Redox Regulation of Autophagy in Dilated and Hypertrophic Cardiomyopathy

Mingjie Yang[1], Yingmei Zhang[1,2], and Jun Ren[1,2]

[1]Department of Cardiology and Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai 210032, China; [2]Center for Cardiovascular Research and Alternative Medicine, University of Wyoming College of Health Sciences, Laramie, WY 82071, USA

Correspondence: ren.jun@zs-hospital.sh.cn (J.R.)

ABSTRACT | Autophagy is an essential intercellular degrading system for protein aggregates and organelle turnover. Disruption of autophagic flux will disturb cellular homeostasis and influence different cardiovascular diseases including cardiomyopathy. However, no agreement has been reached about whether autophagy ameliorates or exacerbates the pathological development of cardiomyopathy. This review will try to compare the different roles of autophagy in dilated and hypertrophic cardiomyopathies. In addition, oxidative stress is interconnected with autophagy in various aspects, and as such, we will also offer some details of this interplay in cardiomyopathy.

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1. DEFINITION AND CLASSIFICATION OF CARDIOMYOPATHY

According to the 2006 American Heart Association definition for cardiomyopathy, "cardiomyopathies are a heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical dysfunction that usually (but not invariably) exhibit inappropriate ventricular hypertrophy or dilation and are due to a variety of causes that frequently are genetic" [1]. Cardiomyopathies are typically classified into primary cardiomyopathies (mainly involving heart) and secondary cardiomyopathies (other organs also afflicted). Primary cardiomyopathies are further divided into genetic (including hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy/dysplasia, left ventricular noncompaction, PRKAG2 and Danon glycogen storage diseases, conduction defects, mitochondrial myopathies, and ion channel disorders), acquired (including myocarditis, stress-induced, peripartum, tachycardia-induced, and infants of insulin-dependent diabetic mothers) and mixed cardiomyopathies [1].

The 2008 European Society of Cardiology working group has offered a different definition for cardiomyopathy as "a myocardial disorder in which the heart muscle is structurally and functionally abnormal in the absence of coronary artery disease, hypertension, valvular disease, and congenital heart disease sufficient to explain the observed myocardial abnormality" [2]. This definition is considered more practical in clinical use. Approximately 50% of sudden cardiac death in young adults may be attributed to cardiomyopathies. Diagnosis of cardiomyopathies is somewhat straight forward with genetic mutations in sarcomeric proteins being the main driving forces. Recent clinical and experimental findings have depicted contributing factors such as aberrant cardiac sarcomeric structure, ion channels, and protein quality control, in particular autophagy, in the onset and transition of full-blown cardiomyopathies [3].

2. AUTOPHAGY

Autophagy, which refers to self-digesting or self-engulfing in Greek, denotes a lysosome-dependent degradation mechanism of wasted proteins and dysfunctional organelles including mitochondria, endoplasmic reticulum, and peroxisomes. It maintains a basal level under normal circumstances to maintain cellular homeostasis and elevates in face of stress such as starvation, hypoxia, endoplasmic reticulum stress, redox stress, and mitochondrial damage [4]. Autophagy can be classified into three different types, including macroautophagy, microautophagy, and chaperone-mediated autophagy, among which macroautophagy is most widely studied and will be referred to in the following passage. In macroautophagy, autophagosomes transfer intracellular components and damaged organelles and fuse with lysosome to generate autolysosomes, where these proteins and organelles are degraded [5]. The degradation products such as lipids and amino acids are released into the cytoplasm and become building materials for new proteins and membranes. Therefore, autophagy can not only eliminate waste, but serve as a recycling mechanism to provide materials and energy for new synthesis to promote cellular survival under stress. The dynamic process of autophagy called autophagic flux includes initiation, nucleation, elongation, autophagosome formation, fusing with lysosomes to form autolysosomes, and materials degradation. This process is typically regulated by Atg-composed protein complexes (**Figure 1**).

2.1. Upstream Regulation and Initiation of Autophagy

Among the various regulators of autophagy, two most important main upstream regulators of autophagy are mTOR (mammalian target of rapamycin) and AMPK (AMP-activated protein kinase). AMPK is an energy sensor and activates autophagy, while mTOR is a negative regulator which collects information of nutrients. ULK1/Atg1 complex (ULK1/Atg1-Atg13-Atg17), responding to upstream signals, is the initiator of autophagosome formation [6]. mTORC1 phosphorylates ULK1 and suppresses initiation of autophagic flux under nutrient-rich conditions, whereas its dissociation from the complex under starvation allows the start of autophagosome nucleation [7]. The activation of AMPK inhibits mTORC1 and promotes autophagy.
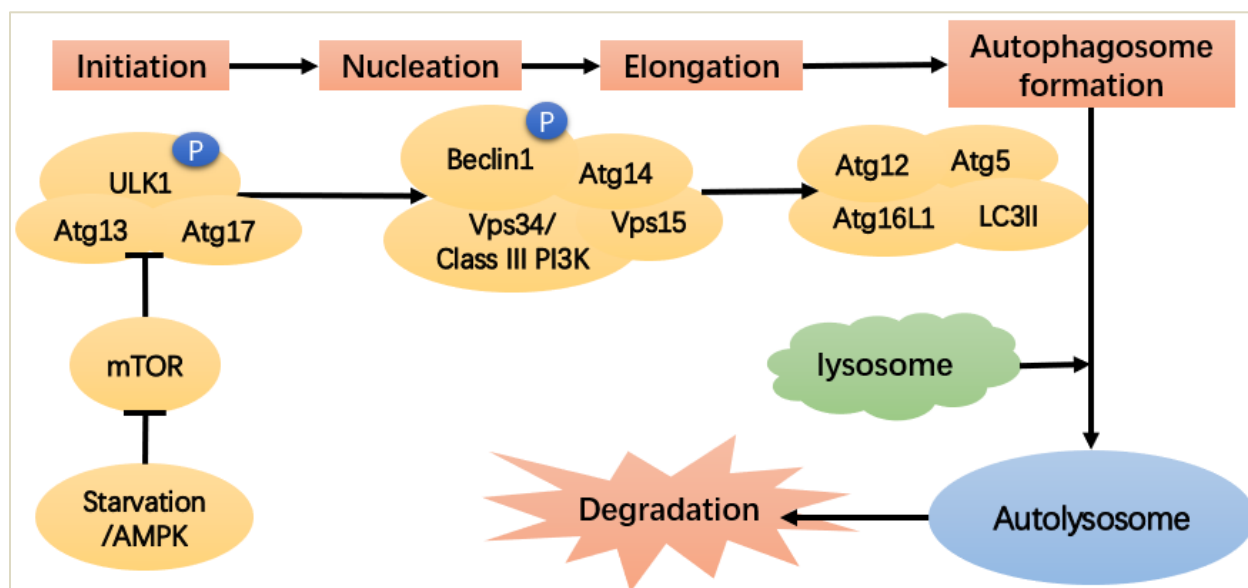
FIGURE 1. Schematic illustration of the general process of autophagy including initiation, nucleation, elongation, autophagosome and autolysosome formation, and materials degradation. ULK1/Atg1 complex is the initiator which is mainly regulated by mTOR and AMPK. Activation of ULK1 complex triggers Beclin1 and promotes autophagosome nucleation and elongation. With more Atg-composed protein complexes involved, mature autophagosomes are generated and then fuse with lysosomes to form autolysosomes, where waste proteins and organelles are degraded and released into the cytoplasm.

2.2. Nucleation, Elongation, and Autophagosome Formation

Activation of ULK1 complex phosphorylates and activates Beclin1 complex (Beclin1/Atg6-Atg14-Vps34/Class III PI3K-Vps15), promoting autophagosome nucleation [8]. Then ubiquitin protein Atg12 is activated by Atg7 and linked to Atg5 by Atg10. The Atg12-Atg5 complex binds to Atg16L1, which contributes to the elongation of autophagosome membranes and the formation of premature autophagosome structures.

2.3. Autophagic Flux Measurement

Atg12-Atg5 complex recruits LC3II/Atg8 into the growing membranes before mature autophagosomes are generated and LC3II remains on the structure until autolysosome formation [9]. Therefore, LC3II accumulation indicates the elevation of autophagic flux [10]. Besides, with an mRFP-GFP-LC3 tandem, au-tophagosomes are tagged with a yellow fluorescent signal while autolysosomes with red, and as such, the increased fluorescent signal shows the increase of autophagic flux [11].

2.4. Autolysosome Formation and Materials Degradation

After mature autophagosomes engulf target materials, fuse with lysosomes, and generate autolysosomes, protein and lipid aggregates and damaged organelles are degraded by different enzymes in autolysosomes. The breakdown products such as amino acids and lipids are then released into the cytoplasm and recycled for new metabolic synthesis [9].

3. AUTOPHAGY IN CARDIOMYOPATHY

Autophagy, a degrading process of protein aggregates and dysfunctional organelles, is crucial for

maintaining cellular homeostasis and defective autophagic flux may lead to cardiac dysfunction and cardiomyopathies. This mini-review will mainly concentrate on the different contributions of autophagy in dilated cardiomyopathy versus hypertrophic cardiomyopathy (as depicted in **Figure 2**).

3.1. Autophagy Exacerbates Doxorubicin-Induced Dilated Cardiomyopathy

Doxorubicin (DOX) is an effective chemotherapeutic agent for various tumors, but its cardiotoxicity limits its use. The molecular mechanism of DOX cardiotoxicity is still under debate despite efforts in past few decades. Among its cardiac side effects, heart failure and dilated cardiomyopathy are most commonly seen and studied by clinicians and scientists. Approximately 10% of pediatric cancer survivors who once utilized DOX developed severe dilated cardiomyopathy after 10 to 20 years [12]. A recent study which compared short-term and long-term duration of DOX treatment found the long-term exposure was easier to trigger dilated cardiomyopathy, which was evidenced by left ventricular dysfunction and structural change. Echocardiography showed left ventricular dilation and ejection fraction decrease, and morphological analysis showed cardiac interstitial fibrosis [13]. This was in line with clinical observations that DOX-induced cardiomyopathy was usually delayed for years or even decades.

As an essential self-digesting process for bulk degradation and organelle turnover, autophagy is believed to influence the development of DOX-induced cardiomyopathy. Some researchers believe that DOX promotes cardiotoxicity by increasing autophagy. DOX activated AMPK and therefore promoted autophagy and apoptosis in H9c2 cells and in mouse hearts. Besides, it increased the level of Atg5 and Atg12 and hyperactivated PI3K involved in autophagy initiation and nucleation in order to trigger autophagy [14]. Using 3-MA to inhibit PI3K suppressed autophagosomal formation and reversed cardiotoxic outcomes following DOX treatment. Another study found that aldehyde dehydrogenase 2 (ALDH2) was a cardioprotective factor for DOX-induced cardiomyopathy by inhibiting 4-hydroxynonenal (4-HNE) formation and autophagic flux [15]. Recently, microRNA is thought to be a possible regulator of cardiac remodeling, and angiotensin-converting enzyme 2 (ACE2) was found to be able to attenuate DOX-induced cardiac dysfunction via microRNA-30e. Beclin-1 and LC3II/I ratio decreased pronouncedly with inhibited autophagy, and left ventricular contractility was obviously elevated [16].

3.2. Autophagy Protects from Dilated Cardiomyopathy

Some other researchers, however, have different opinions on the role of autophagy in DOX cardiotoxicity. In GFP-LC3 transgenic mice and neonatal rat cardiomyocyte (NRCM), diarylhepatanoid curcumin attenuated DOX-induced cardiotoxicity by elevating the level of autophagy and LC3II [14]. Another study found that silencing Nrf2, an essential transcriptional regulator of p62, suppressed autophagy and increased DOX cardiotoxicity [17]. Similarly, macrophage migration inhibitory factor (MIF)-knockout mice showed impaired autolysosome formation and exacerbated DOX cardiotoxicity [18]. Therefore, opposite opinions exist about whether autophagy exacerbates or ameliorates DOX-induced cardiotoxicity. The different conclusions may possibly be attributed to the different models employed, different DOX treatment doses and duration, and different methods to measure autophagic flux.

Apart from DOX, researchers found that defective autophagy led to the development of dilated cardiomyopathy using other models. LMNA gene mutation which encodes lamin A and C was found to alter autophagy signaling pathway and contribute to dilated cardiomyopathy and other muscular dystrophy in the LMNA point mutation mouse model. AKT-mTOR was hyperactivated in the hearts of LMNA mice, and suppressing mTOR pharmacologically restored autophagy and ameliorated cardiomyopathy [19]. It was further showed that overexpressing dual specificity phosphatase 4 (Dusp4) in the hearts of LMNA mice increased the level of AKT-mTOR and thus inhibited autophagy [20]. Another study found that Mst1 phosphorylated threonine 108 in Beclin1 and enhanced the interaction between Beclin1 and Bcl-2, inhibiting PI3K in PI3K-Beclin1-Atg14L-Vps34 complex and suppressing autophagic flux. Autophagy inhibition resulted in accumulation of protein aggregates and cardiac dysfunction after myocardial infarction and dilated cardiomyopathy. It is believed that impaired autophagy results in the aggregation ofmisfolded and used proteins and dysfunctional organelles, which does great harm to cardiomyocytes
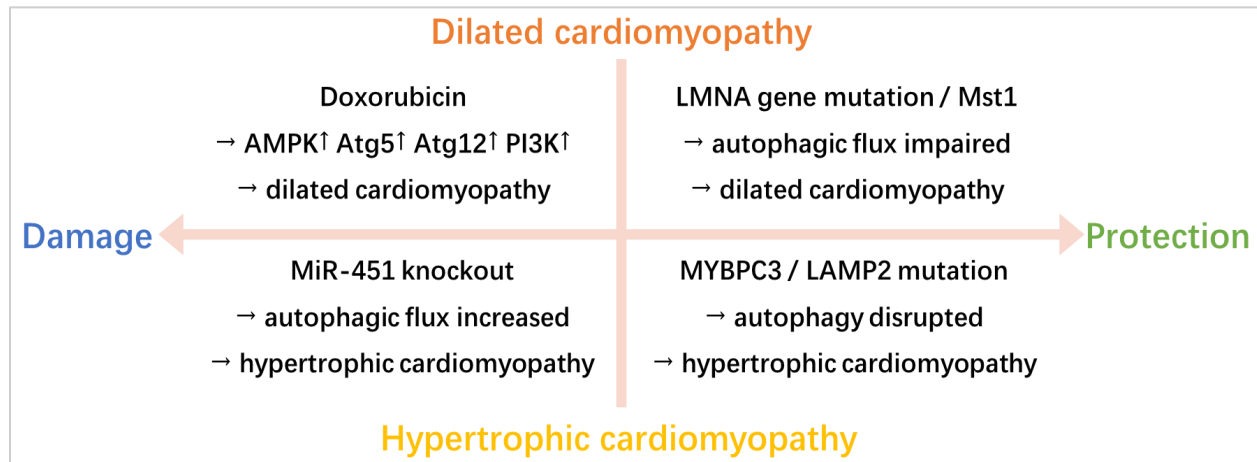
Dilated cardiomyopathy

Doxorubicin

→ AMPK↑ Atg5↑ Atg12↑ PI3K↑

→ dilated cardiomyopathy

LMNA gene mutation / Mst1

→ autophagic flux impaired

→ dilated cardiomyopathy

Damage ←————————————————→ **Protection**

MiR-451 knockout

→ autophagic flux increased

→ hypertrophic cardiomyopathy

MYBPC3 / LAMP2 mutation

→ autophagy disrupted

→ hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy

FIGURE 2. Different roles of autophagy in dilated and hypertrophic cardiomyopathy. Increased autophagy exacerbated doxorubicin-induced cardiomyopathy, while in LMNA gene mutation mice, autophagy protected against the development of dilated cardiomyopathy. On the other hand, most hypertrophic cardiomyopathies resulted from impaired autophagic flux due to specific gene mutations, but some studies showed opposite outcome.

and thus leads to the development of dilated cardiomyopathy [21].

3.3. Impaired Autophagy Contributes to Hypertrophic Cardiomyopathy

Most hypertrophic cardiomyopathies are attributed to genetic mutations encoding sarcomeric proteins, and autophagy plays a role in this process as an essential protein degradation signaling pathway. Mybpc3-encoding cardiac myosin-binding protein c (cMyBP-c) is a commonly seen mutated gene associated with hypertrophic cardiomyopathy. Autophagy dysregulation was found in Mybpc3-targeted knock-in mice, and if treated with rapamycin or caloric restriction, autophagy would be restored and cardiomyopathy caused by Mybpc3 mutation would be ameliorated [22]. LAMP2 (lysosome-associated membrane protein 2) is another important mutated gene related to Danon disease, an X-linked disorder which is characterized by defective autophagy and life-threatening hypertrophic cardiomyopathy. LAMP2 deficiency disturbs lysosome biogenesis and maturation and fusion with autophagosome, all of which are crucial steps of autophagic flux. Biopsies from the diseased individuals showed unusual autophagic vacuoles in cardiomyocytes and skeletal muscles, which proved that autophagy was blocked in Danon disease [23]. Another study reported the development of the phenotype of hypertrophic cardiomyopathy in cardiomyocyte-specific PTEN knockout mice, evidenced by functional and histological alterations. Increased cardiac mTOR and suppressed autophagy were observed in these mice. With rapamycin treatment for a month to inhibit mTOR, the established hypertrophic cardiomyopathy was reversed, which suggested a cardioprotective function of autophagy [24].

3.4. Autophagy Promotes Hypertrophic Cardiomyopathy

As mentioned above, microRNA is nowadays considered a regulator of cellular homeostasis and involved in the development of hypertrophic cardiomyopathy as well. MiR-451 was found to be significantly decreased in the heart tissue from hypertrophic cardiomyopathy patients. Further study showed that downregulation of miR-451 promoted autophagosome formation and upregulated autophagic flux, as evidenced by elevated autophagy markers LC3 and Beclin-1. Moreover, tuberous sclerosis complex 1 (TSC1) was found to be the target of

miR-451. MiR-451 knockout increased the level of TSC1 and autophagy, which eventually resulted in hypertrophic cardiomyopathy [25].

4. OXIDATIVE STRESS AND ITS INTERPLAY WITH AUTOPHAGY IN CARDIOMYOPATHY

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated during cell metabolism. In the physiological state, ROS production and neutralization by antioxidants usually keep a balance and no oxidative stress occurs. In pathological conditions, the balance is disturbed and ROS/RNS accumulation causes severe oxidative damage to various cellular biomolecules and organelles and leads to various diseases including cardiovascular diseases [26].

Given that autophagy is an intracellular degrading system, it is pertinent to understand how oxidative stress and autophagy interplay with each other at both transcriptional and post-translational levels to regulate cellular homeostasis in cardiomyocytes. Autophagy is triggered in response to stress, such us nutrient deprivation, viral infection, and genotoxic stress [27]. Oxidative stress is regarded as a converging point of these stimuli, and the excessively produced ROS act as upstream activators of autophagy. When treated with the antioxidant N-acetylcysteine, autophagic flux was blocked in cardiomyocytes [26]. On the other hand, oxidative stress causes damage to proteins and organelles, and autophagy serves as an indispensable way to degrade toxic components. Besides, cardiomyocytes are in great need of energy and have abundant mitochondria, which are the main source of ROS in the heart. Mitophagy not only degrades damaged mitochondria caused by oxidative stress, but also eliminates the major generation factory of ROS. Ablation of ATG5/6/7 disrupted autophagy and increased the production of ROS. Therefore, autophagy can also be regarded as an antioxidant mechanism to avoid cellular damage in a broader sense [26].

Despite complex connections between autophagy and oxidative stress, we still know little about whether this interplay will affect cardiomyopathies, and most studies concentrate on diabetic cardiomyopathy. Researchers found that diabetes increased oxidative stress and the level of oxidized proteins and lipids, disturbing cellular energy homeostasis. Therefore, autophagy plays an essential role in degrading

protein aggregates to minimize oxidative damage and protect cardiac structure and integrity. In addition, since the majority of ROS was generated in mitochondria, a mitochondrial targeted antioxidant mitoquinone attenuated cardiac dysfunction and brought back defective autophagy in another non-cardiac system [28].

As mentioned above, MYBP3 mutation is a critical genomic change that causes dilated cardiomyopathy. Oxidative stress was elevated in these mouse models, as evidenced by the changes of GSH/GSSG ratio, protein carbonyl, and lipid malondialdehyde [29]. Another study found that elevation of ALCAT1 led to oxidative stress and mitochondrial dysfunction in hypertrophic cardiomyopathy, which was ameliorated by ALCAT1 deficiency, suggesting that ALCAT1 may be the missing link in between [30]. Notably, H_2O_2-induced oxidative stress disrupted autophagic flux, and resveratrol attenuated it through SIRT1/FOXO1/Rab7 axis and thus ameliorated oxidative stress injury in hearts from diabetic mice [31].

5. CONCLUSION

Autophagy and oxidative stress are significant elements in the development of cardiomyopathies though the underlying mechanisms remain to be elucidated. No agreement has been reached about whether autophagy is cardioprotective or cardiotoxic. Most researchers believe that autophagy promotes DOX-induced dilated cardiomyopathy while ameliorates or reverses pathological change in other dilated cardiomyopathies. Many hypertrophic cardiomyopathies are caused by gene mutations, and defective autophagy contributes to the pathological development. Moreover, oxidative stress and autophagy have complex interplay with each other. ROS may act as an initiator of autophagy while autophagy is an essential mechanism to degrade oxidized products and avoid cellular damage. Therefore, we believe that the role of autophagy depends on the etiologies and types of cardiomyopathies and the various research models utilized. In most cases, autophagy is cardioprotective since it is a key mechanism to preserve cellular homeostasis. In some particular circumstances such as DOX-induced dilated cardiomyopathy, autophagy may exacerbate the diseases. Due to the importance and complexity of autophagy, further research is warranted to find possible therapeutic targets to stop

the progression of cardiomyopathy through regulating autophagy.

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REFERENCES

1. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation* 2006; 113(14):1807–16. doi: 10.1161/circulationaha.106.174287.

2. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J* 2008; 29(2):270–6. doi: 10.1093/eurheartj/ehm342.

3. McKenna WJ, Maron BJ, Thiene G. Classification, epidemiology, and global burden of cardiomyopathies. *Circ Res* 2017; 121(7):722–30. doi: 10.1161/CIRCRESAHA.117.309711.

4. Mei Y, Thompson M, Cohen R, Tong X. Autophagy and oxidative stress in cardiovascular diseases. *Biochim Biophys Acta* 2015; 1852(2):243–51. doi: 10.1016/j.bbadis.2014.05.005.

5. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell* 2011; 147(4):728–41. doi: 10.1016/j.cell.2011.10.026.

6. Kuma A, Mizushima N. Physiological role of autophagy as an intracellular recycling system: with an emphasis on nutrient metabolism. *Semin Cell Dev Biol* 2010; 21(7):683–90. doi: 10.1016/j.semcdb.2010.03.002.

7. Jung C, Seo M, Otto N, Kim D. ULK1 inhibits the kinase activity of mTORC1 and cell proliferation. *Autophagy* 2011; 7(10):1212–21. doi: 10.4161/auto.7.10.16660.

8. Russell R, Tian Y, Yuan H, Park H, Chang Y, Kim J, et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat Cell Biol* 2013; 15(7):741–50. doi: 10.1038/ncb2757.

9. Rabinowitz J, White E. Autophagy and metabolism. *Science* 2010; 330(6009):1344–8. doi: 10.1126/science.1193497.

10. Tanida I, Minematsu-Ikeguchi N, Ueno T, Kominami E. Lysosomal turnover, but not a cellular level, of endogenous LC3 is a marker for autophagy. *Autophagy* 2005; 1(2):84–91.

11. Mizushima N, Yoshimori T, Levine B. Methods in mammalian autophagy research. *Cell* 2010; 140(3):313–26. doi: 10.1016/j.cell.2010.01.028.

12. Yarana C, St Clair D. Chemotherapy-induced tissue injury: an insight into the role of extracellular vesicles-mediated oxidative stress responses. *Antioxidants (Basel)* 2017; 6(4):75. doi: 10.3390/antiox6040075.

13. O'Connell J, Romano M, Campos Pulici E, Carvalho E, de Souza F, Tanaka D, et al. Short-term and long-term models of doxorubicin-induced cardiomyopathy in rats: a comparison of functional and histopathological changes. *Exp Toxicol Pathol* 2017; 69(4):213–9. doi: 10.1016/j.etp.2017.01.004.

14. Navarro-Yepes J, Burns M, Anandhan A, Khalimonchuk O, del Razo L, Quintanilla-Vega B, et al. Oxidative stress, redox signaling, and autophagy: cell death versus survival. *Antioxid Redox Signal* 2014; 21(1):66–85. doi: 10.1089/ars.2014.5837.

15. Sun A, Cheng Y, Zhang Y, Zhang Q, Wang S, Tian S, et al. Aldehyde dehydrogenase 2 ameliorates doxorubicin-induced myocardial dysfunction through detoxification of 4-HNE and suppression of autophagy. *J Mol Cell Cardiol* 2014; 71:92–104. doi: 10.1016/j.yjmcc.2014.01.002.

16. Lai L, Chen J, Wang N, Zhu G, Duan X, Ling F. MiRNA-30e mediated cardioprotection of ACE2 in rats with doxorubicin-induced heart failure through inhibiting cardiomyocytes autophagy. *Life Sci* 2017; 169:69–75. doi: 10.1016/j.lfs.2016.09.006.

17. Li S, Wang W, Niu T, Wang H, Li B, Shao L, et al. Nrf2 deficiency exaggerates doxorubicin-induced cardiotoxicity and cardiac dysfunction. *Oxid Med Cell Longev* 2014; 2014:748524. doi: 10.1155/2014/748524.

18. Xu X, Bucala R, Ren J. Macrophage migration inhibitory factor deficiency augments doxorubicin-induced cardiomyopathy. *J Am Heart Assoc* 2013; 2(6):e000439. doi: 10.1161/JAHA.113.000439.

19. Choi J, Worman H. Reactivation of autophagy ameliorates LMNA cardiomyopathy. *Autophagy* 2013; 9(1):110–1. doi: 10.4161/auto.22403.

20. Choi J, Wu W, Muchir A, Iwata S, Homma S, Worman H. Dual specificity phosphatase 4 mediates cardiomyopathy caused by lamin A/C (LMNA) gene mutation. *J Biol Chem* 2012; 287(48):40513–24. doi: 10.1074/jbc.M112.404541.

21. Maejima Y, Kyoi S, Zhai P, Liu T, Li H, Ivessa A, et al. Mst1 inhibits autophagy by promoting the interaction between Beclin1 and Bcl-2. *Nat Med* 2013; 19(11):1478–88. doi: 10.1038/nm.3322.

22. Singh S, Zech A, Geertz B, Reischmann-Düsener S, Osinska H, Prondzynski M, et al. Activation of autophagy ameliorates cardiomyopathy in Mybpc3-targeted knockin mice. *Circ Heart Fail* 2017; 10(10): e004140. doi: 10.1161/CIRCHEARTFAILURE.117.004140.

23. Endo Y, Furuta A, Nishino I. Danon disease: a phenotypic expression of LAMP-2 deficiency. *Acta Neuropathol* 2015; 129(3):391–8. doi: 10.1007/s00401-015-1385-4.

24. Xu X, Roe N, Weiser-Evans M, Ren J. Inhibition of mammalian target of rapamycin with rapamycin reverses hypertrophic cardiomyopathy in mice with cardiomyocyte-specific knockout of PTEN. *Hypertension* 2014; 63(4):729–39. doi: 10.1161/HYPERTENSIONAHA.113.02526.

25. Song L, Su M, Wang S, Zou Y, Wang X, Wang Y, et al. MiR-451 is decreased in hypertrophic cardiomyopathy and regulates autophagy by targeting TSC1. *J Cell Mol Med* 2014; 18(11):2266–74. doi: 10.1111/jcmm.12380.

26. Morales C, Pedrozo Z, Lavandero S, Hill J. Oxidative stress and autophagy in cardiovascular homeostasis. *Antioxid Redox Signal* 2014; 20(3):507–18. doi: 10.1089/ars.2013.5359.

27. Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: the clash between damage and metabolic needs. *Cell Death Differ* 2015; 22(3):377–88. doi: 10.1038/cdd.2014.150.

28. Varga Z, Giricz Z, Liaudet L, Haskó G, Ferdinandy P, Pacher P. Interplay of oxidative, nitrosative/nitrative stress, inflammation, cell death and autophagy in diabetic cardiomyopathy. *Biochim Biophys Acta* 2015; 1852(2):232–42. doi: 10.1016/j.bbadis.2014.06.030.

29. Lynch T, Sivaguru M, Velayutham M, Cardounel A, Michels M, Barefield D, et al. Oxidative stress in dilated cardiomyopathy caused by MYBPC3 mutation. *Oxid Med Cell Longev* 2015; 2015:424751. doi: 10.1155/2015/424751.

30. Liu X, Ye B, Miller S, Yuan H, Zhang H, Tian L, et al. Ablation of ALCAT1 mitigates hypertrophic cardiomyopathy through effects on oxidative stress and mitophagy. *Mol Cell Biol* 2012; 32(21):4493–504. doi: 10.1128/MCB.01092-12.

31. Wang B, Yang Q, Sun YY, Xing YF, Wang YB, Lu XT, et al. Resveratrol-enhanced autophagic flux ameliorates myocardial oxidative stress injury in diabetic mice. *J Cell Mol Med* 2014; 18(8):1599–611. doi: 10.1111/jcmm.12312.