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Cardiac ubiquitin ligases: Their role in cardiac metabolism, autophagy, cardioprotection and therapeutic potential☆

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ABSTRACT

Both the ubiquitin-proteasome system (UPS) and the lysosomal autophagy system have emerged as complementary key players responsible for the turnover of cellular proteins. The regulation of protein turnover is critical to cardiomyocytes as post-mitotic cells with very limited regenerative capacity. In this focused review, we describe the emerging interface between the UPS and autophagy, with E3's regulating autophagy at two critical points through multiple mechanisms. Moreover, we discuss recent insights in how both the UPS and autophagy can alter metabolism at various levels, to present new ways to think about therapeutically regulating autophagy in a focused manner to optimize disease-specific cardioprotection, without harming the overall homeostasis of protein quality control. This article is part of a Special Issue entitled: The role of post-translational protein modifications on heart and vascular metabolism edited by Jason R.B. Dyck & Jan F.C. Glatz.

1. Introduction

There is growing recognition of the role that protein quality control systems play in the maintenance of the heart during health and disease. These include the ubiquitin-proteasome system (UPS), which is responsible for the turnover of many cellular proteins at the molecular level, with the complementary lysosomal autophagy system clearing dysfunctional organelles (e.g. mitochondria), damaged macromolecules, and larger aggregate-prone proteins (pre-amyloid oligomers) [1,2]. With a greater understanding of the diverse role that the UPS and autophagy play in the heart, we have identified new and novel links with metabolism that build upon simpler first constructs. These findings provide additional ways in which cardiac metabolism may be regulated therapeutically through the manipulation of specific ubiquitin ligase activities, or more broadly in disease context-specific short-term regulation of autophagy.

1.1. Why does protein quality control matter?

Mechanisms that regulate protein turnover and prevent protein aggregation either through refolding and/or degradation are critical to the heart in ways that differ from most cells in the body. This is because the cardiomyocytes, like neurons, are post-mitotic cells with very limited regenerative capacity, in contrast to skin or gut epithelial cells [3]. Evolutionarily, the autophagic removal of damaged organelles [4,5] and misfolded proteins by the ubiquitin-proteasome system [6] allows maintenance of cardiac function. Recent studies illustrate that the regulation of these two systems additionally controls cellular metabolism [7–10]. As the mechanisms that link these two systems with metabolism become clearer, opportunities to intervene and protect the heart in disease may more obvious.

2. Metabolism and autophagy

Due to the high energy demands of the heart, the ability to extract energy (i.e. produce ATP) from variable sources is critical. One contributing factor may be the limited energy reserves available to the heart [11]. The

Abbreviations: ATG, autophagy-related gene; AMPK, AMP activated protein kinase; BECN1, beclin1; b-TrCP, beta-transducin repeat-containing protein; Cbl, Casitas B-lineage lymphoma; CHIP, Carboxyl terminus of Hsc70 interacting protein; CHMP2B, charged multivesicular body protein 2B; DEPTOR, DEP-domain-containing mTOR-interacting protein; DFCP1, double FYVE-containing protein 1; HDAC, histone deacetylases; I/R, ischemia/reperfusion; mTOR, mammalian target of rapamycin; MuRF1, muscle ring finger-1; NEDD4, neural precursor cell-expressed developmentally; RNF, RING finger protein; SILAC, stable isotope labeling of amino acids in cell culture; TRAF6, TNF-receptor-associated factor 6; TAC, trans-aortic constriction; TRIM, Tripartite motif-containing protein; UPS, ubiquitin proteasome system; VSP34, vacuolar sorting protein 34.

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links between autophagy and metabolism are most evident in time of crisis and/or adaptation as seen during mammalian fetal development. For example, at birth, the maternal energy source is interrupted [12], leaving the newborn heart to function without nutrition until milk arrives. The activation of autophagy has been reported in the mouse heart within 30 min after birth, staying elevated for at least 24 h [13]. With maternal delivery of nutrition, autophagy is inhibited by the insulin-mediated downregulation of protein degradation [14].

2.1. Autophagy, amino acids, and ATP in starvation

In the adult heart, starvation-induced autophagy can fuel the heart in multiple ways, including the degradation of nucleic acids, the breakdown of proteins, and the lysosomal digestion of lipids and sugars [15]. In as little as 12 h without nutrition, cardiac autophagy is upregulated; however, the significance of this metabolic source can best be seen when it is blocked [16]. Blocking autophagic activity has been reported to accelerate cell death and reduce cardiac performance while decreasing available amino acids and ATP in the heart [16]. Conversely, stimulating autophagy has been reported to protect against starvation by limiting ATP loss and attenuating ER stress [17].

2.2. Autophagy regulation of glucose and lipid metabolism in the heart

While fatty acid is the substrate utilized most in the adult heart (cardiomyocytes), glucose metabolism has important roles in the heart. For example, the fetal heart primarily utilizes glucose as a substrate for ATP production. Similarly, cardiac stress in the adult heart induces a shift away from fatty acid utilization toward glucose as a source of energy. [11], as is seen in human heart failure [18,19], with regulation attributed to inhibition of PPARα activity [20], HIF-1α, and other transcription factors (discussed in more detail next). In parallel, the mammalian target of rapamycin (mTOR) regulates autophagy in the failing heart [21]. During nutrient deprivation, autophagy activity is increased by at least two mechanisms, including AMP activated protein kinase (AMPK) mediated activation of autophagy-initiating kinase Ulk1 [22] and by upregulation of the hexokinase-II enzyme, a glycolytic enzyme that protects against starvation by inhibiting mTORC1 [23]. Interestingly, impaired lipid degradation by autophagy (a process termed lipophagy) has been reported to contribute to the accumulation of toxic lipids [24]. In the context of high-fat diet induced accumulation of lipids, the proper autophagic clearance of lipids has proven important in attenuating reactive oxygen species [25–27]. The attenuation of the lipotoxic cardiomyopathy in pressure overload-induced heart failure [28] similarly demonstrates the importance of autophagy in clearing lipids in cardiac stress, a process regulated by oxidized phospholipids [29].

3. Autophagy in the heart

Autophagy is a highly conserved protein quality control system that catabolizes misfolded and aggregated proteins as well as damaged, worn organelles for recycling and energy homeostasis. Autophagosome nucleation begins with the activation of several autophagy-related gene (ATG) proteins and with the help of VPS34 (class III phosphoinositide 3 kinase vacuolar sorting protein 34) and BECLIN1 (Fig. 1A). A double-membraned isolation membrane elongates and forms around the ubiquitinated substrate of interest (Fig. 1B). Adaptor proteins, like p62 (also known as sequestosome 1), bind ubiquitin and assist with docking substrate cargo inside the phagophore, to later be degraded. Closing and sealing of the membrane completes autophagosome formation (Fig. 1C). Finally, lysosome and autophagosome fuse to form the autolysosome. Acidic hydrolases from the lysosome degrade the contents of the autolysosome (Fig. 1D). The resulting molecular components (carbohydrates, lipids, amino acids, and nucleic acids) are released to support cellular metabolism and homeostasis [30–32]. Autophagic flux refers to the entire process of autophagy, beginning with the formation of an autophagosome around the cargo to be degraded and ending with the release of degraded macromolecules into the cytosol [33].

Under basal conditions or low stress, autophagy occurs at low levels to maintain homeostasis. These low levels of autophagy are critical for cell survival. Cellular homeostasis requires a minimal amount of autophagic flux. Inhibiting autophagy disrupts a cell's homeostasis and can lead to cell death [30]. Alternatively, autophagy responds rapidly to stress, particularly nutrient starvation, which elicits a robust enhancement of autophagic flux to meet the energy needs of the cell [30]. Specifically, cardiac autophagy is required for metabolic adaptation by providing amino acids, glucose, and lipids, as described above.

4. Role of autophagy in common heart diseases

Our understanding of the role of autophagy in cardiac injury has grown tremendously in recent years. It's significance, and context-dependent cardioprotection has recently been reviewed in depth (see Schiattarella and Hill [34]). We briefly summarize the role of autophagy in ischemic heart disease, pathological cardiac hypertrophy, and chemotherapy-induced cardiotoxicity.

4.1. Ischemic heart disease

The number of people living with ischemic heart disease continues to increase; in fact, it was the worldwide leading cause of death in 2010 [35]. During ischemic heart disease, narrowed coronary arteries cause a reduction of blood flow to the heart resulting in ischemia. Injury results from both cardiac ischemia and cardiac reperfusion, each triggering stress in distinct ways, however cardiac autophagy has been shown to be cardioprotective during both phases [36–38]. The energy depletion observed during ischemia triggers autophagic mechanisms to replenish metabolic substrates and to remove damaged organelles [39]. Nutrient depletion results in the activation of AMPK (via increased AMP resulting from utilization of ATP), which then phosphorylates (inactivates) mTOR and thus disinhibits autophagy to enhance flux [40,41]. During reperfusion, restoration of oxygen and nutrients lead to the massive production of reactive oxygen species (ROS). As the electron transport chain becomes active in the presence of reestablished oxygen, mitochondrial ROS amplification results from ROS-induced ROS release [42]. Damaged proteins and organelles from lipid peroxidation-driven ROS enhances autophagy to clear damaged organelles that would further increase oxidative stress and cellular dysfunction [42].

Interestingly, there have been reports for and against the benefits of autophagy during ischemia and reperfusion. Some reports show that enhances in autophagy are cardioprotective during ischemia/reperfusion (I/R) and serve to salvage the myocardium [43]. Similarly, chronic ischemia in a porcine model demonstrated an association between elevated autophagy and reduced apoptosis [44]. Yet, other reports show that autophagy and cell death correlate and that inhibition of autophagy reduces cell death [45,46]. Interestingly, work by Matsui et al. demonstrates that autophagy may have dual roles and may be protective during ischemia but contributes to cell death during reperfusion [40]. In this study, mouse hearts subjected to ischemia resulted in the induction of enhanced autophagy in an AMPK-dependent and cardioprotective manner, but mouse hearts subjected to I/R showed beclin1-dependent upregulation of autophagy that was not beneficial to the heart [40]. These data are confusing, making inferences of the benefits of autophagy during ischemia and reperfusion difficult. The field of autophagy research is young and differing results are likely a result of different models, mechanisms, and tools used to measure and induce or inhibit autophagy at different times during the I/R continuum. At this time, it appears that autophagy may provide differing levels of cardioprotection during the ischemia and reperfusion phases: autophagy during I/R can afford cardioprotective effects by providing ATP during the ischemia

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