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EDITORIAL COMMENT

# ER-Specific Autophagy or ER-Phagy in Cardiac Myocytes Protects the Heart Against Doxorubicin-Induced Cardiotoxicity*

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Anthracyclines, such as doxorubicin (DOX), comprise a broad class of cytotoxic agents used to treat a wide variety of cancers; however, clinical use is limited by dose-dependent cardiotoxicity, and the most prevalent clinical manifestations of DOX-induced cardiotoxicity (DIC) include heart failure and lethal arrhythmias.[1] Although acute DIC can be mitigated somewhat by limiting lifetime cumulative exposure, many patients still present with a delayed appearance of cardiotoxicity, years or even decades after exposure.

Numerous investigations have aimed to decipher mechanisms underlying DIC to better understand the therapeutic limitations of anthracyclines.[2] These studies have been done mostly in cardiac myocytes because they are major targets of DIC-related heart disease, and many have focused on a process occurring in all cells including cardiac myocytes, autophagy, arising from the Greek words *phagy* meaning eat, and *auto* meaning self.[2,3] One function of autophagy is to remove damaged, potentially toxic cellular components; in this way, autophagy can be viewed as protective and required to fend off toxicity resulting from pathology and drug treatment, including anthracyclines. Autophagy-mediated removal of cellular debris begins with engulfment of damaged components into a membranous structure called the autophagosome, which then fuses with the lysosome, a membranous organelle containing enzymes housed at a low internal pH, which facilitates the degradation of damaged cellular components.[2,4] In some cases, autophagy occurs in an organelle-specific manner. Because continuous and robust ATP production by healthy mitochondria is critical for cardiac myocyte contraction and viability, traditionally, investigations in the heart have focused on the role of mitochondrial autophagy, or mito-phagy, the selective degradation of defective mitochondria in cardiac pathologies, including DIC.[2] By contrast, selective autophagy of other damaged organelles, such as the endoplasmic reticulum (ER), have been relatively less studied. Cardiac myocytes harbor an extensive and specialized ER, the sarcoplasmic/endoplasmic reticulum (SR/ER), which is critical for maintaining robust cardiac contractility.[5,6] As such, ER autophagy, or ER-phagy, is of potential importance in DIC pathophysiology.

The SR/ER in cardiac myocytes is the site of crucial cellular processes, most notably the release and reuptake of calcium from the SR/ER lumen into the cytosol, which regulates cardiac myocyte contraction.[6] However, despite the central importance of the SR/ER of cardiac myocytes in heart contraction, no tools have been developed to investigate ER-phagy in the heart of experimental animals, in vivo. Accordingly, in this issue of *JACC: CardioOncology*, Nakagama et al[7] developed a new method to investigate ER-phagy in cardiac myocytes of mice, in vivo. To achieve this, they engineered a protein that could be expressed and retained in the

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SR/ER of cardiac myocytes, which allowed them to visualize and quantify the SR/ER, as well as portions of the SR/ER engulfed by autophagosomes, that is, SR/ER that is undergoing ER-phagy. Their genetically engineered protein was a chimera comprised of green fluorescent protein (GFP) fused to red fluorescent protein (RFP); GFP is readily degraded by lysosomal proteases, whereas RFP is not. They expressed this protein in the hearts of mice treated with or without DOX, then obtained cardiac tissues for fluorescence microscopy to assess ER-phagy. With this new method, Nakagama et al[7] could identify intact SR/ER in cardiac myocytes, whose overlapping RFP and GFP signals exhibited a yellow fluorescence. They could also quantify how much of the SR/ER had been engulfed into lysosomes where only red fluorescence remained as a direct readout of ER-phagy. Thus, the red-fluorescent signal was directly proportional to ER-phagy activity, in vivo, providing the first animal model that enables investigators to visualize ER-phagy in the heart.

Nakagama et al[7] combined their new method with a widely used DOX treatment paradigm, administering it intraperitoneally once per week at 5 mg/kg over 4 weeks, totaling a cumulative exposure of 20 mg/kg. Studies were terminated 1 week after the last administered dose to assess ER-phagy. This assessment revealed a significant increase in the number of red puncta in myocytes of hearts from DOX-treated mice compared with untreated mice, suggesting that DOX increased ER-phagy. ER-phagy is mediated by receptors that direct portions of the ER to autophagic machinery.[4,8] Accordingly, to determine whether the increase in ER-phagy they observed in DOX-treated mice was protective or detrimental, Nakagama et al[7] deleted one such receptor.[8] As anticipated, this greatly diminished ER-phagy in the heart, which was accompanied by an increase in the detrimental effects of DOX on cardiac structure and function. Thus, the investigators were able to conclude that increased ER-phagy in DOX-treated mice is protective in the heart.

The findings presented here by Nakagama et al[7] demonstrate the importance of ER-phagy in the heart for the first time and highlight the potential clinical significance of future research focused on finding ways to enhance ER-phagy in the heart. In the context of DIC, boosting ER-phagy in the hearts of patients treated with DOX may be an effective therapeutic approach for reducing morbidity and mortality from DIC-related cardiac pathologies. Preserving cardiac myocyte viability in DOX-treated patients is crucial, since in adults, cardiac myocytes do not regenerate to a significant degree.[9] It is notable that autophagy-related cell death of cardiac myocytes has been observed in a myriad of cardiac pathologies, including DIC,[2,3] as a form of cell death that is distinct from either apoptosis or necrosis. Previous investigations of autophagy in the setting of DIC have noted robust but transient induction of autophagy in cardiac myocytes treated with DOX; however, on a more protracted, chronic timeframe, autophagy has been observed to be suppressed by DOX.[2,3] Activating autophagy in this context has yielded conflicting findings as some studies have shown that by stimulating the initiating step in autophagy, at a time when autophagic flux is impaired, exacerbated cardiac damage and DIC-related pathology.[2,3] However, in other studies, promoting autophagy at later steps can restore flux and prevent DOX-related declines in cardiac function.[10] Thus, it appears there is an optimal therapeutic window in which activating autophagy may be most efficacious in DIC. Still, although it is currently unknown whether ER-phagy can be augmented therapeutically, Nakagama et al[7] demonstrate that inhibition of ER-phagy via conditional ablation of a key receptor is sufficient to promote DIC.

Thus, there is a need for continued investigation of mechanisms of DIC in order to better understand the time- and dose-dependent dysregulation of autophagy by DOX, so effective treatment strategies can be better defined. Although in the long-term DOX inhibits autophagic flux, there may be continued selective degradation of other organelles.[2] However, whether this is true for the ER and whether ER-phagy is impaired by dysregulated flux remain to be seen. Addressing these gaps in knowledge will be important to determine the therapeutic efficacy of enhancing ER-phagy. How autophagy-related cell death can be therapeutically alleviated in the heart is still an active area of investigation, and our knowledge of ER-phagy is especially limited but warrants deeper investigation in the context of DIC and other initiators of cardiac pathology, given the importance of SR/ER function throughout life in cardiac myocytes.

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