Critical Review

Regulation of Cardiac Autophagy by Insulin-like Growth Factor 1

Rodrigo Troncoso[1]
Jessica Díaz-Elizondo[1]
Sandra P. Espinoza[1]
Mario F. Navarro-Marquez[1]
Alejandra P. Oyarzún[1]
Jaime A. Riquelme[1]
Ivonne Garcia-Carvajal[1]
Guillermo Díaz-Araya[1]
Lorena García[1]
Joseph A. Hill[2]
Sergio Lavandero[1,2,3*]

[1]*Centro de Estudios Moleculares de la Célula, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile*
[2]*Department of Internal Medicine (Cardiology), University of Texas Southwestern Medical Center, Dallas, TX, USA*
[3]*Programa Biología Molecular y Celular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile*

Abstract

Insulin-like growth factor-1 (IGF-1) signaling is a key pathway in the control of cell growth and survival. Three critical nodes in the IGF-1 signaling pathway have been described in cardiomyocytes: protein kinase Akt/mammalian target of rapamycin (mTOR), Ras/Raf/extracellular signal-regulated kinase (ERK), and phospholipase C (PLC)/inositol 1,4,5-triphosphate ($InsP_3$)/Ca^{2+}. The Akt/mTOR and Ras/Raf/ERK signaling arms govern survival in the settings of cardiac stress and hypertrophic growth. By contrast, PLC/$InsP_3$/Ca^{2+} functions to regulate metabolic adaptability and gene transcription. Autophagy is a catabolic process involved in protein degradation, organelle turnover, and nonselective breakdown of cytoplasmic components during nutrient starvation or stress. In the heart, autophagy is observed in a variety of human pathologies, where it can be either adaptive or maladaptive, depending on the context. We proposed the hypothesis that IGF-1 protects the heart by rescuing the mitochondrial metabolism and the energetics state, reducing cell death and controls the potentially exacerbate autophagic response to nutritional stress. In light of the importance of IGF-1 and autophagy in the heart, we review here IGF-1 signaling and autophagy regulation in the context of cardiomyocyte nutritional stress. © 2013 IUBMB Life, 65(7):593–601, 2013

Keywords: *signal transduction; signaling; general bioenergetics*

Introduction

Numerous studies have shown that deregulation of autophagy contributes to the pathogenesis of cardiovascular disease, diabetes, and cancer (1). Autophagy is a catabolic process involved in protein degradation, organelle turnover, and nonselective breakdown of cytoplasmic components during nutrient starvation or stress. Autophagy initiates with formation of the autophagosome, a double-membrane intracellular structure of reticular origin that engulfs cytoplasmic contents and ultimately fuses with lysosomes for cargo degradation. Materials degraded within these newly formed autolysosomes are recruited to anabolic reactions to maintain energy levels and provide macromolecules for the synthesis of higher order structures (nucleic acids, proteins, or organelles), thereby sustaining cell metabolism, homeostasis, and survival (see Fig. 1) (2). Despite its key role in survival, autophagy also contributes to cell death when activated excessively or inefficiently, as occurs during tissue and organ development or in certain pathological states. Indeed, the terms "cell death with autophagy" or "autophagy cell death" have been coined based on death stimulus that results in morphological features of autophagy, where the pharmacological or genetics inhibition of autophagy blocks the morphological features. In the case of "cell death with autophagy," the inhibition of autophagy does not alter the fate of the cell. By contrast, in "autophagy cell death," the inhibition of autophagy changes the fate of the cell and protects the cell from the death stimulus (3).

In the presence of abundant nutrient supply, growth factors stimulate anabolic processes. In this context, autophagic flux is relatively suppressed. In contrast, autophagy is triggered by

Volume 65, Number 7, July 2013, Pages 593-601
**Address for correspondence to: Sergio Lavandero, Facultad de Ciencias QuÚmicas y FarmacÕuticas, Universidad de Chile, Sergio Livingstone 1007, Santiago 838-0492, Chile. Tel: +562-2978-2919. Fax: +562-2978-2912. E-mail: slavander@uchile.cl.*
Received 14 February 2013; accepted 22 March 2013
DOI: 10.1002/iub.1172
Published online 14 May 2013 in Wiley Online Library (wileyonlinelibrary.com)

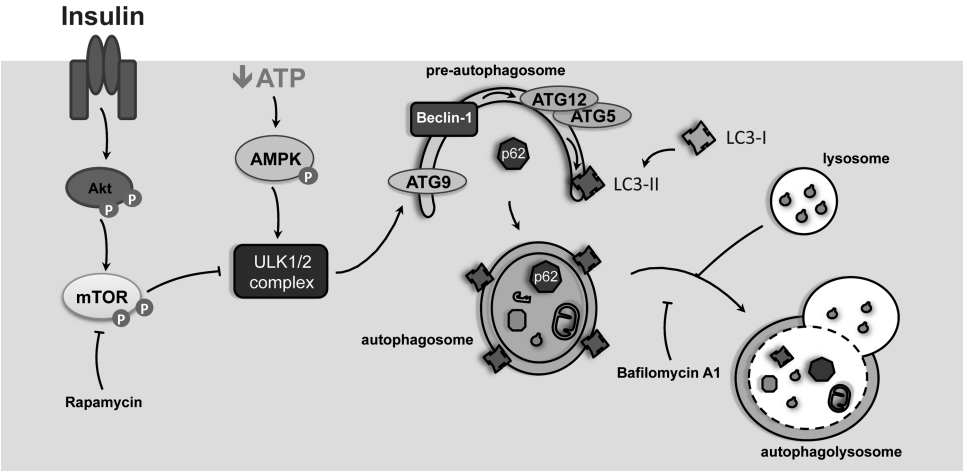

FIG 1 *The autophagy cascade. Nutrient sensing cascades regulates mTOR and AMPK, which sense the presence or absence of energy respectively. These proteins control the initiation of autophagy via their control over the ULK1/2 protein complex. The initial phagophore formation requires Beclin1 and expansion of the membrane is mediated by 2 ubiquitin-like conjugation systems, Atg12-Atg5 and LC3. The membrane elongates until the edges fuse around its target forming the autophagosome. The autophagosome then fuses with the lysosome to form the autophagolysosome. Finally, the content is degraded.*

nutritional stress or by defects in growth factor-dependent signaling to replenish intracellular stores of key components of energy metabolism. Two key growth factors, insulin and insulin-like growth factor (IGF-1), serve as important regulators of autophagy. Particularly, IGF-1 has shown to be cardioprotective. These effects have been attributed to the regulation of metabolism, inhibition of apoptosis, stimulation of cardiac hypertrophy, intracellular Ca^{2+} regulation, direct effects on cardiomyocyte contractility and the recent finding of the regulatory action on autophagy. Regarding to autophagy, IGF-1 avoids nutritional stress-induced autophagy by increasing cellular ATP levels through the increase in mitochondrial metabolism (4).

In this article, we review the literature regarding the cardiac role of IGF-1 with emphasis in the regulation of autophagy and the signaling pathways that governs IGF-1 actions.

IGF-1 Signaling

IGF-1 is a peptide that mediates cellular processes involved mostly in cell growth and protection. IGF-1 is essential for normal growth, development, and differentiation events in specific tissues (5). IGF-1 initiates its signaling cascade through activation of the IGF-1 receptor (IGF-1R). Upon binding of ligand to the receptor, autophosphorylation occurs at a specific tyrosine residue in the IGF-1R, resulting in activation of two downstream signaling pathways: phosphatidylinositol 3-kinase (PI3K)/Akt and Ras/Raf/extracellular signal-regulated kinase (ERK) cascades (6) (see Fig. 2).

The PI3K-Akt pathway commences with tyrosine autophosphorylation of IGF-1R β subunit. This leads to recruitment of insulin receptor substrates-1/2 (IRS1/2) to the receptor phosphotyrosines for subsequent phosphorylation of IRS1/2 by the intrinsic tyrosine kinase activity of the IGF-1R. PI3K binds to IRS1/2 and catalyzes addition of a third phosphate moiety to phosphatidylinositol 4,5-biphosphate (PIP_2) to produce a second messenger of lipid origin, phosphatidylinositol 3,4,5-triphosphate (PIP_3). This latter event is required for activation of the serine/threonine kinase Akt, a critical node in this molecular cascade. When PIP_3 is formed, Akt translocates to the plasma membrane, where it is phosphorylated by 3-phosphoinositide-dependent protein kinase-1. This phosphorylation event leads to incomplete Akt activation. An additional phosphorylation event mediated by mammalian target of rapamycin complex 2 (mTORC2) is necessary to fully activate Akt (7). Recently, it has been shown that ubiquitination of Akt by the E3-ligase TRAF6 is also required to achieve full activation (8).

The Ras/Raf/ERK pathway mainly regulates cell growth, survival, and differentiation. The initial step in the cascade involves binding of growth factor receptor-bound protein-2 (Grb2) and son-of-sevenless to phosphotyrosines on the IRS proteins Grb2-associated-binding protein 1 (Gab1) and Src homology 2 domain-containing protein (Shc). This induces Ras and Raf activity, followed by activation of downstream kinases. Next, phosphorylation of mitogen-activated protein kinase (MAPK)/ERK kinase 1/2 (MEK1/2) leads to phosphorylation of its substrate, ERK 1/2, which then activates its own targets (9). IGF-1 induces cell growth and proliferation through the Ras/Raf/ERK pathway in concert with the Akt/mTOR pathway which mediates protein synthesis (10).

Beyond this classical pathway, our laboratory has studied a third signaling pathway, which involves the increases in cytosolic Ca^{2+} levels through the activation of phospholipase C (PLC)/inositol 1,4,5-trisphosphate ($InsP_3$) pathway (11). In addition, we have observed that IGF-1 induces a rapid increase in nuclear Ca^{2+} that is independent of cytosolic Ca^{2+} and regulates gene transcription by promoting migration of a plasma membrane receptor in close proximity to the nucleus (12).

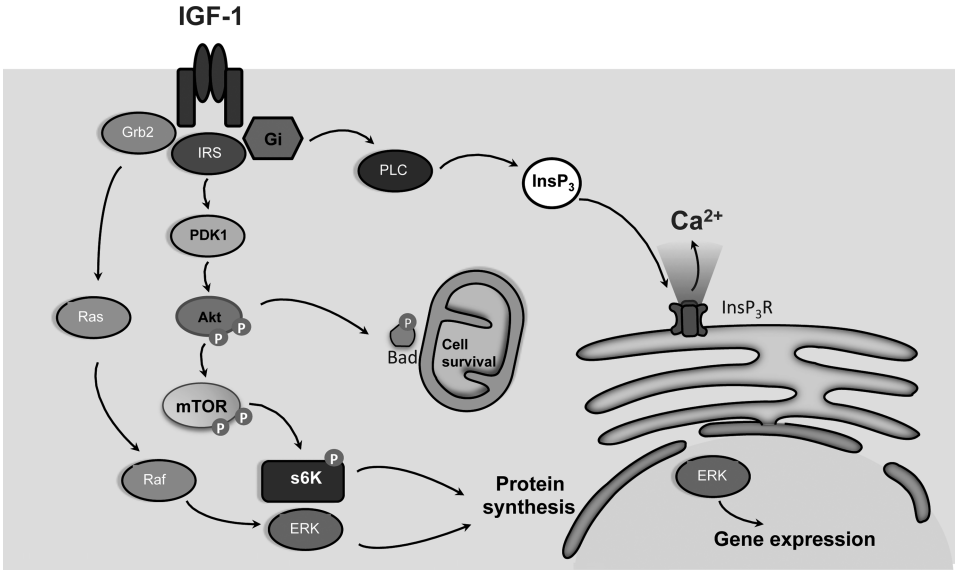

FIG 2 *IGF-1 signaling in cardiomyocytes. IGF-1 acts through a tyrosine kinase receptor called IGF-1R. After the autophosphorylation of the receptor, three key pathways are activated. The first, Ras/Raf/ERK pathway mainly regulates cell growth, survival, and differentiation. Second, Akt/mTOR pathway controls survival and protein synthesis. Third, the PLC/$InsP_3$/Ca^{2+} pathway, which control protein synthesis and metabolism.*

IGF-1 and Cardioprotection

The myocardium comprises long-lived, post-mitotic cardiomyocytes (13). Therefore, despite the ongoing controversy regarding the regenerative capacity of adult heart, elucidation of cellular mechanism underlying cardiomyocyte viability is of fundamental importance to cardiovascular medicine.

Apoptosis, programmed cell death type I, is a key physiological process in embryonic development, cell turnover, and removal of inflammatory cells. Conversely, defects in the apoptotic program contribute to several diseases, including cancer, ischemic damage, neurodegenerative diseases, and autoimmune disorders. A variety of stimuli that are known to participate in the pathogenesis of heart failure induce cardiomyocyte apoptosis, including hypoxia, ischemia and reperfusion, oxidative stress, and hyperosmotic stress (14–16).

Antiapoptotic effects of IGF-1 are mediated by IGF-1R and the consequent activation of PI3K/Akt/mTOR and the Ras/Raf/MEK/ERK signaling pathways (17). Akt activity appears to be of major importance for the antiapoptotic properties of IGF-1, where the targets are the Bcl-2-family member Bad, procaspase-9, the transcription factors nuclear factor-κB, cAMP response element-binding protein (CREB), Forkhead family of transcription factors (FoxO), and glycogen synthase kinase-3β (14).

Several studies reported phosphorylation and inactivation of Bad as one mechanism for Akt-mediated survival (18). Bad exerts its apoptotic function by forming heterodimers with mitochondria-bound Bcl-2 and Bcl-$_{XL}$, neutralizing their protective effects, and promoting cell death. IGF-1-stimulation is followed by PI3K-dependent phosphorylation of Bad by Akt and by MEK1-dependent phosphorylation of ERK1 and ERK2 (17). IGF-I also stimulates phosphorylation of CREB in a PI3K- and MEK1-dependent manner (14). Ectopic overexpression of a dominant-negative mutant of CREB abolished the antiapoptotic effect of IGF-1 (14). Protein levels of the antiapoptotic factor Bcl-2 increased after prolonged periods of IGF-1-stimulation, a phenomenon that could be reversed by pharmacological inhibition of PI3K and by overexpression of dominant-negative CREB (14,19).

Caveolin 1 (Cav-1), the major caveolar protein, is critical to IGF-1 signaling, as it interacts directly with IGF-1R and its intracellular substrates (20). IGF-1 does not prevent apoptosis in Cav-1-siRNA H9C2 cells, suggesting that Cav-1 is required to mediate the antiapoptotic effects of IGF-1 in cardiomyoblasts (21). Cav-1 downregulation suppresses IGF-1R tyrosine phosphorylation, resulting in reduced activation of IRS-1, Shc, and Akt. Cav-1 is also involved in IGF-1R-dependent antiapoptotic signaling after serum deprivation (21).

In addition to its antiapoptotic effects, IGF-1 exerts pleiotropic antioxidant and anti-inflammatory actions. This has been shown to be important in atherosclerosis, a chronic inflammatory disease in which early endothelial dysfunction and subintimal modified lipoprotein deposition progresses to complex, advanced lesions predisposed to erosion, rupture, and thrombosis (22). IGF-1 also suppresses autophagic cell death of plaque-derived vascular smooth muscle cells via Akt-dependent inhibition of LC3 expression (23).

IGF-1 and Cardiac Autophagy

Autophagy occurs at low, basal levels in virtually all cells to maintain homeostatic functions such as protein and organelle

turnover (1). Autophagy can be induced by a wide range of stress conditions, including nutrient starvation, growth factor withdrawal, oxidative stress, infection, hypoxia, and endoplasmic reticulum stress (24). Stress-induced autophagy is controlled primarily by two critical signaling elements: mTOR and 5′-AMP-activated protein kinase (AMPK). mTOR integrates information about availability of nutrients and growth factors and is a central negative regulator of autophagy. AMPK is activated by a high ratio of AMP to ATP and induces autophagy by phosphorylating mammalian Atg1 homolog UNC-51-like kinase 1 (ULK1) and through mTOR inhibition (24).

Cardiomyocyte function and survival rely critically on the presence of basal levels of autophagy (25,26). In a model of controlled cardiomyocyte-specific Atg5-deficiency, autophagy was found to play a beneficial role in the heart under basal, resting conditions and in response to pressure overload (27). In complete absence of autophagy, pressure overload triggered rapid-onset cardiac hypertrophy, left ventricular dilation, and diminished cardiac output. Thus, constitutive autophagy controls cardiomyocyte size and function (27). By contrast, the long-term consequences of Atg5 deficiency in the heart include cardiomyocyte hypertrophy and diminished cardiac output by the age of 10 months in mice, a consequence of accumulation of defective proteins and organelles (28). Deficiency of LAMP2 in Danon disease triggers severe and progressive cardiomyopathy stemming from defective fusion of autophagosomes with lysosomes (29). Together, these facts highlight the vital housekeeping role of cardiomyocyte autophagy as a mechanism of protein and organelle surveillance, recycling, and quality control (25,26).

Autophagy is induced by fasting, consistent with activation the catabolic pathways (4,30). Moreover, 72 h of starvation affects myocardial ATP content without altering cardiac function, and suppression of autophagy by bafilomycin A1 during starvation results in pronounced ATP reductions and impaired heart performance (30). Similar results have been reported in glucose-deprived neonatal cardiomyocytes (4,31). In contrast, pressure-overload stress, a common clinical scenario which can lead to heart failure, induces autophagy, which is required for hypertrophic growth of the myocardium (32). Consistent observations were reported by Nakai et al., noting that autophagy was increased in mice exposed to 1-week of pressure overload (27). Together, these data reinforce the idea that myocyte remodeling requires significant shifts in mechanisms of cell growth and protein degradation, including autophagy.

Robust levels of autophagy have been associated with multiple stressors that induce cardiac pathology, including elevated afterload, chronic ischemia, and ischemia/reperfusion injury (33). Autophagy acts as a double-edge sword; it can either antagonize or promote disease pathogenesis, depending on the context and amplitude of its induction (34). Autophagy induction is protective when cells are starved during ischemia (31). On the other hand, autophagy is maladaptive in the setting of severe afterload stress (35).

IGF-1 plays an important role in the regulation of cell survival, proliferation, differentiation, and metabolism (4). Through activation of the PI3K pathway, IGF-1 activates mTOR, a central negative regulator of autophagy (4,23,36). An inhibitory effect of IGF-1 on autophagy has been observed in human osteocarcinoma cells (37), vascular cells from patients with atherosclerotic lesions (23), mammary epithelial cell involution (38), human fibroblasts (36), and rat cardiomyocytes (4). In addition, mice with diminished production of IGF-1 manifest augmented levels of autophagy in diverse tissues (36). By contrast, IGF-1 promotes autophagy in H9c2 cell lines (39) and in Purkinje neurons (40).

Work from our lab has revealed that short-term treatment with IGF-1 is cardioprotective against nutrient-deprivation stress (28). In this regard, IGF-1 prevented cardiomyocyte stress-induced autophagy by increasing ATP levels and promoting mitochondrial metabolism, including mitochondrial Ca^{2+} uptake and oxygen consumption, through the Akt/mTOR and AMPK/mTOR axis (4). Mitochondrial Ca^{2+} uptake is required to provide optimal bioenergetics maintaining cellular ATP levels and autophagy at basal state (41). Cardenas et al. showed that basal $InsP_3R$ activity is required to provide a physiological Ca^{2+} signal sensed by the mitochondria, thereby controlling mitochondrial bioenergetics. Absence of this Ca^{2+} transfer results in enhanced phosphorylation of pyruvate dehydrogenase and AMPK activation and stimulation of autophagy (42). Thus, we proposed that the control of the Ca^{2+} transfer to the mitochondria by IGF-1 results in ATP production that supports the energetic requirements of the cell, avoiding AMPK activation responsible of autophagy induction.

mTOR Signaling

TOR is an atypical serine/threonine kinase that belongs to the PI3K-related kinase family first identified in yeast *Saccharomyces cerevisiae* and later in humans and other mammals. The mammalian homolog of yeast TOR is called mTOR (43,44). mTOR interacts with other proteins and forms two complexes, referred to as mTORC1 and mTORC2, which have different sensitivities to rapamycin. Both complexes include the catalytic mTOR subunit, the mammalian lethal with sec-13 protein 8, DEP domain containing mTOR-interacting protein (Deptor), and the Tti1/Tel2 complex (45). Regulatory-associated protein of mTOR (Raptor) and proline-rich Akt substrate 40 kDa (PRAS40) participate only in mTORC1 (46,47). By contrast, rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated MAPK-interacting protein 1 (mSin1), and protein observed with rictor 1 and 2 (Protor1/2) are specific to mTORC2 (48). The inhibition of mTORC1 by rapamycin results in growth arrest and lack of a cellular response to growth factor and nutrient stimulation (43).

The TSC1/2 protein complex is an important regulator of mTORC1, acting as a GTPase-activating protein for the Ras homolog enriched in brain (Rheb) GTPase (49). When Rheb

binds GTP, it interacts with mTORC1, stimulating its activity. Thus, the TSC1/2 complex acts as an inhibitor of mTORC1 by inactivating Rheb (44). mTORC1 can be regulated by growth factors, stress, energy status, oxygen, and amino acids. In particular, IGF-1 regulates mTOR to maintain normal levels of nutrients and cell growth and division after mitogen stimulation (50). Regulation of mTORC1 by the IGF-1/Akt pathway occurs through phosphorylation of the TSC1/2 complex, which leads to its inhibition and subsequent activation of mTORC1 (50). Activation of mTORC1 by Akt can also occur in a TSC1/2-independent manner by phosphorylating and promoting dissociation of PRAS40 from raptor, which in turn, inhibits mTORC1 (44). The two major downstream targets of mTORC1 are the eIF4E binding protein (4EBP1) and the p70 ribosomal protein S6 kinase (S6K), which upon activation, promote protein synthesis and cell growth (50). Activation of mTOR by IGF-1 results in the biosynthetic effects mentioned above, but it also negatively regulates autophagy (4,38). Moreover, our group has shown that autophagy and cell death induced by nutritional stress in cardiomyocytes are inhibited by IGF-1 through a mechanism that depends on the Akt/mTOR pathway (4).

Inhibition of autophagy by mTORC1 occurs through phosphorylation and suppression of the ULK1/mammalian autophagy-related gene 13 (Atg 13)/focal adhesion kinase family-interacting protein of 200 kDa (FIP200) protein complex (51,52). Two hypotheses have been proposed to explain mTORC1-dependent regulation of the ULK1 complex. Under normal nutrient conditions, mTORC1 interacts with ULK1 and phosphorylates ULK1 and Atg13, which leads to an open (inactive) conformation of the complex; under conditions of nutrient deprivation, mTORC1 no longer interacts with ULK1, which makes it unable to phosphorylate ULK1 and Atg13, leading to a closed (active) conformation of the ULK1 complex (53). Autophagy can also be regulated by mTORC1 through its modulation of death-associated protein 1 (DAP1), an inhibitor of autophagy. Moreover, mTORC1 can inhibit the biogenesis of lysosomes by phosphorylating the transcription factor EB (TFEB) (54). When mTORC1 is inhibited by nutrient starvation, it allows the translocation of TFEB to the nucleus and its consequent activation, which in turn, promotes the expression of genes involved in autophagosome formation and autophagosome-lysosome fusion (54).

AMPK Signaling

A decrease in cellular energy stores (ATP) activates AMPK and stimulates autophagy (55). This protein is a heterotrimeric kinase comprising $\alpha\beta\gamma$ subunits and is a critical integrator of multiple signals in the control of energy balance (56). Different kinds of stressors elicit decreases in the ATP/AMP ratio, activating liver kinase B1 (LKB1), which then triggers AMPK to phosphorylate the TSC1/2 complex, leading to mTOR inhibition through Rheb (57). However, AMPK can also regulate mTORC1 by an alternative mechanism, as this kinase directly phosphorylates Raptor, leading to mTORC1 inhibition (58). The upstream Ca^{2+}/CaM-dependent protein kinase kinase β (CaMKKβ) also phosphorylates AMPK in an AMP-independent and Ca^{2+}-dependent manner (59). Cytokines and increases in intracellular Ca^{2+} each activate autophagy via this AMPK-dependent mechanism (58).

Finally, AMPK can directly phosphorylate ULK1 on serines 317 and 777 leading to its dissociation from the mTORC1 complex and consequently enhancing autophagic turnover in response to glucose deprivation (60). In addition, the Guan's and Shawn's groups showed that AMPK phosphorylation of ULK1 occurs *in vivo* and *in vitro* in different phosphorylation sites, depicting the requirements of these targets sites for efficient autophagy induction (61,62). Under basal conditions, mTOR phosphorylates ULK1 on serine 757 preventing its activation and interaction with AMPK (56). On the other hand, loss of AMPK or ULK1 induces p62 accumulation and defective mitophagy, revealing that ULK1 is necessary for mitochondrial homeostasis and cell survival (63).

AMPK is an essential regulator of glucose deprivation-induced protective autophagy in cultured cardiomyocytes (31). Also transgenic mice overexpressing a dominant negative AMPK manifest reduced autophagy on exposure to nutrient deprivation (31). Moreover, we showed that IGF-1 inhibits autophagy by increasing ATP levels, which controls AMPK activation, in glucose-deprived cultured cardiomyocytes (4).

Ca^{2+} Signaling

IGF-1 induces a fast and transient increase in Ca^{2+} levels in the nucleus and cytosol of cultured cardiomyocytes by releasing this ion from intracellular stores through an $InsP_3$-dependent signaling pathway (11,12). This increase in Ca^{2+} is dependent on both tyrosine kinase activity and pertussis toxin-sensitive G protein-dependent activation of PI3K (11). This kinase, through indirect activation of PLC, is responsible, in turn, for elevating intracellular $InsP_3$ and consequent activation of InsP3 receptors ($InsP_3R$) (11).

On the other hand, Ca^{2+}-CaMKKβ can also activate AMPK; this provides a link between Ca^{2+} signaling and autophagy. Several reports have described increases in cytoplasmic Ca^{2+} by diverse mechanisms, including ionomycin, thapsigargin, vitamin D, ATP, and Ca^{2+} channel agonists (41). Ca^{2+} signaling has also been linked to mTOR-dependent and independent pathways. Among the mTOR-independent pathways, roles for the $InsP_3R$-Ca^{2+} release channel and Ca^{2+} signaling to mitochondria have been emphasized. The $InsP_3R$ at the ER provides Ca^{2+} to mitochondria constitutively, which in turn promotes conversion of pyruvate into acetyl-CoA, tricarboxylic acid cycle activity, and production of ATP via the electron transport chain (41). By contrast, when Ca^{2+} is not transferred to mitochondria, energy levels drop, which leads to AMPK activation and autophagy induction (42). Findings from our group, consistent with the role of mitochondrial Ca^{2+}, revealed that

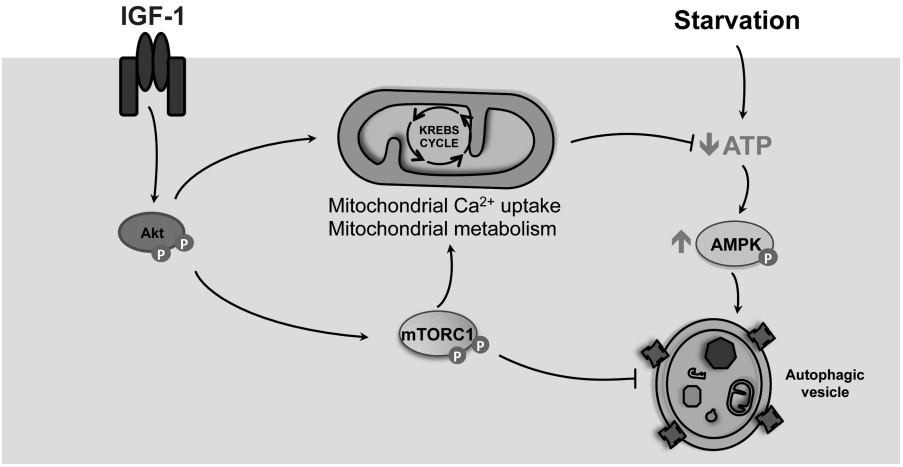

FIG 3 *Proposed model for the cardioprotective effects of IGF-1. Nutritional stress leads to a decay of intracellular ATP levels, activating AMPK and autophagy to restore the energetic state of cardiomyocytes and to prevent cell death. IGF-1 rescues ATP levels and activated the Akt/mTOR pathways inhibiting the activation of autophagy.*

IGF-1 improves mitochondrial bioenergetics through mitochondrial Ca^{2+} uptake in cardiomyocytes in the setting of either nutritional stress or normal conditions, restoring oxygen consumption and thus promoting ATP production (4). In addition, we showed that inhibition of mitochondrial Ca^{2+} uptake by pharmacological inhibition of the mitochondrial Ca^{2+} uniporter significantly reduced the effects of IGF-1 on oxygen consumption and LC3 processing (4).

Metabolic Regulation by IGF-1

IGF-1 and insulin receptors share more than 80% homology in their kinase domains (64), and they share downstream effectors for metabolic utilization (PI3K-Akt). In light of this, a considerable body of literature refers to insulin/IGF-1 signaling as one pathway, assuming insulin to be responsible for metabolic changes and IGF-1 responsible for increases in protein synthesis and growth (65). However, the two peptides have critical dissimilarities in their pathways, as they act through different Akt isoforms (66). Akt1 is required for normal cardiac growth, while Akt2 regulates cardiomyocyte metabolism and survival; thus, IGF-1 exerts its action through the Akt1 isoform, controlling cell growth, whereas insulin preferentially activates Akt2, regulating metabolism. Additionally, preferential stimulation of the Ras-Raf-ERK pathway by IGF-1—and not insulin—may account for distinct net effects of the two hormones (66).

On the other hand, emerging evidence points to the existence of insulin/IGF-1 hybrid receptors (67–69). Hybrid receptors are heterologous, covalently linked IGF-1R and IR α-β hemireceptors that maintain trans-autophosphorylation activity upon ligand binding and are present in a variety of mammalian tissues, including the heart (67). These hybrid receptors can be activated by insulin as well as IGF-1, although IGF-1 has greater affinity (64).

Whereas IGF-1 signaling has not been elucidated completely in cardiac tissue, genetic models in mice have been informative. Reduced IGF-1 levels are associated with obesity, cardiovascular disease, atherosclerosis, and diabetes mellitus; conversely, high-fat feeding reduces cardiomyocyte IGF-1 signaling, leading to mitochondrial dysfunction, intracellular Ca^{2+} deregulation, and abnormal insulin signaling (70). A mouse model of liver-specific inducible inactivation of the IGF-1 gene revealed that absence of circulating IGF-1 reduces myocardial creatine content, a compound essential to cardiac energy homeostasis, by regulating the cardiomyocyte creatine transporter gene (71). Moreover, in a cardiomyocyte-specific IGF-1 transgenic model, IGF-1 overexpression blunted high-fat diet-induced declines in insulin-stimulated glucose uptake and insulin receptor activation and signaling, suggesting that IGF-1 may bypass the membrane insulin receptor by exerting its biological actions through either the IGF-1 receptor or insulin-IGF-1 receptor hybrid (72). Also IGF-1 is critical to the maintenance of cardiac bioenergetics in nutrient stress conditions, because IGF-1 increases mitochondrial metabolism and cellular ATP levels, by a provoking a rise in mitochondrial Ca^{2+} uptake and respiration (4). Additionally, IGF-1 deficiency during starvation increases cardiac AMPK activity, suggesting a distinct role for IGF-1 in preserving cardiac energetics under these conditions (4).

Conclusions

An expanding body of evidence highlights the cardioprotective role of IGF-1 (4,15,17,72). These effects have been attributed to regulation of metabolism, inhibition of apoptosis, increases in cardiomyocyte size, intracellular Ca^{2+} regulation, and direct effects on cardiomyocyte contractility (17). Yet, little is known regarding the effects IGF-1 on autophagy, a major adaptive

mechanism in most cells. Thus, in light of our recent work, and that of others, we propose that IGF-1 primarily protects from nutritional insult to the heart. The inhibitory effects of IGF-1 on autophagy can be explained by stimulation of the IGF-1R/Akt/mTOR axis and by increasing mitochondrial Ca^{2+} levels, oxygen consumption, and cellular ATP levels that together lead to AMPK inhibition. Additionally, we have observed that IGF-1 deficiency increases cardiac AMPK activity during starvation conditions, suggesting a distinct role for IGF-1 in preserving cardiac energetics in this setting. Thus, by maintaining ATP levels, IGF-1 additionally regulates autophagy by inhibiting the AMPK/mTOR axis during nutritional stress (see Fig. 3).

In conclusion, IGF-1 exerts important cardioprotective actions through the well-known Akt/mTOR pathway, as well as via regulation of autophagy. However, governance of the cellular energetic state has emerged as a novel mechanism for the beneficial actions of IGF-1. Thus, when autophagy becomes activated during nutritional challenge, IGF-1 upregulates the energetic state of the cardiomyocyte, obviating the need for autophagy to replenish nutrient stores. Thereby, IGF-1 may reduce permanent cellular damage elicited during nutrient stress-related pathological states, such as ischemic heart disease or myocardial infarction.

Acknowledgements

This work was supported by FONDECYT (grant 1120212 to S.L., 3110114 to R.T.), CONICYT (grant Anillo ACT 1111 to J.A.H., L.G., and S.L.; Redes 120003 to S.L. and J.A.H.), NIH (grants HL-075173, HL-080144, and HL-090842 to J.A.H.), AHA (grant 0640084N to J.A.H.), and AHA-Jon Holden DeHaan Foundation (grant 0970518N to J.A.H.). We are thankful for the PhD fellowships from CONICYT, Chile to M.N, I.G.C., and J.R.

REFERENCES

[1] Levine, B. and Kroemer, G. (2008) Autophagy in the pathogenesis of disease. Cell 132, 27–42.

[2] Mizushima, N. (2007) Autophagy: process and function. Genes Dev. 21, 2861–2873.

[3] Kroemer, G. and Levine, B. (2008) Autophagic cell death: the story of a misnomer. Nat. Rev. Mol. Cell Biol. 9, 1004–1010.

[4] Troncoso, R., Vicencio, J. M., Parra, V., Nemchenko, A., Kawashima, Y., et al. (2012) Energy-preserving effects of IGF-1 antagonize starvation-induced cardiac autophagy. Cardiovasc. Res. 93, 320–329.

[5] Laron, Z. (2001) Insulin-like growth factor 1 (IGF-1): a growth hormone. Mol. Pathol. 54, 311–316.

[6] Becker, A. B. and Roth, R. A. (1990) Insulin receptor structure and function in normal and pathological conditions. Annu. Rev. Med. 41, 99–115.

[7] Sarbassov, D. D., Guertin, D. A., Ali, S. M., and Sabatini, D. M. (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307, 1098–1101.

[8] Yang, W. L., Wang, J., Chan, C. H., Lee, S. W., Campos, A. D., et al. (2009) The E3 ligase TRAF6 regulates Akt ubiquitination and activation. Science 325, 1134–1138.

[9] Pouyssegur, J., Volmat, V., and Lenormand, P. (2002) Fidelity and spatio-temporal control in MAP kinase (ERKs) signalling. Biochem. Pharmacol. 64, 755–763.

[10] Fuentes, E. N., Bjornsson, B. T., Valdes, J. A., Einarsdottir, I. E., Lorca, B., et al. (2011) The IGF-I/PI3K/Akt and the IGF-I/MAPK/ERK pathways in vivo in skeletal muscle are regulated by nutrition and contribute to somatic growth in the fine flounder. Am. J. Physiol. 300, R1532–R1542.

[11] Ibarra, C., Estrada, M., Carrasco, L., Chiong, M., Liberona, J. L., et al. (2004) Insulin-like growth factor-1 induces an inositol 1,4,5-trisphosphate-dependent increase in nuclear and cytosolic calcium in cultured rat cardiac myocytes. J. Biol. Chem. 279, 7554–7565.

[12] Ibarra, C., Vicencio, J. M., Estrada, M., Lin, Y., Rocco, P., et al. (2012) Local control of nuclear Ca^{2+} signalling in cardiac myocytes by perinuclear microdomains of sarcolemmal igf-1 receptors. Circ. Res. 112, 236–245.

[13] Soonpaa, M. H. and Field, L. J. (1998) Survey of studies examining mammalian cardiomyocyte DNA synthesis. Circ. Res. 83, 15–26.

[14] Mehrhof, F. B., Muller, F. U., Bergmann, M. W., Li, P., Wang, Y., et al. (2001) In cardiomyocyte hypoxia, insulin-like growth factor-I-induced antiapoptotic signaling requires phosphatidylinositol-3-OH-kinase-dependent and mitogen-activated protein kinase-dependent activation of the transcription factor cAMP response element-binding protein. Circulation 104, 2088–2094.

[15] Eisner, V., Criollo, A., Quiroga, C., Olea-Azar, C., Santibanez, J. F., et al. (2006) Hyperosmotic stress-dependent NFkappaB activation is regulated by reactive oxygen species and IGF-1 in cultured cardiomyocytes. FEBS Lett. 580, 4495–4500.

[16] Chiong, M., Wang, Z. V., Pedrozo, Z., Cao, D. J., Troncoso, R., et al. (2011) Cardiomyocyte death: mechanisms and translational implications. Cell Death Dis. 2, e244.

[17] Saetrum Opgaard, O. and Wang, P. H. (2005) IGF-I is a matter of heart. Growth Horm. IGF Res. 15, 89–94.

[18] Kurmasheva, R. T. and Houghton, P. J. (2006) IGF-I mediated survival pathways in normal and malignant cells. Biochim. Biophys. Acta 1766, 1–22.

[19] Foncea, R., Galvez, A., Perez, V., Morales, M. P., Calixto, A., et al. (2000) Extracellular regulated kinase, but not protein kinase C, is an antiapoptotic signal of insulin-like growth factor-1 on cultured cardiac myocytes. Biochem. Biophys. Res. Commun. 273, 736–744.

[20] Maggi, D., Biedi, C., Segat, D., Barbero, D., Panetta, D., et al. (2002) IGF-I induces caveolin 1 tyrosine phosphorylation and translocation in the lipid rafts. Biochem. Biophys. Res. Commun. 295, 1085–1089.

[21] Salani, B., Briatore, L., Garibaldi, S., Cordera, R., and Maggi, D. (2008) Caveolin-1 down-regulation inhibits insulin-like growth factor-I receptor signal transduction in H9C2 rat cardiomyoblasts. Endocrinology 149, 461–465.

[22] Higashi, Y., Sukhanov, S., Anwar, A., Shai, S. Y., and Delafontaine, P. (2010) IGF-1, oxidative stress and atheroprotection. Trends Endocrinol. Metab. 21, 245–254.

[23] Jia, G., Cheng, G., Gangahar, D. M., and Agrawal, D. K. (2006) Insulin-like growth factor-1 and TNF-alpha regulate autophagy through c-jun N-terminal kinase and Akt pathways in human atherosclerotic vascular smooth cells. Immunol. Cell Biol. 84, 448–454.
[24] Murrow, L. and Debnath, J. (2012) Autophagy as a stress-response and quality-control mechanism: implications for cell injury and human disease. Annu. Rev. Pathol. 8, 105–137.
[25] Nemchenko, A., Chiong, M., Turer, A., Lavandero, S., and Hill, J. A. (2011) Autophagy as a therapeutic target in cardiovascular disease. J. Mol. Cell Cardiol. 51, 584–593.
[26] Xie, M., Morales, C. R., Lavandero, S., and Hill, J. A. (2011) Tuning flux: autophagy as a target of heart disease therapy. Curr. Opin. Cardiol. 26, 216–222.
[27] Nakai, A., Yamaguchi, O., Takeda, T., Higuchi, Y., Hikoso, S., et al. (2007) The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. Nat. Med. 13, 619–624.
[28] Taneike, M., Yamaguchi, O., Nakai, A., Hikoso, S., Takeda, T., et al. (2010) Inhibition of autophagy in the heart induces age-related cardiomyopathy. Autophagy 6, 600–606.
[29] Maron, B. J., Roberts, W. C., Arad, M., Haas, T. S., Spirito, P., et al. (2009) Clinical outcome and phenotypic expression in LAMP2 cardiomyopathy. JAMA 301, 1253–1259.
[30] Kanamori, H., Takemura, G., Maruyama, R., Goto, K., Tsujimoto, A., et al. (2009) Functional significance and morphological characterization of starvation-induced autophagy in the adult heart. Am. J. Pathol. 174, 1705–1714.
[31] Matsui, Y., Takagi, H., Qu, X., Abdellatif, M., Sakoda, H., et al. (2007) Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circ. Res. 100, 914–922.
[32] Cao, D. L., Wang, Z. V., Battiprolu, P. K., Jiang, N., Morales, C., et al. (2011) HDAC inhibitors attenuate cardiac hypertrophy by suppressing maladaptive autophagy. Proc. Natl. Acad. Sci. USA 108, 4123–4128.
[33] Wang, Z. V., Rothermel, B. A., and Hill, J. A. (2010) Autophagy in hypertensive heart disease. J. Biol. Chem. 285, 8509–8514.
[34] Rothermel, B. A. and Hill, J. A. (2007) Myocyte autophagy in heart disease: friend or foe? Autophagy 3, 632–634.
[35] Zhu, H., Tannous, P., Johnstone, J. L., Kong, Y., Shelton, J. M., et al. (2007) Cardiac autophagy is a maladaptive response to hemodynamic stress. J. Clin. Invest. 117, 1782–1793.
[36] Bitto, A., Lerner, C., Torres, C., Roell, M., Malaguti, M., et al. (2010) Long-term IGF-I exposure decreases autophagy and cell viability. PLoS One 5, e12592.
[37] Gu, Y., Wang, C., and Cohen, A. (2004) Effect of IGF-1 on the balance between autophagy of dysfunctional mitochondria and apoptosis. FEBS Lett. 577, 357–360.
[38] Sobolewska, A., Gajewska, M., Zarzynska, J., Gajkowska, B., and Motyl, T. (2009) IGF-I, EGF, and sex steroids regulate autophagy in bovine mammary epithelial cells via the mTOR pathway. Eur. J. Cell Biol. 88, 117–130.
[39] Aki, T., Yamaguchi, K., Fujimiya, T., and Mizukami, Y. (2003) Phosphoinositide 3-kinase accelerates autophagic cell death during glucose deprivation in the rat cardiomyocyte-derived cell line H9c2. Oncogene 22, 8529–8535.
[40] Bains, M., Florez-McClure, M. L., and Heidenreich, K. A. (2009) Insulin-like growth factor-I prevents the accumulation of autophagic vesicles and cell death in Purkinje neurons by increasing the rate of autophagosome-to-lysosome fusion and degradation. J. Biol. Chem. 284, 20398–20407.
[41] Cardenas, C. and Foskett, J. K. (2012) Mitochondrial Ca^{2+} signals in autophagy. Cell Calcium 52, 44–51.
[42] Cardenas, C., Miller, R. A., Smith, I., Bui, T., Molgo, J., et al. (2010) Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca^{2+} transfer to mitochondria. Cell 142, 270–283.
[43] Bai, X. and Jiang, Y. (2010) Key factors in mTOR regulation. Cell Mol. Life Sci. 67, 239–253.
[44] Laplante, M. and Sabatini, D. M. (2012) mTOR signaling in growth control and disease. Cell 149, 274–293.
[45] Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Ruegg, M. A., et al. (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat. Cell Biol. 6, 1122–1128.
[46] Hara, K., Maruki, Y., Long, X., Yoshino, K., Oshiro, N., et al. (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell 110, 177–189.
[47] Sancak, Y., Thoreen, C. C., Peterson, T. R., Lindquist, R. A., Kang, S. A., et al. (2007) PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol. Cell 25, 903–915.
[48] Oh, W. J. and Jacinto, E. (2011) mTOR complex 2 signaling and functions. Cell Cycle 10, 2305–2316.
[49] Inoki, K., Li, Y., Zhu, T., Wu, J., and Guan, K. L. (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat. Cell Biol. 4, 648–657.
[50] Feng, Z. and Levine, A. J. (2010) The regulation of energy metabolism and the IGF-1/mTOR pathways by the p53 protein. Trends Cell Biol. 20, 427–434.
[51] Ganley, I. G., Lam du, H., Wang, J., Ding, X., Chen, S., et al. (2009) ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J. Biol. Chem. 284, 12297–12305.
[52] Hosokawa, N., Hara, T., Kaizuka, T., Kishi, C., Takamura, A., et al. (2009) Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. Mol. Biol. Cell 20, 1981–1991.
[53] Jung, C. H., Ro, S. H., Cao, J., Otto, N. M., and Kim, D. H. (2010) mTOR regulation of autophagy. FEBS Lett. 584, 1287–1295.
[54] Settembre, C., Zoncu, R., Medina, D. L., Vetrini, F., Erdin, S., et al. (2012) A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. EMBO J. 31, 1095–1108.
[55] Meley, D., Bauvy, C., Houben-Weerts, J. H., Dubbelhuis, P. F., Helmond, M. T., et al. (2006) AMP-activated protein kinase and the regulation of autophagic proteolysis. J. Biol. Chem. 281, 34870–34879.
[56] Mihaylova, M. M. and Shaw, R. J. (2011) The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nat. Cell Biol. 13, 1016–1023.

[57] Inoki, K., Zhu, T., and Guan, K. L. (2003) TSC2 mediates cellular energy response to control cell growth and survival. Cell 115, 577–590.
[58] Shaw, R. J. (2009) LKB1 and AMP-activated protein kinase control of mTOR signalling and growth. Acta Physiol. (Oxf) 196, 65–80.
[59] Steinberg, G. R. and Kemp, B. E. (2009) AMPK in health and disease. Physiol. Rev. 89, 1025–1078.
[60] Hardie, D. G. (2011) AMPK and autophagy get connected. EMBO J. 30, 634–635.
[61] Lee, J. W., Park, S., Takahashi, Y., and Wang, H. G. (2010) The association of AMPK with ULK1 regulates autophagy. PLoS One 5, e15394.
[62] Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., et al. (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol. Cell 30, 214–226.
[63] Egan, D. F., Shackelford, D. B., Mihaylova, M. M., Gelino, S., Kohnz, R. A., et al. (2011) Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science 331, 456–461.
[64] DeBosch, B. J. and Muslin, A. J. (2008) Insulin signaling pathways and cardiac growth. J. Mol. Cell Cardiol. 44, 855–864.
[65] Nagoshi, T., Yoshimura, M., Rosano, G. M., Lopaschuk, G. D., and Mochizuki, S. (2011) Optimization of cardiac metabolism in heart failure. Curr. Pharm. Des. 17, 3846–3853.
[66] Muslin, A. J. (2011) Akt2: a critical regulator of cardiomyocyte survival and metabolism. Pediatr. Cardiol. 32, 317–322.
[67] Bailyes, E. M., Nave, B. T., Soos, M. A., Orr, S. R., Hayward, A. C., et al. (1997) Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting. Biochem. J. 327, 209–215.
[68] Slaaby, R., Schaffer, L., Lautrup-Larsen, I., Andersen, A. S., Shaw, A. C., et al. (2006) Hybrid receptors formed by insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) have low insulin and high IGF-1 affinity irrespective of the IR splice variant. J. Biol. Chem. 281, 25869–25874.
[69] Soos, M. A., Field, C. E., and Siddle, K. (1993) Purified hybrid insulin/insulin-like growth factor-I receptors bind insulin-like growth factor-I, but not insulin, with high affinity. Biochem. J. 290, 419–426.
[70] Marsh, S. A. and Davidoff, A. J. (2012) Heart smart insulin-like growth factor 1. Hypertension 59, 550–551.
[71] Tang, M. S., Redfors, B., Lindbom, M., Svensson, J., Ramunddal, T., et al. (2012) Importance of circulating IGF-1 for normal cardiac morphology, function and post infarction remodeling. Growth Horm. IGF Res. 22, 206–211.
[72] Zhang, Y., Yuan, M., Bradley, K. M., Dong, F., Anversa, P., et al. (2012) Insulin-like growth factor 1 alleviates high-fat diet-induced myocardial contractile dysfunction: role of insulin signaling and mitochondrial function. Hypertension 59, 680–693.