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Autophagy and p62 in cardiac protein quality control

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This is an addendum to a recent report which demonstrates for the first time that autophagic flux is increased in the heart of a well-established mouse model of cardiac proteinopathy and p62 is transcriptionally upregulated in cardiomyocytes and hearts overexpressing human cardiomyopathy-linked misfolded proteins. The p62 plays a critical and protective role in aggresome formation and autophagic activation in cardiomyocytes overexpressing misfolded proteins.

Keywords: autophagy, p62, cardiomyocytes, protein aggregation, desmin-related cardiomyopathy

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By facilitating protein folding/refolding or by removing terminally misfolded proteins in the cell, protein quality control (PQC) is vital to the functioning and the fate of the cells, especially the postmitotic cells, such as cardiomyocytes and neurons. PQC depends upon intricate collaborations between chaperones and targeted proteolysis. PQC-associated proteolysis is primarily performed by the ubiquitin-proteasome system. However, it is increasingly suggested that (macro)autophagy may also play a role, at least a supplemental role, in removing terminally misfolded proteins, especially when the proteasome is impaired or overwhelmed.

The most expressed chaperone protein in cardiac and skeletal myocytes is αB-crystallin (CryAB). The importance of cardiac PQC is best illustrated by the linkage of a missense mutation (R120G) of CryAB (CryAB^{R120G}) to human desmin-related myopathy (DRM), a heterogeneous group of myopathies that can also be caused by mutations in the gene encoding desmin or its partner proteins. The cardiac aspect of DRM, known as desmin-related cardiomyopathy (DRC), is often the life-threatening part of DRM. The pathological hallmark of DRC is the presence of desmin-positive aggregates in cardiomyocytes, identifying DRC as a bona fide cardiac proteinopathy. In the past decade, studies on DRC have contributed remarkably to elucidating the pathophysiology of proteinopathy and given rise to a provocative hypothesis that PQC inadequacy is a major pathogenic factor in the progression of a large subset of common heart diseases that lead to congestive heart failure, the final common pathway of virtually all heart diseases and a leading cause of death.

As evidenced by the presence of abundant pre-amyloid oligomers and markedly elevated levels of ubiquitinated proteins in most explanted failing human hearts, aberrant protein aggregation and PQC inadequacy are likely common pathological processes in the development of heart failure. DRC/DRM per se are not common but mouse models of DRC created via cardiomyocyte-restricted overexpression of DRM-linked mutant genes, such as CryAB^{R120G} and a 7-amino acid (R172 through E178) deletion mutation of the desmin gene (D7-des), have served as invaluable platforms for studying cardiac PQC and proteinopathy. As a result, we and others have unraveled proteasome functional insufficiency, mitochondrial dysfunction, cardiomyocyte cell death, and perhaps reductive stress in DRC pathogenesis.

Prior to our report, for which this addendum is written, increased autophagosomes in CryAB^{R120G}-based DRC mouse hearts were described, but autophagic flux had not been determined in a model of cardiac proteinopathy. p62 was purported to mediate the formation

of inclusion bodies and activate selective autophagy but this had not been demonstrated in cardiomyocytes. The role of p62 in PQC in the cell appears to be cell type-dependent, but p62 had virtually not been studied in the heart. Moreover, the molecular mechanisms of PQC, particularly in cardiomyocytes, remain incompletely delineated. Hence, we sought to fill the void and were able to discern for the first time that (1) autophagic flux is adaptively increased in a bona fide mouse model of cardiac proteinopathy; (2) pharmacological enhancement of autophagy facilitates the removal of misfolded proteins in cardiomyocytes; (3) p62 is transcriptionally upregulated in cardiomyocytes and the heart by overexpression of $CryAB^{R120G}$ or D7-des; and (4) p62 plays a likely pivotal and protective role in cardiomyocytes in mediating two major defending mechanisms against increased misfolded proteins: aggresome formation and the activation of selective autophagy. In addition to our conclusions mentioned above, we have also come across a couple of intriguing phenomena deserving further elaboration and discussion.

First, a striking discrepancy is observed between the abundant biochemical markers and the very modest morphological evidence of autophagic vesicles revealed by transmission electron microscopy (TEM) in D7-des transgenic mouse hearts. The protein levels of p62 are substantially increased in both $CryAB^{R120G}$ and D7-des hearts. Immunofluorescence labeling shows that the upregulated p62 is almost exclusively in the protein aggregates in D7-des hearts. When GFP-LC3 is expressed, p62 also colocalizes largely with GFP-LC3. Increased lysosomal protease cathepsin D activity is observed in D7-des hearts and the majority of GFP-LC3 puncta show positive immunostaining for cathepsin D. However, we can only rarely detect autolysosome-like vesicles in some of the cells using TEM. The prevalence of autophagosomes in the D7-des hearts is indeed increased after the mice are treated with bafilomycin A_1 to inhibit lysosomal removal of autophagosomes; but still the prevalence of autophagic vesicles in the D7-des heart is much lower than what we expected from the degree of upregulation of p62 or LC3-II. This discrepancy raises a possibility that p62, LC3-II, and perhaps cathepsin D function in an undocumented pathway, in addition to macroautophagy, to handle aberrant protein aggregates.

Second, autophagosomes do not appear to engulf the large protein aggregates or aggresomes. Reports from studies of non-cardiac cells have often claimed that p62 promotes the formation of large protein aggregates or aggresomes, which not only segregates the toxic misfolded proteins but also makes the misfolded proteins more easily removed by autophagy. This gives an impression that aggresomes are directly removed by autophagy. Indeed, many large protein aggregates, both perinuclear and scattered throughout the cell, are observed in cardiomyocytes overexpressing D7-des, as is the case in hearts overexpressing $CryAB^{R120G}$. However, we cannot find a single autophagic vacuole that contains large protein aggregates, even after blocking the fusion with lysosomes. Instead, the autophagic vesicles are usually much smaller than the aggresome. They are located in the vicinity of the large aggregates, but engulfed small aggregates or amorphous electron-dense materials. These observations suggest two nonexclusive possibilities: (1) autophagy may catch small aggregates on their way to coalesce with the aggresome; (2) proteins in aggresomes need to be broken off the main hub before being removed by autophagy.

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