Review Article

Maladaptive autophagy in diabetic heart disease

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Abstract

Diabetic cardiomyopathy (DCM) is a distinct phenomenon associated with the development of cardiac dysfunction and loss of cardiovascular cells in the absence of common predisposing factors, such as atherosclerosis and hypertension. However, the exact mechanisms mediating augmented cellular death in DCM remain to be elucidated. Autophagy is a physiological cellular degradation pathway that plays an important role in the cellular homeostasis, which could become maladaptive with stress. The role of this maladaptive autophagy in the evolution of DCM is unknown. This review describes the molecular mechanisms involved in DCM and autophagy. Based on the available evidence, the review discusses the possible key role of autophagy in DCM.

Key words: Autophagy, cardiovascular disease, diabetes, molecular mechanisms

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INTRODUCTION

Diabetes mellitus (DM) is one of the most abundant chronic diseases worldwide which continue to increase rapidly and significantly. According to the World Health Organization, diabetes will be the seventh leading cause of death by the year 2030.[1,2] In New Zealand, 4% of the adult population has diagnosed diabetes and further 3% is estimated to have undiagnosed diabetes.[3]

Among different types of DM, Type-2 DM (T2DM) comprises >90% of individuals with diabetes all over the world.[2,4] T2DM is a result of the body not producing sufficient insulin, or the cells in the body do not recognize the insulin that is produced. The result of the imbalance between insulin responsiveness and insulin production leads to hyperglycemia (or high glucose levels in the blood).[4] Hyperglycemia eventually leads to many systemic effects such as neuropathy, nephropathy, retinopathy, and cardiovascular diseases (CVDs).[5]

CVD is the most common cause of death in individuals with T2DM, with 60% of the diabetic deaths are due to cardiovascular complications.[6] According to clinical, epidemiological, and pathological studies, incidence of heart failure (HF) in diabetic individuals is significantly higher than their nondiabetic peers due to a high incidence of diastolic and/or systolic left ventricular (LV) dysfunction.[7] Furthermore, individuals with T2DM are at a higher risk of developing CVD as compared to those with Type-1 DM.[6,7] A 7-year follow-up study on diabetic individuals reported the prevalence of HF as 3.7% in individuals with Type-1 DM, and in contrast, the prevalence of HF in individuals with T2DM was 11.8%.[8] This high prevalence of HF associated with T2DM is mainly due to a lack of understanding of the pathophysiological mechanisms involved in the progression of diabetic cardiomyopathy (DCM).

Autophagy is a catabolic process involving self-digestion of cellular organelles such as proteins, carbohydrates, and lipids as a measure to remove the waste products of metabolism and provide nutrition during demand.[9] Therefore, autophagy plays a vital role under physiological conditions.[9,10] In addition, autophagy also plays a protective or a pathological role depending on the type and duration of the stress conditions. However, the role of autophagy in diabetes-induced CVD is not clear.

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In this review, we discuss the pathogenesis and molecular mechanisms of DCM and autophagy, and based on the available evidence, we will discuss the possible key role for maladaptive autophagy in diabetic heart.

DIABETIC CARDIOMYOPATHY

DCM was first reported in 1972 by Rubler *et al*., and DCM as a clinical entity remains ambiguous despite decades of investigations.[11] DCM can be defined as structural and functional abnormalities of the myocardium without any associated coronary artery disease (CAD) or hypertension in diabetic individuals, leading to a progressive loss of cardiac cells that are replaced by fibrotic matrix.[11-13] Several pathophysiological mechanisms have been proposed to explain the structural and functional changes associated with DCM [Figure 1].

PATHOGENESIS OF DIABETIC CARDIOMYOPATHY

Functional changes

Cardiac dysfunction can be categorized as either diastolic dysfunction (DD) or systolic dysfunction (SD) depending on the phase of cardiac cycle that is impaired.[13,14] DD is an early manifestation of the DCM which is characterized by impaired relaxation in the ventricles along with reduced LV compliance.[15] Impaired LV relaxation is defined as reduced early and increased late diastolic flow. This is measured by mitral valve flow, where there are two distinct peaks, named early ventricular filling (E-wave) and late (A-wave) ventricular filling. The ratio of these two peaks (E/A ratio) is a good index for DD.[14,16] Individuals with an E/A ratio <0.6 are likely to have impaired relaxation, whereas E/A ratios >1.5 are likely to have restrictive patterns.[17] In addition to E/A ratio, E/E′ ratio (E′ is the early diastolic mitral annular velocity) has also been validated as another index of DD.[18] Evidence suggests that these indices are impaired in T2DM individuals.[6] This could be due to a number of molecular alterations listed in Table 1. SD is characterized by the inability of the ventricle to adequately maintain the cardiac output and this is associated with LV dilation, hypertrophy, and reduced ejection fraction.[16,19] Using tissue Doppler strain

Table 1: Summarization of factors leading to diastolic dysfunction and systolic dysfunction

Myocardial dysfunction	Factors leading to myocardial dysfunction
Diastolic dysfunction	Altered calcium handling Increased cardiac lipid accumulation Elevated ROS
Systolic dysfunction	Altered calcium handling Activation of RAS Cell death-apoptosis, necrosis, autophagy

ROS: Reactive oxygen species, RAS: Renin-angiotensin system

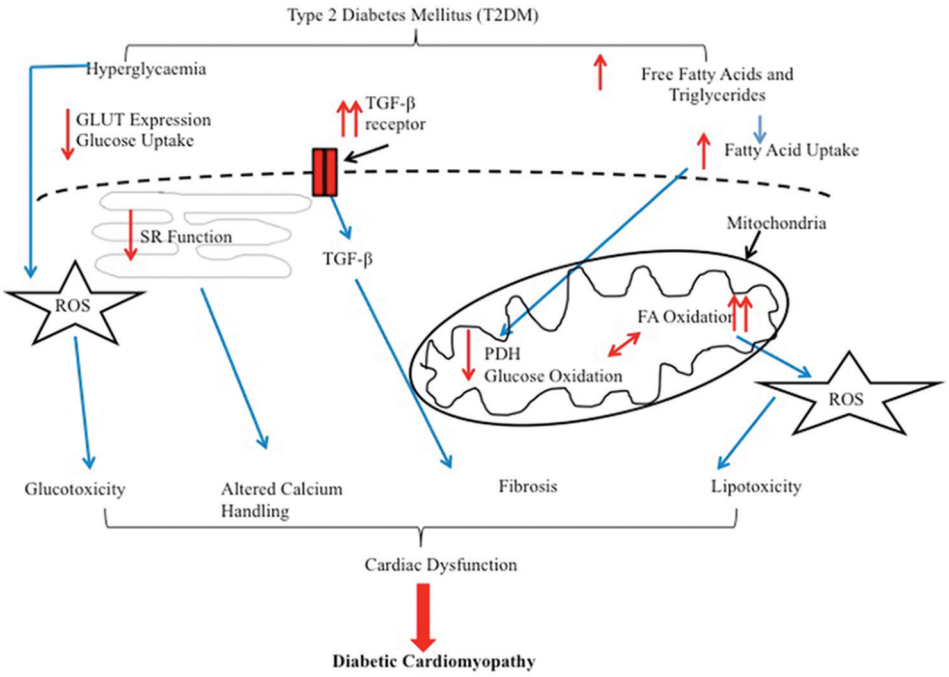

Figure 1: Overall schematic diagram representing the pathogenesis of DCM. Increased FFAs activate the transcription of many genes involved in FA oxidation. Increased FA oxidation leads to the generation of ROS at the level of the electron transport chain. ROS play a critical role in several pathways involved in the pathogenesis of DCM, including lipotoxicity, cell death, and tissue damage. TG: Triglyceride, GLUTs: glucose transporters, PDH: pyruvate dehydrogenase, TGF-β: Transforming growth factor β, SR: Sarcoplasmic reticulum, FFA: Free fatty acid, DCM: Diabetic cardiomyopathy, FA: Fatty acid, ROS: Reactive oxygen species

analysis, Redfield *et al*. detected SD in 24% of randomly selected T2DM individuals without CADs.[20] Molecular alterations leading to SD is listed in Table 1.[12] Each of the molecular alterations will be discussed further.

MOLECULAR CHANGES

Impaired calcium (Ca^{2+}) handling

Cardiac contractility is regulated by the intracellular calcium (Ca^{2+}) and studies show that Ca^{2+} homeostasis is disrupted in diabetic cardiomyocytes.[21] This could be due to mechanisms such as decreased ability of the sarcoplasmic reticulum (SR) to take up Ca^{2+} and reduced activities of Na^{+}/Ca^{2+} exchanger and the sarcolemmal Ca^{2+} ATPase. The reduction in SR function was associated with decreased ryanodine receptor proteins and increased nonphosphorylated phospholamban proteins.[22] This was confirmed in a db/db mouse model of T2DM, which showed a depression in SR Ca^{2+} load and ryanodine receptor expression.[23] In addition, Ca^{2+} efflux through the Na^{+}/Ca^{2+} exchangers was increased. However, further studies are required to characterize the mechanisms responsible for altered calcium handling in individuals with DCM.

MYOCARDIAL LIPOTOXICITY

In T2DM, the heart is exposed to abnormal substrate and hormone levels. Insulin cannot suppress the hormone sensitive lipase (responsible for mobilization of stored lipids) in the adipose tissue and the secretion of very low-density lipoproteins in liver.[24,25] This increases the circulating lipid levels, exposing the heart to elevated concentrations of fatty acids (FAs). Since the heart is exposed to a modified lipid profile, FA utilization in the diabetic heart is altered as compared to a nondiabetic heart. In T2DM, FA uptake into the cardiac tissue is upregulated through sarcolemmal transporters (FAT/CD36 and FABPpm) due to the elevated FA levels in the plasma.[26] This leads to a downstream signaling cascade increasing the intracellular long-chain fatty acyl coenzyme A (CoA), thereby increasing the mitochondrial β-oxidation, which contributes to the noncarbohydrate-derived pool of acetyl-CoA.[26]

Despite the increased glucose level in plasma, glucose transporter-1 and -4 (GLUT-1 and -4)-mediated glucose uptake is impaired in diabetic hearts.[27] This impairment was partially due to a decreased protein and mRNA level of GLUT-1 and -4 in the myocardium.[27] Consequently, energy metabolism in the diabetic heart is shifted from glucose metabolism toward FA metabolism.[28] In addition, free FA inhibits pyruvate dehydrogenase, which blunts myocardial energy production, leading to the accumulation of glycolytic intermediates [Figure 1].[28]

Furthermore, high FA uptake and metabolism not only accumulate toxic intermediates but also increase the oxygen demand and reactive oxygen species (ROS).[26] Regan *et al*. found a marked increase in triglyceride and cholesterol content in the LV biopsies collected from T2DM individuals.[29] Increase of myocardial FA uptake and oxidation is evident across individuals suffering from T2DM and in T2DM animal models.[19] The exact mechanisms are still not completely understood. Studies have shown that long-chain FA can change the dynamics of plasma and mitochondrial membranes by altering phospholipid composition.[30] In addition, lipotoxic conditions cause changes in the composition of endoplasmic reticulum (ER) membrane phospholipids, leading to ER swelling and ER stress.[31] Therefore, T2DM leads to myocardial lipotoxicity that may contribute to cell death and thus to cardiac dysfunction.

MYOCARDIAL FIBROSIS

DCM is characterized by interstitial and perivascular fibrosis.[12] Fibrosis is characterized by the accumulation of extracellular matrix (ECM), and transforming growth factor-β (TGF-β) is a growth factor that regulates ECM formation. A recent study reported an increase in TGF-β receptor density in the diabetic myocardium that eventually leads to fibrosis associated with DCM.[32] In concordance, Regan *et al*. found a significant increase in collagen in endomyocardial biopsies and collagen deposition in heart biopsies collected from T2DM individuals who did not have CAD.[29] Similarly, the presence of procollagen type I carboxyterminal peptide was also correlated with DD detected in T2DM, suggesting a mechanistic link between myocardial fibrosis and DCM.[33] However, mechanisms for increased cardiac fibrosis in the diabetic heart are not completely understood.

REACTIVE OXYGEN SPECIES

Increased ROS production in the diabetic heart is a contributing factor to the development of DCM. In uncontrolled diabetes, enzymes such as superoxide dismutase (enzyme responsible for inactivating superoxide radicals) and erythrocyte catalase (enzyme responsible for removal of hydrogen peroxide) are decreased.[34] Such deficiencies in enzymes lead to the accumulation of ROS, including superoxide (O_2^-), hydroxyl (OH•), peroxyl (•RO_2), hydroperoxyl (•HRO_2^-) as well as nonradical species such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl).[35] Under physiological states, the majority of ROS are generated in the mitochondria. Under hyperglycemic insult, mitochondrial ROS generation increases and this increase of ROS activates maladaptive signaling pathways, which may lead to cell death. This eventually contributes to the progression of

DCM. In obese (ob/ob) and diabetic (db/db) mice hearts, increased ROS production was associated with increased caspase-3 activation (marker of apoptosis).[36] In addition, increased DNA damage and loss of activity in DNA repair pathways were also observed in these models compared to controls. Therefore, increased ROS-mediated cell death could promote abnormal cardiac remodeling, which ultimately contributes to the characteristic structural and functional abnormalities associated with DCM.

CELL DEATH

Diabetes-induced myocardial cell death has been demonstrated in samples collected from T2DM heart, which shows an increase in both apoptosis and necrosis.[37] The molecular mechanisms responsible for apoptotic cell death in the diabetic heart have been well established. Increased accumulation of ROS as described above can change the membrane potential in the mitochondrial membrane, leading it to depolarize which opens the mitochondrial permeability transition pore (PTP).[38] This causes the release of proapoptotic factors such as cytochrome which then activates caspase-3, inducing apoptosis as outlined in Figure 2. In addition, binding of ligands such as tumor necrosis factor-α and Fas to death receptors also causes caspase activation through a signaling mechanism to induce apoptosis [Figure 2].[36,39]

Moreover, opening of the mitochondrial PTP also causes adenosine triphosphate (ATP) depletion.[38] This ATP depletion increases intracellular calcium (Ca^{2+}) entry, leading to the development of a state of hypercontracture, causing extreme cell shortening, eventually leading to necrosis [Figure 2].[40] In addition, cytochrome C released from the opening of PTP also decreases the activity of the electron transport chain, leading to the depletion of ATP, which in turn activates necrosis [Figure 2].[38] Collectively, it appears that multiple signaling pathways are involved in diabetes-induced myocardial cell death. As described above, apoptosis- and necrosis-mediated cell death in diabetic are well defined. In contrast, the role of another major type of cell death named autophagy in the diabetic heart still remains to be elucidated. The current studies show a critical and a central role of autophagy in the complicated signaling cascades, suggesting its major role in diabetes-induced myocardial cell death.

AUTOPHAGY

Autophagy is a catabolic process involving self-digestion of cellular organelles such as proteins, carbohydrates, and lipids as a measure to remove the waste products of metabolism and provide nutrition during demand.[9] Therefore, autophagy plays a vital role under physiological conditions.[9,10] In addition, autophagy also plays a protective or a pathological role depending on the type and duration of the stress conditions. There are three types of autophagy, which will be discussed further with the major focus on the most common form of macroautophagy (MA).

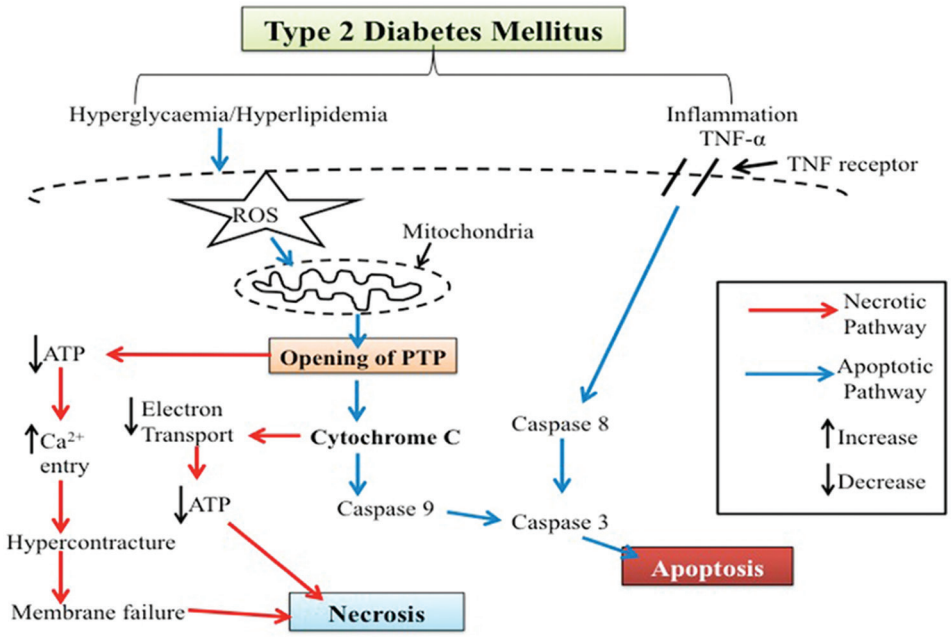

Figure 2: Overall schematic diagram representing the cell death mechanism in diabetic cardiomyopathy. ROS alter the membrane potential increasing MPT. This causes the opening of the mitochondrial PTP, releasing proapoptotic factors such as cytochrome C. Cytochrome C activates caspase-3, and this causes depletion of ATP, which induces apoptosis and necrosis, respectively. ROS: Reactive oxygen species, MPT: Mitochondrial permeability transition, PTP: Permeability transition pore, ATP: Adenosine triphosphate

TYPES OF AUTOPHAGY

There are three main autophagy pathways: Chaperone-mediated autophagy (CMA), microautophagy (MI), and MA.[41] CMA pathway is activated by physiological stresses such as prolonged starvation. The process is initiated with the identification of cytosolic proteins (substrate) with particular amino acid motifs by a complex of molecular chaperones,[42,43] which then binds it to a receptor in the lysosomal membrane, the lysosome-associated membrane protein (lamp) type 2a to form a translocation complex.[43] A second chaperone complex located in the lysosomal lumen helps the translocation complex to reach the lysosomal matrix where they undergo complete degradation [Figure 3].[42-44] Hence, unlike the MA and MI (*vide infra*), there is no vesicular transport involved in the protein degradation; rather, it is mainly through selective protein degradation.

The MI pathway involves internalization of cytosolic cargo through invaginations of the lysosomal membrane itself directly engulfing the portions of cytoplasm and any constituents [Figure 3].[41,45] This allows the lysosomal hydrolases to access the internalized substrates for degradation of the engulfed cargo. While the MI has been well described in yeast, the molecular mechanisms of this process in the eukaryotic cells remain unknown.[45-47]

MA, or more commonly autophagy, is an evolutionarily conserved process which occurs in virtually all eukaryotic cells ranging from yeast to mammals.[10] During this process, the cytosolic elements that need to be degraded are sequestered by an isolating membrane of nonlysosomal origin [Figure 3]. Isolating membranes then seal to form a unique structure named an autophagosome, which fuses with lysosomes before degrading the sequestered components.[10] As indicated above, this review will focus only on MA (referred to as autophagy hereafter).

STEPS INVOLVED IN AUTOPHAGY PROCESS

There are several sequential steps involved in autophagy such as nucleation, sequestration, degradation, and the generation of amino acids and FAs.[48]

Nucleation and assembly of autophagy proteins are mediated by autophagy-related genes (Atg) and require the class III phosphatidylinositol 3-kinase (PI3-K) complex [Figure 4]. The class III PI3-K complex is composed of PI3-K vacuolar protein sorting 34 (Vps34), a serine/threonine kinase Vps15, Atg14, and beclin-1 [Figure 4]. Beclin-1 is a mammalian ortholog of Atg6/Vps30 and is regulated by Bcl-2 (B-cell lymphoma/leukemia-2), an antiapoptotic protein. Dissociation of

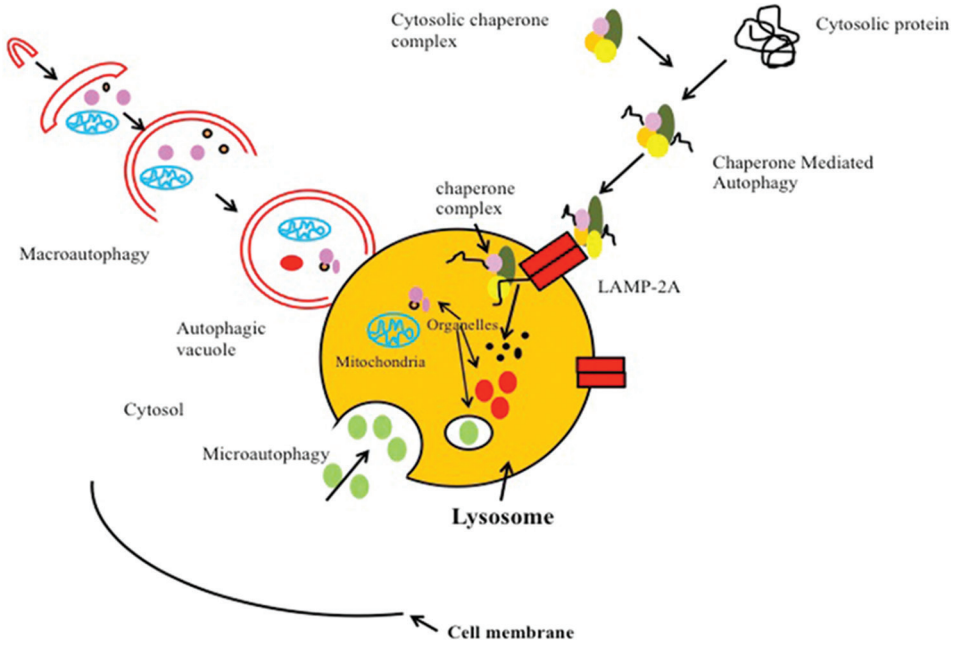

Figure 3: Types of autophagy in mammalian cells. CMA involves the degradation of cytosolic organelles (circles) through the binding of LAMP type 2A-LAMP-2A receptor in the lysosome. The microautophagy pathway involves internalization of cytosolic cargo through invaginations of the lysosomal membrane itself, directly engulfing the portions of cytoplasm and any constituents. The macroautophagy pathway involves the isolation of cytosolic organelles in a unique structure named "autophagosome," which fuses with lysosomes before degrading the sequestered components. Single proteins are represented as strings. CMA: Chaperone-mediate autophagy, LAMP: Lysosome-associated membrane protein

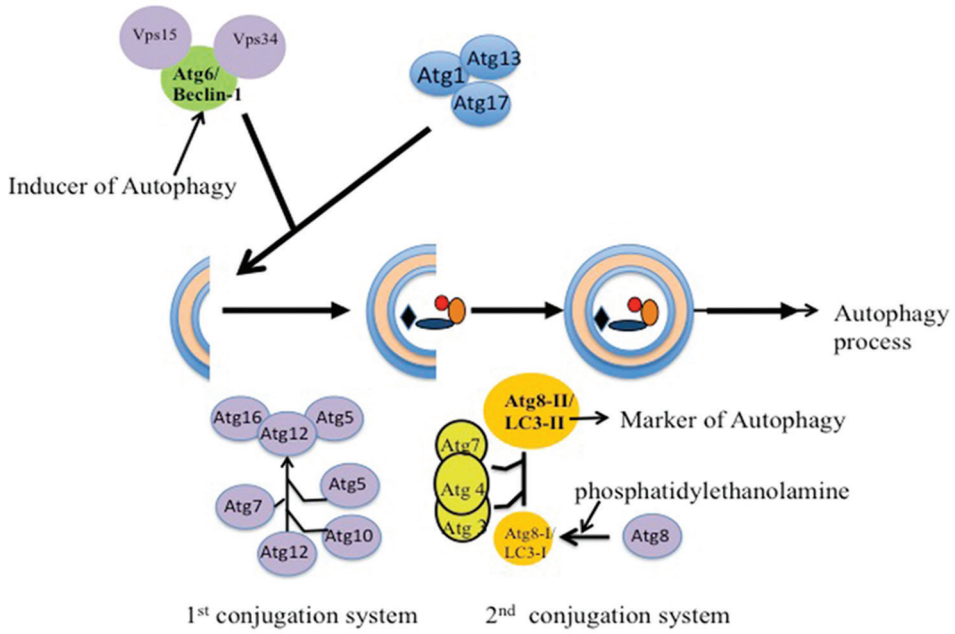

Figure 4: Schematic representation of autophagy-related gene assembly in the autophagy process. Nucleation and assembly of autophagy proteins are mediated by Atg and require the class III PI3-K complex. Class III PI3-K complex is composed of PI3-K Vps34, a serine/threonine kinase Vps15, Atg14, and Atg6 or beclin-1. Beclin-1 is the inducer of autophagy and involved in the vesicle nucleating step of the autophagosome membrane. Autophagosome membrane elongation process features two conjugation systems: 1st conjugation of Atg5 to Atg12 by the collective assistant of Atg7-Atg10, 2nd Conjugation of phosphatidylethanolamine to Atg8 (also known as microtubule-associated protein 1 light chain 3; LC3) by the sequential action of Atg3, Atg4, and Atg7. This conjugation system converts soluble form of LC3 (LC3-I) to the autophagic vesicle-associated form (LC3-II), which is used as a marker of autophagy. PI3-K: Phosphatidylinositol 3-kinase, Vps34: Vacuolar protein sorting 34, Atg: Autophagy-related genes

beclin-1 from Bcl-2 is required for autophagy induction, leading to the formation of an isolation membrane.[48] Isolation membrane then forms the double membrane vesicular autophagosome.

Sequestration of damaged cytoplasmic organelles and constituents is mediated by the autophagosome.[48,49] The autophagosome membrane elongation process features two conjugation systems; the first being the conjugation of Atg5 to Atg12 by the collective assistant of Atg7–Atg10 as shown in Figure 4[49-51] and the second system being the conjugation of phosphatidylethanolamine to Atg8 (also known as microtubule-associated protein 1 light chain 3; LC3) by the sequential action of Atg3, Atg4, and Atg7 [Figure 4]. Second conjugation system also converts the soluble form of LC3 (LC3-I) to the autophagic vesicle-associated form (LC3-II), which is used as a marker of autophagy.[51,52]

Degradation is carried out by the fusion of the outer membrane of the autophagosome with the lysosome, thus forming the structure named autophagolysosome. Cytoplasmic organelles sequestered in the autophagosome structure are then degraded by lysosomal hydrolases.[53] Generation of amino acids and FAs is followed by degradation as the recycling of nutrients is important for the physiological functioning of the cell.

MOLECULAR SIGNALING PATHWAYS REGULATING AUTOPHAGY

Pathways inhibiting autophagy

Signaling pathways inhibiting autophagy include the target of rapamycin (TOR), which is an atypical serine/threonine kinase highly conserved from yeast to human. In mammalian cells, mammalian TOR (mTOR) integrates several stimuli to regulate protein synthesis, nutrient uptake, and autophagy in response to growth factors, amino acids, and cellular energy.[54] mTOR serves as the master regulator of autophagy. mTOR integrates upstream activating signals that inhibit autophagy through class I PI3-K-protein kinase B (PKB, also known as Akt) pathway. On association with growth factors, tyrosine kinase receptors undergo autophosphorylation and become activated. This leads to the stimulation of two key signal transducing components: Small GTPase Rag and class I-PI3-K.[45,55]

The small GTPase Rag directly binds and activates mTOR in response to amino acids.[56] Rag A or B interacts with Rag C or D to form a heterodimer that is anchored to the surface of the lysosome.[55,56] In the absence of amino acids, Rag A/B is GDP-loaded and Rag C/D is GTP-loaded and the resulting Rag heterodimer is unable to bind mTOR.[45,56] Addition of amino acids induces the exchange

of GDP with GTP in Rag A/B and the conversion of GTP to GDP in Rag C/D, allowing the Rag heterodimer to bind and thereby activating mTOR to inhibit autophagy.[55,56]

PI3-K catalyzes the production of phosphatidylinositol 3-phosphate at the plasma membrane and hence increases membrane recruitment and activation of Akt.[50,57] Akt further activates mTOR through inhibition of the downstream protein complex, the tuberous sclerosis complex 1/2 (TSC1/TSC2).[45,50,58] The TSC1/TSC2 heterodimer, which is a stable complex, senses the upstream inputs from the kinase Akt. Phosphorylation of TSC2 by Akt leads to the disruption of its complex with TSC1 and results in mTOR activation. Thus, PI3-K-Akt activation suppresses autophagy in mammalian cells through activation of mTOR.[45,58]

In addition to mTOR, interaction of Bcl-2 with beclin-1 also inhibits autophagy under nutrient-rich conditions.[59,60] Dissociation of beclin-1 from Bcl-2 involves the phosphorylation of Bcl-2 by the stress-induced c-Jun N-terminal Kinase.[56,60,61]

PATHWAYS ACTIVATING AUTOPHAGY

Adenosine 5'-monophosphate-activated protein kinase (AMPK) is the major energy sensor in the cell which is activated by conditions such as low energy levels.[62,63] Once activated, AMPK directly phosphorylates and activates TSC1/TSC2 and inhibits mTOR activity.[63] Thus, the phosphorylation of TSC1/TSC2 by AMPK and Akt has opposite effects on mTOR. Recent reports stated that AMPK regulates mTOR by directly phosphorylating Raptor, a regulatory associated protein of mTOR. Raptor phosphorylation is important for the inhibition of mTOR signaling by AMPK.[45] Thus, AMPK serves as a positive regulator of autophagy. Moreover, myocardial infarction and ischemia/reperfusion cause an increase in ROS and a depletion of ATP. This induces autophagy through AMPK activity providing the cardiomyocytes with nutrients bringing cytoprotection.[45,63]

Rapamycin is a macrocyclic antibiotic and was discovered as a potent antifungal agent.[64] Rapamycin binds to the intracellular receptor FK506 binding protein 12 (FKBP12) and interferes with the FKBP12 rapamycin binding domain of mTOR.[45] FKBP12/rapamycin interaction inhibits mTOR autophosphorylation and phosphorylation, thus activating autophagy. In addition, rapamycin has also been proposed to inhibit mTOR by destabilizing the mTOR-Raptor complex.[64]

DUAL ROLE OF AUTOPHAGY

The increasing number of physiological functions linked to autophagy has led to a better understanding of its role, and research in this topic has been growing exponentially. In particular, the term autophagy has been linked to human pathologies, such as neurodegenerative diseases, CVD, and different forms of cancer.[65,66] However, determining whether autophagy protects or contributes to cell damage is a challenge. Under extreme conditions, autophagy is crucial for cell adaptation and survival. When nutrients are scarce, breakdown of intracellular macromolecules provides the energy required for minimal cell functioning. Consequently, activation of autophagy could play a protective role in early stages of several diseases. Recent reports reconcile these opposing roles of autophagy in disease and show that autophagy can act as both protector and killer of the cell. This depends on the stage of the disease, surrounding cellular environment, or the therapeutic interventions attempted. The initial activation of autophagy in response to cellular damage is likely to aim to protect the cell by degrading the altered macromolecules or organelles.[67] Once a certain level of intracellular damage is reached, autophagy might instead become an effective way of removing the injured cell from a tissue. Examples of this dual role of autophagy and the different outcomes of the activation or inactivation of autophagy in cancer, neurodegenerative, infectious, and CVD are discussed in Table 2.

Table 2: Dual role of autophagy

Disease	Activation of autophagy	Inactivation of autophagy
Cancer: Early stages	Inhibits the growth of tumor[67,68]	Increases the tumor growth Cells lose the ability to undergo autophagy following anticancer treatments cancer[67-69]
Cancer: Late stages	Increases the survival rate of low-vascularized tumor cells[67] Removal of damaged intracellular organelles following anticancer treatments (radiation, chemotherapy)[67,69]	Decrease the survival rate in low-vascularized tumor cells[67-69] Increase the efficiency of anticancer treatments as the damaged organelles cannot be eliminated[67,69]
Neurodegeneration	Increase the removal of cytoplasmic protein aggregates[66,70,71]	Increase the accumulation of cytoplasmic protein aggregates[66,72]
Infectious disease	Eliminate bacterial and viral particles[73]	Provide a "shelter" for the bacteria to replicate and inhibit[73]
Cardiovascular disease	Adaptive response to ischemia/reperfusion injury[6,74] Excessive loss of cardiomyocytes leads to heart failure[12]	Ventricular dilation, contractile dysfunction, and disorganized sarcomere[75]

In addition, autophagy can also play a detrimental role. Autophagy as a maladaptive process is a novel idea and has placed this maladaptive process in the center of current research in major human disorders such as DCM.

MALADAPTIVE AUTOPHAGY IN CARDIOVASCULAR DISEASE

The possibility that autophagy may play a maladaptive role in response to cardiac stresses is a novel idea. Ischemia or pressure overload are frequently encountered in individuals with CAD, hypertension, aortic valvular disease, and congestive HF. Cardiac biopsies collected from these individuals showed an accumulation of autophagosomes, suggesting a maladaptive phase of autophagy. Consistently, rodent models of these cardiac diseases and isolated stressed cardiomyocytes also showed an increase of autophagosomes.[76] This accumulation of autophagosomes could be either due to an activation of the autophagy process or due to a blockade of downstream steps in autophagy, such as insufficient fusion or decreased lysosome degradation. Therefore, it is critical to evaluate the overall autophagic system, rather than detecting the number of autophagosomes and the presence of LC3-II processing only. The term "autophagic flux" represents the entire process of autophagy.[77] Before molecular studies, it was largely assumed that autophagy invariably contributed to cardiomyocyte cellular homeostasis and had a cytoprotective role in the cardiac stress settings.[78] In a study done in beclin-1 (autophagy inducer) mice, lack of beclin-1 resulted in a maladaptive autophagy during reperfusion and a protective role during ischemia.[79] Furthermore, inhibition of beclin-1 enhanced the cardiac cell survival following ischemic/reperfusion in rats. Collectively, these studies support the maladaptive autophagy in response to cardiac stresses.

CROSSTALK BETWEEN APOPTOSIS AND AUTOPHAGY

Currently, intensive investigations are carried out to look into the crosstalk between apoptosis and autophagy due to clear molecular connections. Bcl-2 proteins play an important role in the regulation of apoptosis by inhibiting the release of cytochrome C from mitochondria.[80] Recently, beclin-1 was found to interact with Bcl-2 protein. Bcl-2 binding to beclin-1 inhibits beclin-1-mediated autophagy through sequestration of beclin-1. Therefore, this interaction may be an important mechanism involved in the regulation of both autophagy and apoptosis [Figure 5].[80] Bim-1 is another proapoptotic protein that belongs to Bcl-2 family. In response to death stimuli, bim-1 is activated, initiating mitochondrial-dependent apoptosis [Figure 5].[81] Bim-1 also inhibits autophagy by interacting with beclin-1. These data suggest that bim-1 switches

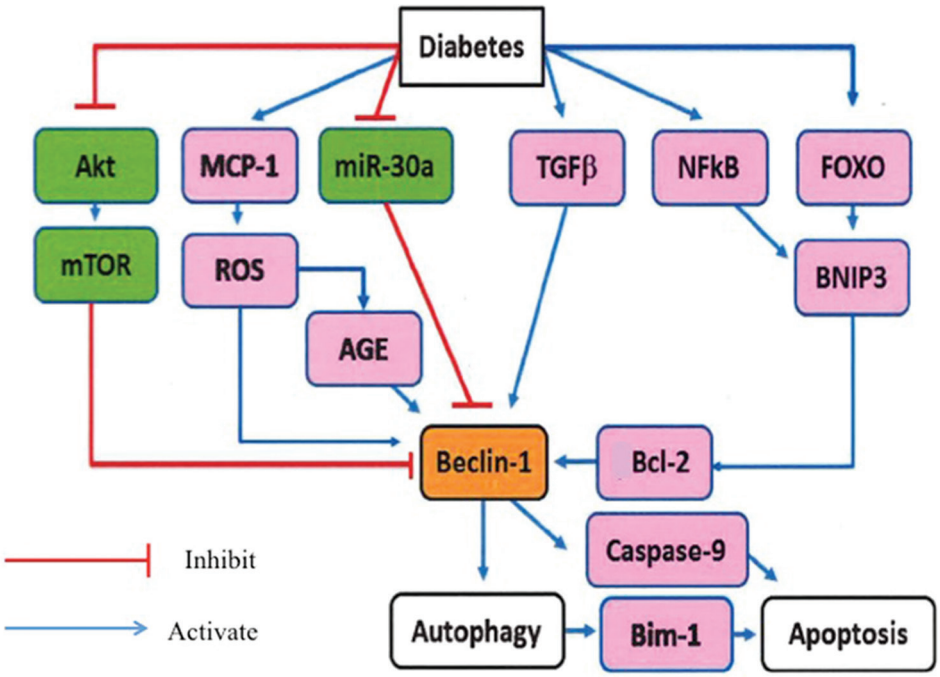

Figure 5: Positive and negative regulators of autophagy. Beclin-1-dependent autophagy is activated by inhibiting beclin-1 negative regulators (Akt, miR-30a) and inducing beclin-1 activators (ROS, AGE, TGF-β, NFκB, FOXO). Akt: Protein kinase B, mTOR: mammalian target of rapamycin, MCP-1: Monocyte chemoattractant protein-1, ROS: Reactive Oxygen Species, AGE: Advanced glycated endproducts, TGF-β: Transforming growth factor β, NFkB: nuclear factor kappa-light-chain-enhancer of activated B cells, FOXO: Forkhead box protein O, BNIP3: BCL2/adenovirus E1B 19 kDa protein-interacting protein, Bcl-2: B-cell lymphoma/leukemia-2

between apoptosis-inactive to autophagy inhibitory and apoptosis-active to autophagy-permissive sites.[81] Taken together, it is evident that there is a molecular link between apoptosis and autophagy.

DIABETES AND AUTOPHAGY

Several studies suggest that diabetes-mediated hyperglycemia induces several cellular signaling pathways. For example, Akt signaling is reduced or impaired in adipocytes collected from T2DM individuals and Zucker rats, respectively.[82] In addition, Younce *et al*. confirmed glucose-induced production of monocyte chemotactic protein-1, ROS, ER stress in H9c2 cardiomyoblasts and in isolated cardiomyocytes.[83] Importantly, these cellular signaling pathways also activate autophagy through beclin-1 as shown in Figure 5. However, maladaptive role of autophagy in DCM still remains unclear.

CONCLUSION

Autophagy is an essential cellular process which plays both physiological and protective roles. While this is illustrated by many studies, autophagy can also play a detrimental role. Autophagy as a maladaptive process is a novel idea and has placed this maladaptive process in the center of the current research in major human disorders. Diabetes is a multisystem disorder with the recent studies showing the key role for molecular alterations as the major cause for pathological consequences in diabetes. Based on the provided evidence, it is highly likely that maladaptive autophagy plays a major role in the pathogenesis of diabetes-induced cardiovascular complications. Future studies focusing on establishing the role of autophagy in diabetes-induced cardiovascular complications will improve our understanding and importantly form a basis for innovative mechanistic therapeutic ideas to treat CVD in patients with diabetes.

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Conflicts of interest

There are no conflicts of interest.

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