Review

New insights into IGF-1 signaling in the heart

Rodrigo Troncoso[1,2*], Cristián Ibarra[3*], Jose Miguel Vicencio[4], Enrique Jaimovich[2], and Sergio Lavandero[1,2,5]

[1] Facultad de Ciencias Quimicas y Farmaceuticas & Facultad Medicina, Universidad de Chile, Santiago 838049, Chile
[2] Centro de Estudios Moleculares de la Célula, Facultad de Ciencias Quimicas y Farmaceuticas & Facultad de Medicina, Universidad de Chile, Santiago 838049, Chile
[3] Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm 17177, Sweden
[4] Hatter Cardiovascular Institute, University College London, London WC1E 6HX, UK
[5] Department of Internal Medicine (Cardiology Division), University of Texas Southwestern Medical Center, Dallas, TX, 75390-8573, USA

Insulin-like growth factor 1 (IGF-1) signaling regulates contractility, metabolism, hypertrophy, autophagy, senescence, and apoptosis in the heart. IGF-1 deficiency is associated with an increased risk of cardiovascular disease, whereas cardiac activation of IGF-1 receptor (IGF-1R) protects from the detrimental effects of a high-fat diet and myocardial infarction. IGF-1R activates multiple pathways through its intrinsic tyrosine kinase activity and through coupling to heterotrimeric G protein. These pathways involve classic second messengers, phosphorylation cascades, lipid signaling, Ca^{2+} transients, and gene expression. In addition, IGF-1R triggers signaling in different subcellular locations including the plasma membrane, perinuclear T tubules, and also in internalized vesicles. In this review, we provide a fresh and updated view of the complex IGF-1 scenario in the heart, including a critical focus on therapeutic strategies.

IGF-1 and the heart

The hormone insulin-like growth factor 1 (IGF-1) is a small peptide of 7.6 kDa, which is composed of 70 amino acids and shares 50% homology with insulin [1]. IGF-1 plays key roles in regulating proliferation, differentiation, metabolism, and cell survival. It is mainly synthesized and secreted by the liver in response to hypothalamic growth hormone (GH); its plasma concentration is finely regulated (Box 1). However, other tissues also produce IGF-1, which acts locally as an autocrine and paracrine hormone. IGF-1 exhibits pleiotropic effects in many organs and is also involved in the development of several pathologies. At the cellular level, IGF-1 can act in different subcellular compartments. It is therefore important to understand the complexity of IGF-1 signaling and its role in the onset and progression of disease. Particularly in the heart, IGF-1 regulates several cellular processes including metabolism, apoptosis, autophagy, aging, and growth [2–4].

Cardiovascular disease (see Glossary) is the primary cause of death throughout the world and epidemiologic studies have projected mortality due to cardiovascular disease increasing worldwide (up to 23.3 million people by 2030) because of the aging population [5]. The end result of many forms of cardiovascular disease is heart failure [6]. By contrast, IGF-1 deficiency has been described in patients with Laron syndrome, liver cirrhosis, intrauterine

Glossary

Apoptosis: also known as programed cell death type I in which the cell uses specialized cellular machinery to kill itself; a cell suicide mechanism that enables metazoans to control cell number and eliminate cells that threaten the animal's survival.
Autocrine: denoting a mode of hormone action in which a hormone binds to receptors and affects the function of the same cell type that produced it.
Autophagy: catabolic process that delivers substrates for lysosomal turnover; involved in protein and organelle degradation as well as nonselective breakdown of cytoplasmic components during nutrient starvation or stress.
Bioenergetics: term that describes the biochemical or metabolic pathways through which the cell ultimately obtains energy.
Cardiac progenitor cells: stem-like cells that differentiate into cardiomyocytes or fibroblasts.
Cardiovascular disease: refers to any disease that affects the cardiovascular system, including cardiac disease, vascular disease of the brain and kidney, and peripheral arterial disease.
Heart failure: occurs when the heart is unable to provide sufficient pump action to maintain blood flow to meet the needs of the body.
Hyperplasia: increase in the volume of an organ in which the cells maintain their size but increase in number.
Hypertrophy: increase in the size of an organ or tissue due to the enlargement of its component cells and not due to their proliferation.
Laron syndrome: autosomal recessive disorder characterized by short stature; it results from failure to generate insulin-like growth factor 1 (IGF-1) in response to hypothalamic growth hormone (GH) due to dysfunction of the GH receptor.
Metabolism: term used to describe all chemical reactions comprising anabolsim (or synthesis) and catabolism (or degradation) that are involved in maintaining the living state of the cells and the organism.
Paracrine: denoting a type of hormone action in which a hormone is synthesized and released from cells to act upon neighboring cells or cell types.
rhIGF-1: recombinant human IGF-1.

Corresponding author: Lavandero, S. (slavander@uchile.cl, sergiolavandero@gmail.com).
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[*]These authors contributed equally to this work.

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Box 1. IGF-1 synthesis and biodisponibility

Insulin-like growth factor 1 (IGF-1) is a 70 amino acid peptide hormone with endocrine, paracrine, and autocrine effects. It shares >60% structure homology with IGF-2 and 50% with pro-insulin. IGF-1 is mainly synthesized in the liver in response to hypothalamic growth hormone (GH). In the peripheral circulation it exerts negative feedback on the somatotrophic axis suppressing pituitary GH release. IGF-1 can also be generated in almost all tissues, but liver synthesis accounts for nearly 75% of circulating IGF-1 levels. As a hormone with a wide range of physiological roles, IGF-1 circulating levels must be strictly controlled. Around 98% of circulating IGF-1 is bound to insulin-like growth factor binding protein (IGFBP). Six forms of high affinity IGFBP have been described, with IGFBP3 binding approximately 90% of circulating IGF-1. Also, IGFBP1–6 and their fragments have significant intrinsic biological activity independent of IGF-1 interaction.

growth restriction, and age-related neurological and cardiovascular disease [7]. Circulating IGF-1 levels correlate negatively with the risk of developing cardiovascular disease [3]. A low circulating level of IGF-1 is associated with an increased risk of coronary artery disease [8] and has been used as a prognostic indicator for ischemic heart disease [9]. Similarly, elderly patients exhibiting low IGF-1 levels present with an increased risk of ischemic stroke and congestive heart failure [10]. IGF-1-binding proteins (IGFBPs; Box 1) have high affinity for IGF-1 and play key roles in transport and storage of IGF-1. IGFBP-3 is the most abundant IGFBP and the ratio IGF-1:IGFBP-3 has been proposed to reflect the activity of IGF-1 in individuals, thereby representing a valuable tool as a predictor of clinical outcomes in heart failure [11]. In this respect, the cardioprotection by IGF-1 is well established [2,12–15] and in some populations low IGF-1 levels are associated with ischemic heart disease and mortality [16,17].

In this review we discuss the complexity of IGF-1 receptor (IGF-1R) signaling in the heart, from its canonical ability to signal through tyrosine kinase activity to noncanonical pathways involving heterotrimeric G protein. We also highlight the impact of IGF-1R signaling on survival, growth, metabolism, aging, and regeneration, and the potential application as a treatment for cardiovascular disease.

IGF-1 receptor signaling

The complexity of the IGF-1 receptor

The cardiac effects of IGF-1 are mediated by activation of the plasma membrane IGF-1R, which belongs to the receptor tyrosine kinase (RTK) family. IGF-1R comprises a $\alpha_2\beta_2$ heterotetrameric complex of approximately 400 kDa. Structurally, IGF-1R has two extracellular α-subunits that contain the ligand-binding sites. Each α-subunit couples to one of two membrane-spanning β-subunits, which contain an intracellular domain with intrinsic tyrosine kinase activity [18]. Both subunits of IGF-1R are the product of one single gene, which is synthesized as a 180 kDa precursor [19]. The immature IGF-1R full peptide is further glycosylated, dimerized, and proteolytically processed for assembly of the mature receptor isoforms α and β. In neonatal and adult rat cardiomyocytes, the IGF-1R precursor peptide and the processed α and β receptor subunits have been detected [20]. Binding of IGF-1 to its receptor initiates a complex signaling cascade in cardiomyocytes [21]. Activation starts by triggering the kinase domain in the β subunits, leading to receptor autophosphorylation and tyrosine phosphorylation of multiple substrates [18]. Through these initial events, IGF-1-encoded information is transduced to a complex network of intracellular lipids, second messengers, and serine/threonine kinases that ultimately links IGF-1 to the regulation of cardiomyocyte proliferation, differentiation, metabolism, hypertrophy, and protection from cell death.

Canonical and noncanonical IGF-1 signaling pathways

Activation of IGF-1R requires the sequential phosphorylation of three conserved tyrosine residues within the activation loop of the catalytic domain [22]. From these phosphorylated motifs, tyrosine 950 contained in an NPXY motif provides a docking site for the recruitment of adaptor proteins, such as insulin receptor substrate-1 (IRS-1) and Shc, as an obligatory step to initiate signaling cascades. Two canonical pathways are activated by IGF-1R in cardiomyocytes – the phosphatidylinositol-3 kinase (PI3K)/Akt pathway and the extracellular signal-regulated kinase (ERK) pathway. Both pathways have been extensively studied, and their involvement in the pro-hypertrophic [23] and pro-survival [24] actions in cardiomyocytes is well established. Interestingly, a noncanonical signaling mechanism for IGF-1R in cardiomyocytes has been described in several recent studies [25–28]. These studies show that some of the effects of IGF-1 are inhibited by the heterotrimeric Gi protein blocker Pertussis toxin (PTX) in several cell lines [25–28], suggesting that IGF-1R is a dual-activity receptor that triggers tyrosine-kinase-dependent responses as well as Gi-protein-dependent pathways. This duality has been reported in cultured neonatal cardiomyocytes; IGF-1R can activate ERK and Akt but also phospholipase C (PLC), which increases inositol 1,4,5 triphosphate ($InsP_3$; IP3) leading to nuclear Ca^{2+} signals [29]. These effects can be fully abolished by incubating the cells with PTX or by overexpressing the Gβγ dimmer-scavenger β-adrenergic receptor kinase carboxy terminal peptide (βARKct) [20,29]. Immunoprecipitation studies indicate that, under basal conditions, there is an interaction between IGF-1Rβ and the subunits Gαi and Gβ of Gi protein, and this interaction increases after binding of IGF-1 [20]. Moreover, activation of the noncanonical IGF-1R/Gi/PLC/$InsP_3$/Ca^{2+} pathway crosstalks with and regulates the canonical ERK pathway, because separate incubation with PTX, overexpression of βARKct, and inhibition of PLC dramatically decrease IGF-1-induced ERK phosphorylation [29]. These studies provide clear evidence of the existence of the noncanonical signaling mechanism of IGF-1R in cardiomyocytes, mediated by a PTX-sensitive heterotrimeric G protein. A summarized diagram of canonical and noncanonical pathways of IGF-1R is presented in Figure 1. Nevertheless, the complexity of IGF-1R signaling not only relies on the activation of multiple intracellular signaling pathways but is also determined by a particular structural organization of IGF-1R signaling microdomains in cardiomyocytes.

Atypical subcellular distribution of the IGF-1 receptor

As for many other classical receptors, it is assumed that the IGF-1R is functionally located at the plasma membrane. In

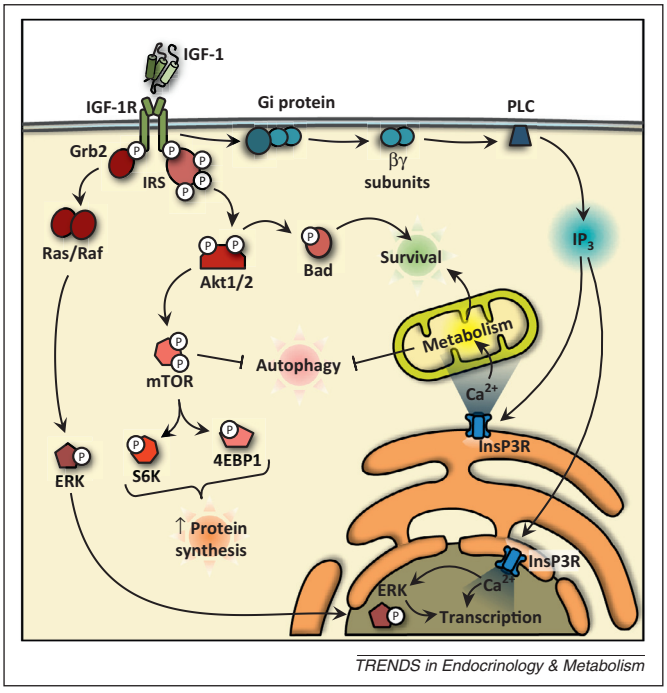

Figure 1. Canonical and noncanonical signaling pathways activated by insulin-like growth factor 1 (IGF-1) in cardiomyocytes. Binding of IGF-1 to plasma membrane IGF-1 receptor (IGF-1R) leads to receptor autophosphorylation in the intracellular β-subunits. Docking of Grb2 to the phosphorylated IGF-1Rβ subunits leads to extracellular signal-regulated kinase (ERK) phosphorylation through the Ras/Raf/Mitogen-activated protein kinase (MEK) axis. Phosphorylated ERK can translocate to the nucleus to control gene expression. Phosphorylated β-subunits also provide docking sites for insulin receptor substrate-1 (IRS-1), which mediates phosphatidylinositol-3 kinase (PI3K) activation and Akt phosphorylation. Downstream targets of activated Akt are mechanistic target of rapamycin (mTOR), which suppresses autophagy and promotes protein synthesis by activating S6K and eukaryotic translation initiation factor 4E binding protein 1 (4EBP1). Akt also phosphorylates and inactivates Bad, thus inhibiting apoptosis. IGF-1R activation also promotes its interaction with a Pertussis-toxin-sensitive heterotrimeric Gi protein, which mediates the activation of phospholipase C (PLC) and hydrolysis of plasma membrane phosphatidylinositol 4,5 biphosphate (PIP_2) to form inositol 1,4,5 triphosphate ($InsP_3$; IP3) which activates $InsP_3$ receptors located at the endoplasmin reticulum (ER)/nuclear envelope Ca^{2+} store, producing nucleoplasmic and cytoplasmic Ca^{2+} increases. The former is involved in the regulation of specific target genes and the latter promotes mitochondrial Ca^{2+} uptake, which increases mitochondrial respiration and metabolism, further preventing apoptosis and regulating autophagy. Canonical signaling pathways include the ERK and Akt axes, and are shown in red, whereas the noncanonical G protein pathway is shown in blue. Both pathways interact as Ca^{2+} contributes to ERK activation and additionally both Akt and ERK can compensate each other's activation. Abbreviations: MEK, Mitogen-activated protein kinase; mTOR, mechanistic target of rapamycin; 4EBP1, eukaryotic translation initiation factor 4E binding protein 1; PIP_2, phosphatidylinositol 4,5 biphosphate.

cardiomyocytes, however, increasing evidence indicates that surface receptors can localize to the nuclear membranes, where they can undergo activation by similar mechanisms to their plasma membrane counterparts [30]. Other sarcolemmal receptors, such as endothelin-1 [31], angiotensin II [32], or β-adrenergic receptors [33], have been detected in cardiomyocyte nuclei. This nuclear-delimited pool of receptors is functional and can control signaling and gene expression through Ca^{2+} and cAMP compartmentation [20,34]. This effect correlates with the atypical localization of the IGF-1R complex. The α and β subunits can be detected in plasma membrane protein fractions; a significant amount, however, exists in nuclear fractions, indicating two segregated pools of IGF-1R – a sarcolemmal pool and a nuclear pool. This raises the question as to which IGF-1R pool is instrumental for the fast nuclear actions of IGF-1. In cardiomyocytes, IGF-1R is located at the plasma membrane; a significant amount of IGF-1R complex, however, is recruited to the perinuclear region. This apparently contradictory localization is explained by the strategic distribution of IGF-1R in perinuclear T tubules, which are in direct apposition to the nuclear envelope. This interesting pattern of plasma membrane receptors located either in deep T tubules or in peripheral sarcolemma has been observed for β-adrenergic receptors as well [34], suggesting that T tubules may be the platform for several sarcolemmal receptors that require internal compartmentation. The architecture of T tubules is critically important for the efficacy of Ca^{2+}-induced Ca^{2+} release during normal cardiomyocyte contraction and the morphology and density of T tubules are altered in some cardiac pathologies, such as ischemic heart disease, idiopathic dilated cardiomyopathy, and hypertrophic obstructive cardiomyopathy [35].

Although the above mentioned distribution requires a mature T-tubule network, which is only present in adult cardiomyocytes, the invaginated distribution of IGF-1R has also been described in neonatal cardiomyocytes [20]. These immature sarcolemmal invaginations exhibit markers of T tubules and lipid rafts, and can be denominated as premature T tubules or pre-T-tubules. They are likely to have two main functions: acting as organizers of the excitation–contraction coupling machinery during cardiomyocyte differentiation and serving as a perinuclear platform for sarcolemmal receptors that promote gene expression of cardiac-committed genes. Thus, in cardiomyocytes, IGF-1R signaling has a privileged subcellular localization that allows a fast and direct interorganelle communication between the cell surface and the nuclear signaling machinery [36]. These novel findings establish a new level of complexity in receptor signaling where, depending on location, the same receptor can regulate different functions. Additionally, this model for the local regulation of nuclear Ca^{2+} provides a new insight into Ca^{2+} signaling in the heart. Classically, it has been considered that $InsP_3$-dependent nuclear Ca^{2+} signals are generated as a result of the solubility of $InsP_3$, which accounts for its fast diffusion from the plasma membrane to the nuclear $InsP_3$ receptors [36]. Recent findings using nuclear-restricted buffers of Ca^{2+} [20] or $InsP_3$ [37] have, however, revealed that these signaling events take place inside the nucleus, which is dependent on the perinuclear activation of IGF-1R. This novel view of nuclear Ca^{2+} signaling explains how nuclear Ca^{2+} changes can be insulated from the cytosolic Ca^{2+} oscillations that regulate excitation–contraction coupling in the cytosol. A comparison between these two views is presented in Figure 2. The compartmentation of receptor signaling in deep T tubules may have important implications in disease states such as heart failure or during ischemia, where the structure of T tubules is severely affected [38].

Pleiotropic actions of IGF-1 on the cardiovascular system

Cell death

The heart has a low capacity for regeneration and repair after injury so that it is susceptible to numerous stresses

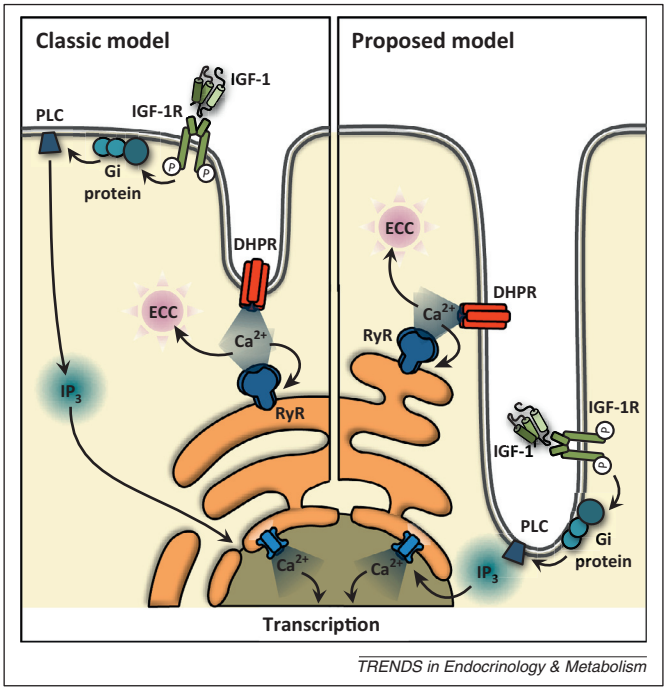

Figure 2. Classical versus proposed models of nuclear Ca^{2+} signaling in cardiomyocytes. The insulin-like growth factor 1 receptor (IGF-1R) can specifically regulate nuclear Ca^{2+} signaling independently of the role of Ca^{2+} on excitation–contraction coupling. On the classic model, inositol 1,4,5 triphosphate ($InsP_3$; IP3) produced after IGF-1R activation travels from the peripheral plasma membrane to the nucleus, where it activates $InsP_3$ receptors. In this model $InsP_3$ bypasses its receptors present on the sarcoplasmic reticulum, which would lead to cytosolic Ca^{2+} signals. The novel model that we propose is based on recent findings [20,37], where the IGF-1R signaling complex is present in T-tubule invaginations toward the nucleus. In these compartments, IGF-1R activation leads to locally restricted $InsP_3$ production that allows nuclear Ca^{2+} signals to regulate gene expression of genes associated with the development of cardiomyocyte hypertrophy. Abbreviations: RyR, ryanodine receptor; ECC, excitation–contraction coupling; PLC, phospholipase C; DHPR, dihydropyridine receptor.

that lead to cardiomyocyte death. Upon injury, the heart must respond to adapt its function to ever-changing workload demands. Cell death, either progressive or acute, is a hallmark of various cardiac diseases, including heart failure and myocardial infarction [39]. A variety of stimuli are known to participate in the progression of heart failure including hypoxia, ischemia/reperfusion, oxidative stress, and osmotic stress, all of which are potent inducers of cardiomyocyte cell death [39]. The antiapoptotic and pro-survival properties of IGF-1 are fundamentally important in cardiovascular medicine. At the cellular level, antiapoptotic effects of IGF-1 are mediated by activation of the PI3K/Akt/mechanistic target of rapamycin (mTOR) and the Ras/Raf/Mitogen-activated protein kinase (MEK)/ERK signaling pathways (Figure 1) [2]. Activation of Akt appears as an obligate step for the antiapoptotic actions of IGF-1. Some of the downstream targets of Akt are the Bcl-2 family member Bad, procaspase-9, the transcription factors nuclear factor-κB (NFκB), cAMP response-element-binding protein (CREB), Forkhead family transcription factor (FoxO), and glycogen synthase kinase-3β (GSK-3β) [40]. Several studies have reported phosphorylation-mediated inactivation of Bad as a mechanism for Akt-dependent survival [41]. Nonphosphorylated Bad exerts its apoptotic function by forming heterodimers with mitochondria-bound Bcl-2 and Bcl-X_L, neutralizing their protective effects on mitochondria and promoting cell death. Activation of IGF-1R is followed by Akt-dependent phosphorylation of Bad and by MEK1-dependent phosphorylation of ERK1 and ERK2, which in turn activates pro-survival pathways [2].

Growth

IGF-1 is a positive regulator of protein synthesis and cell growth. Systemic and local IGF-1 are essential for appropriate organ growth during embryonic development. Mice lacking the *Igf-1* gene are born with a 40% lower bodyweight in comparison with wild type mice [42]. Besides supporting physiological growth, IGF-1 also promotes hypertrophy of tissues with high energy demands. For instance, IGF-1 and IGF-1R are upregulated during differentiation of skeletal muscle cells, whereas selective overexpression of IGF-1 in skeletal or cardiac muscle results in increased protein content and cell size [43]. Although IGF-1 promotes activation of several signaling pathways, muscle hypertrophy has mostly been described as dependent on the activation of the PI3K/Akt/mTOR axis [43]. However, Song *et al.* showed that the overexpression of IGF-1 in skeletal muscle results in hypertrophy mediated via the mTOR/p70S6K pathway, but in a manner independent of Akt [44]. Other pathways such as the ERK and G protein axis are, however, thought to be required for hypertrophy in response to different stimuli [45,46]. In addition, the ERK axis crosstalks with the Akt pathway to compensate for Akt deficiency, leading to hypertrophy [47]. Mice overexpressing cardiomyocyte-specific IGF-1R subjected to exercise show that IGF-1-dependent cardiac hypertrophy mimics physiological, not pathological, cardiac growth by promoting protein translation rather than by activating maladaptative cardiac gene expression [48]. Moreover, *Igf-1r* deletion in adult cardiomyocytes did not affect the baseline growth phenotype of the heart, although it induced resistance to exercise-induced hypertrophy in mice [23]. By contrast, overexpression of a different IGF-1 transgene, using the α-skeletal actin promoter, which increases the expression of human IGF-1 in skeletal and cardiac muscle, produced hypertrophy and heart failure. Despite initially inducing physiological hypertrophy, this contradictory final outcome could be related to the different transgene used [49].

Autophagy

Autophagy occurs at low basal levels in virtually all cells maintaining homeostatic functions such as turnover of proteins and organelles [50]. Autophagy can be induced by a wide range of stress conditions, including nutrient starvation, withdrawal of growth factors, oxidative stress, infection, hypoxia, and endoplasmic reticulum stress [50]. A greater extent of autophagy has been associated with multiple stressors inducing pathological cardiac remodelling, including elevated mechanical afterload, chronic ischemia, and ischemia/reperfusion injury [51]. Autophagy acts as a double-edged sword; it can either antagonize or promote disease progression depending on the organ and the magnitude of its activation [52]. Induction of autophagy is protective when cells are starved during ischemia [53], but can be maladaptive with severe afterload stress [54].

One of the canonical pathways activated by IGF-1 is the PI3K/mTOR axis (Figure 1). Because mTOR is a key negative regulator of autophagy [12,55,56], short-term treatment with IGF-1 protects against nutrient-deprivation stress in the heart by decreasing autophagy [4,12]. In this regard, it has been shown that IGF-1 prevents starvation-induced cardiac autophagy by increasing intracellular ATP levels, mitochondrial metabolism, mitochondrial Ca^{2+} uptake, and oxygen consumption, through the Akt/mTOR and AMP-activated protein kinase (AMPK)/mTOR axes [12].

Metabolism

IGF-1 and insulin receptors share more than 80% homology in their kinase domains [57]; they also share downstream effectors for metabolic substrate utilization (PI3K/Akt). A considerable body of evidence refers to insulin/IGF-1 signaling as one single pathway, assuming that insulin is responsible for metabolic changes and IGF-1 responsible for increased protein synthesis and growth [58]. However, the two peptides have critical dissimilarities in their downstream pathways, because they signal through different Akt isoforms [59]. Akt1 is required for normal cardiac growth, whereas Akt2 regulates cardiomyocyte metabolism and survival so that IGF-1 exerts its action through the Akt1 isoform, controlling cell growth. Insulin preferentially activates Akt2, regulating metabolism [59]. Moreover, an additional level of complexity in IGF-1 signaling arises from the identification of insulin and IGF-1 hybrid holoreceptors [60–62]. These chimeras can be activated by insulin as well as IGF-1. Insulin activated this hybrid receptor at supraphysiological doses, whereas IGF-1 principally activated the insulin/IGF-1 hybrid receptor [57]. Expression of hybrid receptors has been detected in human skeletal muscle, heart, coronary artery smooth muscle cells, endothelial cells, adipose tissue, fibroblasts, spleen, red and white blood cells, and placenta [63]. This hybrid receptor consisting of an $\alpha\beta$ subunit pair of both the insulin receptor (IR) and IGF-1R has been described in normal and pathological conditions [64]. The abundance of insulin/IGF-1 hybrid receptors is increased in skeletal muscle of obese patients. This effect is associated with a decrease in insulin receptors caused by hyperinsulinemia [65,66]. In addition, this hybrid receptor may also play a role in several types of cancer [64]. The functional consequences of insulin/IGF-1 hybrid receptor activation in cardiomyocytes remain elusive and further investigations are necessary to unveil the specific signaling pathways controlled by this hybrid receptor.

Reduced IGF-1 levels are independently associated with glucose intolerance, type 2 diabetes mellitus, abdominal obesity, and atherogenic dyslipidemia [7]. Moreover, patients with type 1 or type 2 diabetes mellitus treated with rhIGF-1 enhance protein and glucose metabolism, and improve glucose tolerance, hyperinsulinemia, and hypertriglyceridemia [67,68]. In addition, rhIGF-1 enhances insulin sensitivity and increases oxidative and nonoxidative metabolism [69]. Conversely, high-fat feeding impairs cardiomyocyte IGF-1 signaling, leading to mitochondrial dysfunction, intracellular Ca^{2+} deregulation, and abnormal insulin signaling [70]. Three recent works have approached the mechanism of such control. First, *Igf-1* deletion in liver reduces myocardial creatine, an essential substrate for cardiac energy homeostasis, by regulating the cardiomyocyte creatine transporter gene [71]. Second, in a cardiomyocyte-specific IGF-1 transgenic model, IGF-1 overexpression blunted high-fat-diet-induced insulin resistance, suggesting that IGF-1 may compensate for insulin receptor signaling impairment through the activation of either the IGF-1 receptor or insulin/IGF-1 receptor hybrids [14]. Third, IGF-1 is critical for the maintenance of cardiac bioenergetics during nutrient stress conditions, because IGF-1 promotes mitochondrial metabolism and ATP production by increasing mitochondrial Ca^{2+} uptake and respiration [12]. Additionally, IGF-1 deficiency during starvation increases cardiac AMPK activity, suggesting a distinct role for IGF-1 in preserving cardiac bioenergetics under these conditions maybe through crosstalk with the mTOR pathway [12].

Aging

Ablation of the GH/IGF-1 signaling axis increases lifespan in invertebrates, whereas in mammals this effect remains controversial [3]. The GH/IGF-1 axis progressively declines with aging in elderly humans and laboratory animals, and the reduced IGF-1 levels correlate with a decline in cardiovascular function and the progression of cardiovascular disease. In humans, deficiency of GH and/or IGF-1 has been associated with increased risk of cardiovascular disease, stroke, and diabetes mellitus type 2 [3]. Ecuadorian patients with Laron syndrome have a reduced life expectancy as a result of stroke and cardiovascular disease [72]. Untreated Laron patients show reduced cardiac dimensions and output at rest, alterations that can be improved by supplementation with IGF-1 [73]. In rodents, however, the association between the GH/IGF-1 system and longevity has been extensively demonstrated in Ames dwarf, Snell dwarf, and GH receptor knockout mice. These long-lived rodent models have lower levels of IGF-1 signals and reduced growth and body size. Restoration of GH levels in Ames dwarf mice restores the increased longevity [74]. Lewis dwarf rats, which exhibit an GH/IGF-1 deficiency similar to that in humans, present an increased incidence of late-life stroke but no change in longevity [75]. Lewis dwarf rats also undergo cardiac atrophy, impaired cardiac contractility, and diastolic function [76]. Adeno-associated mediated knockdown of the *Igf-1* gene in the liver of adult mice results in a decrease in circulating IGF-1 levels of approximately 50%, which significantly impairs cardiomyocyte contractility [77]. In experimental aortic constriction studies in mice, liver-specific *Igf-1* deficiency results in a reduced compensatory hypertrophic response and an impaired functional adaptation to pressure overload [78]. Moreover, these mice exhibit dysregulation of Nrf2-dependent antioxidant responses in the vasculature, which leads to marked endothelial dysfunction and endothelial cell apoptosis in the presence of oxidative stress overload [79]. Reduced circulating IGF-1 caused left ventricular dilatation under physiological conditions; it also adversely affected post-myocardial infarction remodelling, as indicated by more-severe deterioration of cardiac function coupled to a markedly reduced content of creatine in the liver *Igf-1*$^{-/-}$ mice [71].

Sepsis

The systemic inflammatory response syndrome to infection has high incidence and mortality rates around the world [80,81]. Myocardial dysfunction is a well-established manifestation of sepsis and septic shock, with myocardial depression occurring in 40–50% of patients [81]. Several mechanisms have been proposed for this myocardial dysfunction, including excessive cardiac inflammation, mitochondrial dysfunction, cardiomyocyte death by apoptosis or necrosis, impaired contractility secondary to the generation of tumor necrosis factor α (TNFα), and induction of inducible nitric oxide synthase [82].

Circulating levels of IGF-1 are drastically reduced in patients with sepsis. IGF-1 supplementation exerts beneficial effects on survival, possibly improving hepatic bacterial clearance and cellular immune response [83,84]. The cardiac-specific overexpression of IGF-1 rescued lipopolysaccharide (LPS)-induced cardiac contractile dysfunction and intracellular Ca^{2+} mishandling [85]. In skeletal muscle, local administration of IGF-1 prevented the sepsis-induced increase in muscle interleukin-6 and atrophic response seemingly by increasing muscle protein synthesis and potentially decreasing proteolysis [86]. Thus, the local increase in IGF-1 might be effective in ameliorating sepsis-induced cardiac dysfunction or skeletal muscle atrophy.

IGF-1 and cardiac progenitor cells

The role of IGF-1 in cell regeneration has been extensively described in other organs, such as skeletal muscle [87], bone [88], and brain [89]. However, for the heart the evidence has been more elusive. Mammalian cardiomyocytes were thought to cease replication shortly after birth. Subsequent growth of the heart results from cardiomyocyte hypertrophy rather than hyperplasia. New evidence, however, reveals that cardiomyocytes turnover continuously [90,91]. In humans, measurements of cardiomyocyte nuclear content of carbon 14 by retrospective isotope dating studies have established a rate of cardiomyocyte turnover of approximately 1.5% per year [91]. It is still unclear whether new cardiomyocytes derive from endogenous cardiac progenitor cells [92] or from pre-existing cardiomyocytes that re-enter the cell cycle [93]. The majority of regenerative approaches for cardiac therapy have involved transplantation of stem cells expanded *in vitro*, including embryonic stem cells, induced pluripotent stem cells, bone-marrow-derived stem cells, or cardiac progenitors into the infarcted myocardium. Although some of these studies have resulted in improvements in cardiac function, extensive data indicate that transplanted cells do not survive in the myocardium [94], and this has led to the postulation of a paracrine mechanism for the observed beneficial effects [95]. Several molecules have been proposed [96], among which IGF-1 mediates important effects on cardiac progenitors [97]. Most of these effects have been described for c-Kit^+ cardiac progenitor cells [98]. It has been shown that cultured c-Kit^+ cells can secrete IGF-1, improving cardiomyocyte survival and contractility in a co-culture model [99]. Expression of IGF-1R in c-Kit^+ cells isolated from human hearts was correlated with higher telomerase activity, proliferation, cardiac differentiation, and decreased apoptosis, whereas absence of IGF-1R led to cell senescence and increased apoptosis [100], effects that are probably mediated by the activation of the Akt pathway, as suggested in other models [101]. Addition of IGF-1 to cultured c-Kit^+ cells isolated from dog [102] or rat [99] hearts promotes cell migration, proliferation, and survival. In experimental myocardial infarction, direct myocardial administration of IGF-1 along with hepatocyte growth factor (HGF) in mice [103] or rats [104], or administration of biotinylated IGF-1 nanofibers in rats [105], promotes c-Kit^+ cell activation *in vivo*, leading to cardiomyogenesis and decreased scarred tissue, less fibrosis, and reduced hypertrophy. Co-administration of IGF-1 and HGF after myocardial infarction upregulates mRNA and protein levels of c-Kit in the entire heart [106]. Stimulation of c-Kit^+ cells with IGF-1 before their transplantation into the infarcted myocardium increases the recovery of cardiac function parameters and myocardial structure [103,104].

The utility of IGF-1 as a co-adjuvant for stem cell therapy to the heart is likely to be context dependent. The activation of the endogenous pool of c-Kit^+ cells after acute heart failure may suffice to repair damage to the myocardium. However, when the endogenous pool of c-Kit^+ cells is depleted in chronic heart failure patients, transplantation of exogenous c-Kit^+ cells can sustain cardiomyocyte replacement [107]. Therefore, depending on the clinical scenario, local administration of IGF-1 into the heart or IGF-1 analogs with enhanced cardiac tropism could be useful to boost endogenous c-Kit^+ cells after acute heart failure. Alternatively, exposure of exogenous c-Kit^+ cells to IGF-1 before transplantation may represent a reasonable approach in patients with chronic heart failure.

The signaling pathways activated by IGF-1 in cardiac progenitors are poorly studied. Recent findings indicate that IGF-1 promotes intracellular Ca^{2+} oscillations through a PLC/$InsP_3$/$InsP_3$R pathway in c-Kit^+ cells. Inhibition of Ca^{2+} oscillations decreases cell proliferation induced by IGF-1 [108], indicating that the Ca^{2+} signaling axis of the IGF-1R exists in c-Kit^+ cells. In other relevant stem cell populations such as embryonic stem cells [109] or mesenchymal stem cells [110,111], IGF-1 improves stem cell 'transplantability', engraftment, and survival, suggesting that activation of IGF-1 signaling is a common step for the cardiac commitment of stem cells. Altogether this evidence indicates that IGF-1 may be an important endogenous paracrine factor regulating the fate of cardiac progenitor cells *in vivo* through mechanisms that are incompletely understood.

IGF-1 and treatment of cardiovascular disease

The beneficial roles of IGF-1 in the cardiovascular system largely explain the interest in the development of new IGF-1-based treatments for cardiovascular disease. So far the FDA has approved two drugs for the treatment of IGF-1 deficiency: mecasermin (Increlex®), a human recombinant IGF-1 analog; and mecasermin rinfabate (IPLEX®), a binary protein complex of human recombinant IGF-1 and human recombinant IGBP-3. The safety of a chronic systemic IGF-1 therapy is open to question because it could promote severe adverse effects, such as an increased risk of cancer [112]. To avoid these problems, several researchers have selectively overexpressed IGF-1 and IGF-1R in the heart [14,113–115]. Local expression of

IGF-1 in cardiomyocytes protects the heart from oxidative stress [114] and promotes functional recovery after myocardial infarction [115]. Moreover, cardiac overexpression of IGF-1 attenuates or even prevents the contractile and metabolic dysfunction induced by high-fat feeding [14]. Furthermore, cardiomyocyte overexpression of IGF-1R prevents type-1-diabetes-induced cardiac fibrosis and diastolic dysfunction [113]. Altogether, this evidence still supports the notion that IGF-1-based cardiac-specific therapies could become a strong beneficial tool for preventing cardiovascular disease.

Concluding remarks and future perspectives

Abundant evidence supports the key physiological roles of IGF-1 in the heart. In cardiomyocytes, IGF-1 activates multiple downstream signaling pathways for controlling cell death, metabolism, autophagy, differentiation, transcription, and protein synthesis (Figure 1). Of great interest are the findings that the entire IGF-1R complex is strategically located in perinuclear sarcolemmal invaginations that locally control nuclear Ca^{2+} signaling and transcriptional upregulation (Figure 2). This novel evidence changes the classical paradigm of IGF-1 signaling and adds a new level of complexity that may be relevant for other signaling receptors in the heart: interorganelle communication between plasma membrane invaginations and the nucleus. The strategic localization of IGF-1R in these structures and the association with heterotrimeric G proteins may explain the differences in the phenotypic response induced by IGF-1 and others agonists, like endothelin-1 and angiotensin II, that also signal through intracellular Ca^{2+}. By activating a noncanonical, selective mechanism of nuclear Ca^{2+} release, IGF-1 can regulate the expression of a specific set of cardiac genes via the generation of a particular signal-encoding pattern, leading to adaptive cardiac hypertrophy, antiapoptotic effects, and metabolic adaptation. Future research should address whether these new signaling mechanisms of IGF-1 connect to other important signaling activated by different agonists.

Deficiency of IGF-1 in humans and animal models is associated with an elevated risk of cardiovascular disease. In addition, the correlation between low circulating IGF-1 levels and the progression of cardiovascular disease observed during aging evidences the protective role of IGF-1 in the heart. Moreover, reduced IGF-1 levels are associated with obesity and diabetes mellitus suggesting also that cardiac metabolism could be controlled by IGF-1. Short treatment with IGF-1 increased mitochondrial metabolism in nutrient-deprived cardiomyocytes. Altogether, this evidence extends the repertory of pleiotropic effects of IGF-1 in the heart and the therapeutic potential in the treatment of cardiovascular disease.

Despite the many novel findings summarized in this article, there are still many questions left unaddressed (Box 2). First, further investigation is necessary to dissect the signaling pathways regulating different cellular processes such as growth, survival, and metabolism. Additionally, we still do not know whether cytoplasmic and nuclear IGF-1 pathways regulate each other and contribute to orchestrate these processes. More work is needed to sort the cellular effects of IR and IGF-1R or hybrid receptor in the cardiovascular system. Second, the molecular mechanisms that regulate metabolic cardiac adaptation in response to IGF-1 are still incompletely understood; we need to clarify the metabolic pathways activated by IGF-1 and its integration into cardiomyocyte bioenergetics. Third, the signaling pathways activated by IGF-1 in cardiac stem cells and the role of these pathways in controlling survival, proliferation, differentiation, and functional integration of transplanted or endogenous stem cells remain to be elucidated. Finally, evaluation of local cardiac IGF-1 therapy should take into account the acute versus chronic effects, local versus systemic effects, cardiomyocyte versus cardiac progenitor effects, and, overall and perhaps the most challenging, cardioprotective versus oncogenic effects.

Box 2. Outstanding questions

Insulin-like growth factor 1 (IGF-1) is an old friend of the heart. Despite the well-known protective effects of IGF-1 on cardiac function and the antiapoptotic effects of this peptide, novel evidence opens new questions to this longstanding relationship.

- How do the multiple signaling pathways triggered by IGF-1 receptor (IGF-1R) interact with each other? What lies further than extracellular signal-regulated kinase (ERK)/Akt/Ca^{2+} activation toward heart function? Do these signaling pathways regulate cardiac fibroblast or endothelial cell function?
- Which are the specific downstream signaling pathways of the different pools of IGF-1R and their role in regulating cardiomyocyte survival, hypertrophy, metabolism, proliferation? What drives IGF-1R to such specific subcellular compartments?
- What is the relevance of the hybrid IGF-1R/insulin receptors on cardiovascular disease?
- Does a crosstalk exist between insulin receptor and IGF-1R in the heart under physiological and pathological conditions? Is one pathway more beneficial than the other?
- Will stem cell therapy of cardiac progenitors be able to provide concrete treatment opportunities? Is IGF-1 a key regulator of this outcome?

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