAC and rescues mitophagy and mitochondrial abnormalities. These results suggest that STX17 plays a critical role in cardiac homeostasis in unstressed conditions and may also represent a candidate target for reducing HF progression. The authors also investigated the molecular mechanisms through which STX17 promotes cardiac mitophagy. STX17 interacts with Drp1 and promotes its mitochondrial translocation to induce mitophagy. In fact, levels of phospho S616-Drp1, which positively correlates with mitochondrial translocation of Drp1 to the OMM, decreases in parallel with STX17 expression after TAC. STX17 overexpression rescues levels of phospho S616-Drp1 in mitochondrial fraction and in MAMs. The protective effects of STX17 are abrogated by inhibition of autophagy by 3-MA or by Mdivi-1, a pharmacological Drp1 inhibitor.

Another interesting finding of the study is the demonstration that STX17 recruits the protein kinase CDK1 to phosphorylate Drp1 at S616 and to promote mitophagy. Pharmacological inhibition of CDK1 reduces cardiac protection exerted by STX17 overexpression in mice after surgery.

Although the study by Xu et al[3] provides a previously uncharacterized mechanism involved in the promotion of Drp1-dependent mitophagy, some open issues remain to be addressed. It would be interesting to check whether mitophagy reactivation rescues cardiac dysfunction in STX17 knockout mice, eg, by the administration of Tat-Beclin 1, because STX17 may also be important for other mitophagy-independent functions in the heart. The effects of STX17 knockout on mitophagy flux in vivo should also be evaluated. STX17 belong to the SNARE proteins that are required for autophagosome-lysosome fusion, although STX17 was previously found to be dispensable for autophagy flux regulation in cardiomyocytes.[5] The molecular mechanism by which CDK1 induces Drp1-dependent mitophagy should be further characterized. Interestingly, previous work demonstrated that STX17 interacts with the phosphatase PGAM5, which in turn dephosphorylates Drp1 at serine 637 at MAMs, thereby promoting mitochondrial fission in cell lines in unstressed conditions. On the other hand, PGAM5 is cleaved in response to stress, dissociates from STX17, and interacts with FUNDC1 in an STX17-dependent manner. PGAM5 dephosphorylates FUNDC1 at serine 13 and allows LC3 and Drp1 mitochondrial recruitment by FUNDC1, which is critical for stress-induced mitophagy activation.[6] Conversely, after its dissociation from PGAM5 in response to stress, STX17 initiates autophagosome formation by binding to Atg14L and recruiting the PI3-kinase complex to the MAMs. It will be interesting to test in the future how PGAM and CDK1 cooperate in regulating Drp1 phosphorylation and Drp1-dependent fission and mitophagy in cardiomyocytes at baseline and in response to PO. The role of STX17 in the promotion of autophagosome formation during cardiac stress also warrants further investigation. Xian et al[7] previously showed that STX17-dependent mitophagosome formation is triggered by Fis1 depletion in cell lines, which leads to mitochondrial accumulation of STX17. The role of Fis1 in the regulation of STX17-dependent recruitment of Drp1 at MAMs in the heart during stress should be addressed in the future. Whether CDK1 also phosphorylates STX17 remains to be elucidated.

In future studies, it would be interesting to evaluate whether STX17 and CDK1 also play a role in the promotion of the alternative form of mitophagy during TAC. The fundamental role of Drp1 in the promotion of alternative forms of mitophagy in response to ischemia or in obesity cardiomyopathy has been previously described. For example, after 2 hours of myocardial ischemia in mice, receptor-interacting serine/threonine protein kinase 1 allows mitochondrial translocation of Drp1 and promotes

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Drp1-dependent mitophagy.[1,2] Future studies should investigate the role of STX17 and CDK1 in these experimental conditions, as well as in other relevant models of HF, such as those resulting chronic myocardial ischemia or during aging.

In conclusion, the study by Xu et al[3] strengthens the evidence that boosting mitophagy is a feasible therapy to reduce HF progression in patients at high risk. It also points out that the up-regulation of STX17 may represent a potential strategy to achieve this goal. The development of pharmacological activators of STX17 level should be reached in a near future.

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ADDRESS FOR CORRESPONDENCE: Dr Sebastiano Sciarretta, Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Corsa della Repubblica 79, Latina LT 04100, Italy. E-mail: sebastianosciarretta@uniroma1.it.

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