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INVITED EDITORIAL

Regulation of calcium handling by autophagy: a novel mechanism limiting cardiac hypertrophy and dysfunction?

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Despite the increasing strategies to prevent and manage heart failure, this condition still has a high mortality and morbidity rate.[1,2] Therefore, the identification of new therapeutic targets to treat this disease is urgently needed.[3]

In recent years, autophagy has emerged as one of the major cellular mechanisms regulating cardiac homeostasis. Autophagy is a catabolic process that digests and recycles senescent and dysfunctional cytoplasmic elements, including whole organelles.[4] Preclinical studies demonstrated that autophagy preserves cardiac function and limits myocardial damage in several conditions.[5] In particular, boosting autophagy exerts protective effects against heart failure.[6] Previous landmark work showed that inducible cardiac-specific deletion of ATG5 gene during adulthood in mice leads to cardiac hypertrophy and left ventricular dilation, with disorganized sarcomeres and mitochondrial abnormalities observed in KO mice at ultrastructural analyses. Conversely, mice with constitutive ATG5 deficiency show no signs of cardiac abnormalities in unstressed conditions, while they develop cardiac dysfunction in response to pressure overload or β-adrenergic stimulation.[7] These results demonstrated for the first time that autophagy represents an adaptive mechanism in response to haemodynamic stress. Subsequent studies also showed that autophagy reduces chronic cardiac remodelling after myocardial infarction. However, the exact mechanisms by which autophagy preserves cardiac function and limits adverse remodelling during stress warrant additional study. The possible involvement of autophagy defects in the transition from hypertrophy to heart failure also needs to be further clarified. Ljubojevic-Holzer *et al.*[8] elucidated for the first time the role of autophagy defects in the development of Ca^{2+} cycling and signalling abnormalities, which represent crucial determinants of cardiac hypertrophy and heart failure. They studied the phenotype of mice with constitutive cardiac-specific ATG5 gene deletion (cATG5−/−) in response to the β-adrenergic stimulation and exercise tolerance test, two conditions of increased workload and heart rate. These mice were obtained by crossing ATG5 floxed mice with transgenic mice overexpressing CRE recombinase under the control of the a-myosin light chain-2 promoter, and they show mild hypertrophy and normal cardiac function at baseline. In contrast, cATG5−/− mice display impaired contractility in response to β-adrenergic stimulation and reduced exercise tolerance, as evaluated by total workload and maximum run distance after exercise testing. These data are consistent with previous evidence indicating that autophagy inhibition leads to early cardiac alterations in response to increased workload before overt cardiac dysfunction develops. At the molecular level, Ljubojevic-Holzer *et al.*[8] found that intracellular Ca^{2+} stores and Ca^{2+} cycling in cardiomyocytes from cATG5 −/− mice are not affected by slow pacing or by acute β-adrenergic stimulation. In contrast, under high pacing frequencies, peak systolic Ca^{2+} amplitude in cytosol and nucleus is reduced, whereas time-averaged nucleoplasmic, but not cytosolic, Ca^{2+} load is significantly increased. Interestingly, increased nuclear calcium load was found to be associated with a significant CaMKII activation in the nucleus of cATG5 −/− cardiomyocytes. Overall, these results show that autophagy disruption significantly affects frequency-dependent calcium cycling and signalling in cardiomyocytes, which is associated with a significant impairment of functional reserve in response to increased heart rate and workload.

Other significant abnormalities were also observed in cATG5−/− mice in this study.[8] A reduced PKA-dependent phosphorylation of Troponin I, a marker of myofilament alterations, was detected in these animals in response to β-adrenergic stimulation. Consistently, sarcomere disarray was also observed. In addition, cATG5−/− mice display reduced cardiac mitochondrial abundance and volume without significant alterations of mitochondrial membrane potential and function. Actually, mitochondria of cATG5−/− cardiomyocytes appear to be in an overcompensatory state at baseline, which may lead to energy depletion in response to increased workload and explain the earlier exhaustion. This result is really intriguing and may be clarified by the recent discovery of an alternative form of mitophagy, a mitochondrial-specific type of autophagy. Alternative mitophagy is regulated by ULK1 and Rab9 but not by ATG5/ATG7, and it was found to be activated in conditions in which conventional autophagy is suppressed, such as pressure overload and diabetes. Alternative mitophagy activation would degrade dysfunctional mitochondria and promote their turnover, thereby limiting mitochondrial defects.[9,10] This mechanism would explain the reduced mitochondrial mass but preserved function observed in cATG5−/− mice. Future studies are encouraged to test this hypothesis.

Ljubojevic-Holzer *et al.*[8] also translated their results to the human disease. They found a reduced expression of ATG5 and other markers of

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autophagy in left ventricular biopsies of patients with compensated hypertrophy and end-stage heart failure, when compared with donor control subjects. The extent of autophagy inhibition is correlated to the increase in wall thickness, suggesting that autophagy inhibition may contribute to the development of cardiac hypertrophy and its transition to maladaptive cardiac remodelling and heart failure. This notion is in line with previous preclinical work demonstrating that autophagy is progressively inhibited in response to pressure overload, paralleling the development of cardiac hypertrophy and contributing to adverse cardiac remodelling and heart failure.[6] Importantly, Ljubojevic-Holzer *et al.* found that ventricular myocytes isolated from non-failing and hypertrophied human hearts show increased nucleoplasmic Ca^{2+} load in response to high pacing frequencies, thus recapitulating the results shown in cATG5 −/− mice. This result suggests that autophagy impairment may affect calcium handling in the human heart, thereby predisposing to the development of cardiac dysfunction. It would be important to test this hypothesis in future studies.

Overall, the study by Ljubojevic-Holzer *et al.*[8] significantly increases our knowledge regarding the importance of autophagy in cardiac adaptation to increased workload and stress by suggesting that part of its beneficial cardiac effects may be mediated by the preservation of calcium cycling and signalling. A deregulated activation of nuclear CaMKII may also keep the genetic programme of hypertrophy active and play an important role in the development and progression of cardiac hypertrophy and remodelling in the presence of autophagy defects during stress. In this regard, previous seminal evidence demonstrated that systemic disruption of CaMKII δ, the most expressed CaMKII isoform in the heart, blocks the transition to decompensated cardiac hypertrophy, cardiac dysfunction, and death in response to pressure overload.[11]

Several important aspects regarding this study remain to be clarified. The mechanisms through which autophagy regulates calcium cycling were not addressed. It is unclear whether this is a direct or an indirect effect. Of note, acute inhibition of autophagy flux by leupeptin was not found to affect calcium cycling, suggesting that chronic and complete inhibition of autophagy is required. This may suggest that calcium cycling derangements in the presence of autophagy impairment are secondary to other cellular modifications, although sarcoplasmic reticulum (SR) structure, SERCA2a levels, intracellular Ca^{2+} stores, caffeine-induced Ca^{2+} transients, and RyR2-mediated SR calcium leak were not affected in cATG5−/− mice. Since autophagy disruption is associated with oxidative stress development, it will be interesting to investigate in the future whether redox modifications of channels, receptors, and proteins regulating calcium handling are involved in these mechanisms.

The real impact of calcium cycling and signalling abnormalities in the development of cardiac derangements associated with autophagy inhibition is also unclear. Sarcomere disarray, mitochondrial dysfunction, energy deprivation, and misfolded protein accumulation may play a prominent role in the induction of cardiac remodelling and dysfunction, as also demonstrated in this study (*Figure 1*). It would be interesting to check whether normalization of calcium cycling, if feasible, or CaMKII inhibition attenuates cardiac abnormalities in cATG5−/− mice.

Finally, it will be important to check whether reactivation of autophagy in conditions in which it is inhibited, such as heart failure, cardiac

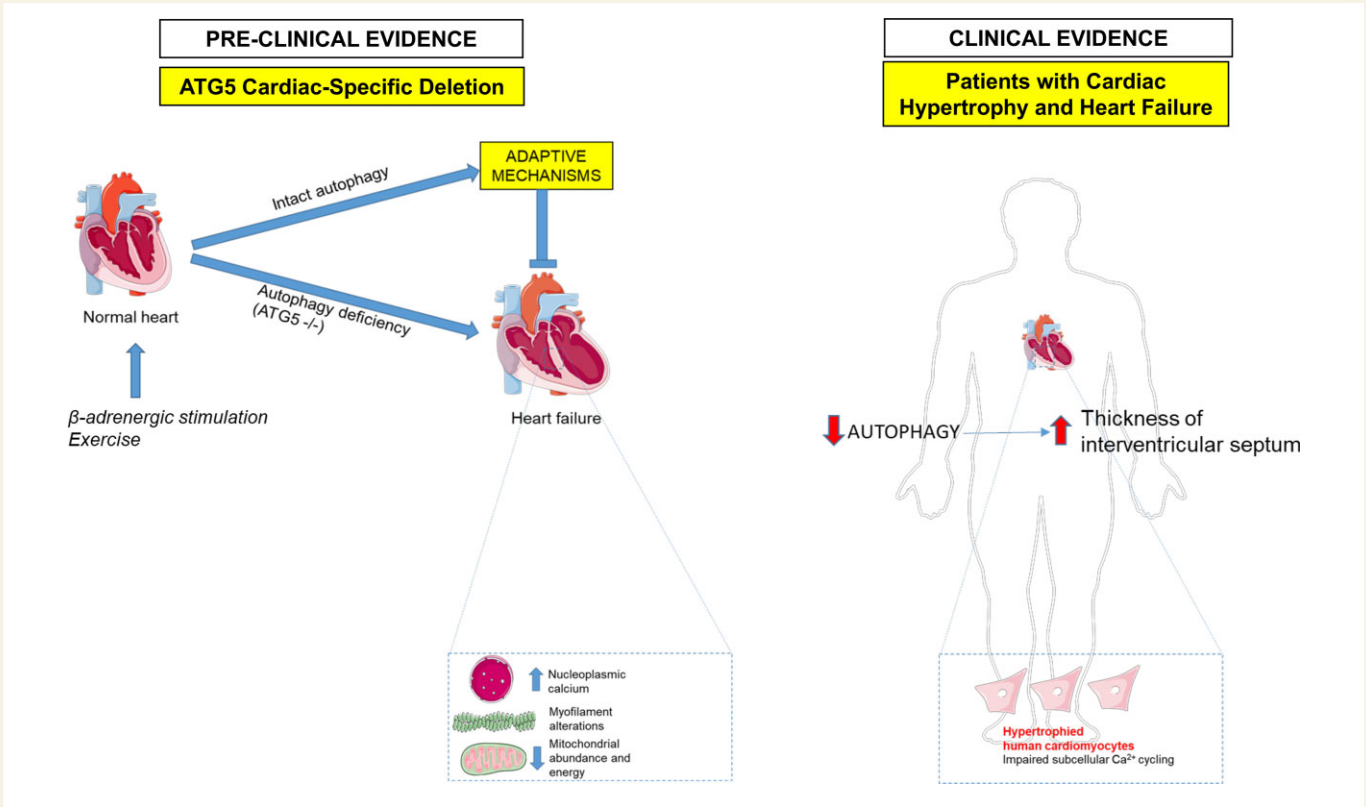

Figure 1 Possible mechanisms underlying the association between autophagy deficiency and heart failure in mouse and human cardiomyocytes. Autophagy inhibition leads to cardiac energy exhaustion and reduction of cardiac reserve and exercise tolerance, which in turn contribute to heart failure. Calcium cycling abnormalities, sarcomere disarray, mitochondrial dysfunction, energy deprivation, and misfolded protein accumulation underlie the pathological effects of autophagy inhibition. The figure was made using tools provided by Servier Medical Arts, amongst others

hypertrophy, ageing, or diabetes, improves cardiac functional reserve by the normalization of calcium handling and signalling. This hypothesis may also be tested soon in clinical trials using natural activators of autophagy, such as trehalose and spermidine, which are FDA-approved molecules with very limited side effects and which were proved to have extraordinary beneficial cardiovascular effects in several pathological conditions.

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