Review

Cardiomyocyte health: adapting to metabolic changes through autophagy

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Autophagy is important in the heart for maintaining homeostasis when changes in nutrient levels occur. Autophagy is involved in the turnover of cellular components, and is rapidly upregulated during stress. Studies have found that autophagy is reduced in metabolic disorders including obesity and diabetes. This leads to accumulation of protein aggregates and dysfunctional organelles, which contributes to the pathogenesis of cardiovascular disease. Autophagy is primarily regulated by two components: the mechanistic target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK). Although mTOR integrates information about growth factors and nutrients and is a negative regulator of autophagy, AMPK is an energy sensor and activates autophagy when energy levels are low. These pathways therefore present targets for the development of autophagy-modulating therapies.

Alterations in metabolism impact autophagy in the heart

Autophagy (see Glossary) is an important mechanism that tissues utilize to maintain cellular homeostasis, and defective autophagy has been implicated in a wide range of pathologies including heart disease. The heart utilizes autophagy to maintain cellular homeostasis under both baseline conditions and in response to stress [1–4]. Although many tissues of the body are susceptible to changes in nutrient supply, it is particularly important for cardiomyocytes to be able to adapt to changes in metabolite supply to sustain contraction. Changes to nutrients, energy status, oxygen levels, or external stresses all have the potential to disrupt heart function, and it is crucial that the heart continues to function despite these major metabolic changes. Changes in metabolism that can directly affect autophagy in the heart include both chronic and acute conditions. Chronic conditions include obesity and metabolic syndrome, resulting in elevated circulating lipid and insulin levels [5]. Acute events such as a myocardial infarction (MI) result in insufficiency of oxygen and glucose supply to a region of the heart, and many studies suggest that upregulation of autophagy in response to acute cardiac stress is cardioprotective and important for minimizing myocardial damage [6,7]. By contrast, the reduced autophagic flux that is observed in mouse models of obesity [8], diabetes [9], and metabolic syndrome [10] is thought to

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Glossary

Akt: a serine/threonine kinase that regulates numerous cellular survival processes including cell proliferation, apoptosis, and autophagy. An important survival kinase in the heart, Akt is activated via phosphorylation by PI3K and participates in activation of mTOR in response to insulin and growth factors.

AMP-activated protein kinase (AMPK): a serine/threonine kinase that is important for sensing changes in energy levels in the cell. AMPK responds to low cellular energy levels by upregulating autophagy through activation of ULK1, disinhibition of Beclin 1, or activation of FoxO transcription factors. Activation of AMPK occurs in response to a decline in ATP:AMP ratio and results in decreasing fatty acid and protein synthesis, and increased glucose transport.

Autophagy: a catabolic cellular process that removes macromolecular structures by lysosomal degradation. Autophagy is important for the constitutive turnover of long-lived proteins and dysfunctional organelles under baseline conditions, and is rapidly upregulated for stress adaptation. Impaired autophagy leads to rapid heart failure.

Beclin 1: an autophagy-promoting protein that forms a complex with VPS34 and AMBRA1 to initiate phagophore formation. Beclin 1 is a BCL-2 homology domain 3 (BH3)-containing protein that is maintained in an inactive state by binding to BCL-2.

Forkhead box protein O (FoxO): a family of transcription factors that regulate the transcription of genes associated with cell proliferation and homeostasis. FoxO1 and FoxO3 proteins are activated by AMPK and activate the transcription of autophagy-promoting genes.

Metformin: an antidiabetic drug that activates AMPK to decrease hepatic glucose production, increase insulin sensitivity, and reduce circulating triglyceride and LDL–cholesterol levels. Metformin activates autophagy in several cell types.

mTOR (mechanistic target of rapamycin)/mTORC1: mTOR is part of the mTOR complex 1 (mTORC1), a master regulator of autophagy. The mTORC1 complex is activated by signaling of insulin/growth factors, amino acids, and nutrients to inhibit autophagy. Conversely, deficiency of nutrients leads to induction of autophagy by mTORC1 deactivation.

Phagophore: a double membrane that encloses and isolates the cytoplasmic components during macroautophagy.

RAGs (Ras-related GTPases): these GTPases form a complex and respond to changes in intracellular amino acid levels. In the presence of amino acids, the active (RAG–GTP) complex promotes mTORC1 localization to the lysosome, where it can interact with RHEB to suppress autophagy. Amino acid deficiency deactivates the RAG complex and promotes mTORC1 deactivation and movement to the cytosol.

Ras homolog enriched in brain (RHEB): a GTP-binding protein that activates mTORC1 in response to both growth factor/insulin signaling and amino acid sensing. GTP-bound RHEB activates mTORC1, thereby inhibiting autophagy. RHEB is inactivated by TSC1/2, and inactive GDP-bound RHEB promotes autophagy via mTORC1 deactivation.

Tuberous sclerosis 1/2 (TSC1/2): membrane proteins that form a complex that is inhibited by Akt. Inhibition of TSC1/2 by Akt promotes RHEB activation, activation of mTORC1, and autophagy suppression.

ULK1 (unc-51-like autophagy activating kinase 1): a kinase that regulates autophagy in response to signals from mTORC1 and AMPK. AMPK phosphorylates and activates ULK1 to promote autophagy, whereas phosphorylation by mTORC1 inhibits ULK1 activation by AMPK.

Vacuolar protein sorting (VPS) 34: class III phosphatidylinositol 3-kinase that forms a complex with Beclin 1 and AMBRA1 to promote autophagosome formation. Vps34 converts phosphatidylinositol (PI) to phosphatidylinositol 3-phosphate (PI3P).

contribute to disease pathology, including the development of heart failure. Long-term dyslipidemia, defective insulin signaling, and other chronic metabolic changes therefore impact the cellular stress response of the heart in ways distinct from acute damage.

In this review we discuss how changes in energy levels, nutrients, and growth factor availability regulate autophagy in the heart, as well as how repression of autophagy in disease states contributes to cardiovascular diseases. We also address the feasibility of specifically targeting autophagy, and how this represents a new avenue in the treatment or prevention of heart disease.

Molecular pathways involved in autophagy

Autophagy begins with phagophore nucleation that is promoted by a complex composed of three proteins: Beclin 1, vacuolar protein sorting 34 (VPS34), and VPS15. The phagophore then elongates via a mechanism that depends on autophagy (Atg) proteins and microtubule-associated protein 1 light chain 3 (LC3) [5,11]. The mature autophagosome engulfs its target material before fusing with a lysosome and degrading the cargo. The amino acids and other components of the degraded material are then transported to the cytosol and reused. Autophagy occurs continuously under baseline conditions in the heart, and impairment of this process results in rapid accumulation of protein aggregates and dysfunctional organelles, leading to heart failure [1,2,7]. Autophagy is rapidly upregulated in the myocardium in response to stress, or to changes to nutrient supply, in an effort to maintain homeostasis [3,4,6,7]. In addition, mitochondria are responsible for the production of ATP for cellular energy through oxidative phosphorylation, but dysfunctional mitochondria generate excessive reactive oxygen species (ROS) and can promote cell death by releasing death-promoting factors. Degradation of dysfunctional mitochondria by autophagy not only prevents cell damage [7,8,12] but also streamlines energy production and utilization [13,14], and this is particularly important in cardiomyocytes that are densely packed with mitochondria.

mTOR is a central regulator of autophagy and metabolism

mTOR is a conserved serine/threonine kinase that regulates cell growth and autophagy by integrating growth factor and nutrient signals [15]. mTOR is activated under nutrient-rich conditions and promotes cell growth in part by suppressing autophagy. Insufficiency of nutrients or growth factors results in mTOR inactivation and induction of autophagy. mTOR is part of the mTORC1 signaling complex which includes the scaffolding protein RAPTOR (regulatory-associated protein of mTOR) as well as PRAS40 (proline-rich Akt substrate 40 kDa), DEPTOR (DEP domain-containing mTOR-interacting protein), and the mTOR associated protein, LST8 homolog (MLST8).

mTOR regulates metabolism by activating specific transcription factors that regulate the expression of enzymes involved in metabolic pathways. For instance, mTORC1 activates peroxisome proliferator-activated receptor γ (PPARγ), a nuclear receptor that regulates the expression of genes involved in fatty acid synthesis and uptake [16]. mTORC1 also activates hypoxia-inducible factor 1α (HIF1α), which promotes the expression of genes that regulate glucose transport and glycolysis [17]. In addition, mTORC1 activates PPARγ coactivator-1 (PGC1α), a nuclear cofactor that regulates mitochondrial biogenesis and the expression of proteins involved in mitochondrial metabolism [18].

Growth factor-dependent mTOR activation

The presence and binding of growth factors and/or insulin to their membrane receptors activates signaling pathways that lead to mTORC1 activation and subsequent inactivation of autophagy. The activation of mTORC1 can occur through an AKT–TSC1/2- or an AKT–PRAS40-dependent manner. In the former, Akt phosphorylates and inactivates the tuberous sclerosis (TSC) 1/2 complex [19]. The TSC1/2 complex is a GTPase-activating protein that promotes the conversion of active, GTP-bound RHEB (Ras homolog enriched in brain) to the inactive GDP-bound RHEB. GTP-bound RHEB is a crucial activator of mTORC1, and inhibition of TSC1/2 by Akt results in increased GTP-bound RHEB, activation of mTORC1, and suppression of autophagy (Figure 1). Akt can also promote activation of mTORC1 in a TSC1/2-independent manner. PRAS40 is a RAPTOR-interacting protein and a constitutive inhibitor of mTORC1. Phosphorylation of PRAS40 by Akt leads to dissociation of PRAS40 from RAPTOR, and subsequent activation of mTORC1 [20].

Amino acid-dependent mTOR activation

The activity of mTORC1 is also independently regulated by intracellular levels of amino acids [21]. When the levels of amino acids present in the cell are sufficient, mTORC1 receives signals that promote its activity and suppress autophagy [22]. In contrast to growth factors, amino acids do not rely on inhibition of TSC1/2 to activate mTORC1 [23]. Instead, the Ras-related GTP-binding (RAG) GTPases play a key role in transducing amino acid signaling to mTORC1 [21]. In the presence of amino acids, active RAG GTPases recruit mTORC1 to the exterior of the lysosome, where it is activated by RHEB [24]. RHEB is responsible for coupling both growth factor signaling and amino acid levels to mTORC1 [25]. However, growth factors in the absence of amino acids do not induce translocation of mTORC1 to lysosomes, and RNAi-mediated knockdown of RHEB abrogates mTORC1 activation by amino acids but does not interfere with the amino acid-induced movement of mTOR to lysosomes [24]. This suggests that translocation of mTORC1 to the lysosomal surface occurs independently of mTORC1 activity and does not require RHEB or growth factors.

mTORC1 activity is crucial for preservation of heart function

The importance of amino acid-dependent activation of mTORC1 was recently demonstrated by Efeyan *et al.* [26]. Upon disruption of the maternal nutrient supply at birth, neonates activate autophagy to adapt to starvation until suckling is established [27]. Mice expressing a constitutively active RAG GTPase have reduced early postnatal survival due to a failure to induce autophagy in tissues