IGF-1 AS A TARGET IN EMERGING HEART FAILURE THERAPEUTICS

by

Paulina Joanna Ramos

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SIGNED:

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

5/9/18

Diana Darnell
Associate Professor,
Cellular and Molecular Medicine
(Educator Scholar Track)

Date

5-13-18

Steven Goldman
Sarver Heart Center
Research Scientist
Professor, Medicine

Date

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Abstract:

Insulin-like growth factor 1 (IGF-1) signaling plays a role in the regulation of heart disease. While most therapeutics target the consequences of cell loss and tissue necrosis, the underlying pathophysiology is not addressed. Emerging therapeutics are exploring the potential effects of IGF-1 in cardiac repair and regeneration. IGF-1 has established a role as a cardioprotective cytokine with pleiotropic effects regulating cell growth, proliferation, metabolism, and survival in the heart. Local expression of IGF-1 in cardiac cells constitutes protection from irreversible loss of cell function post cardiac injury. Its deficiency is implicated in dysregulation of IGF-1 signaling and associated with reductions in heart function. Methods to directly increase endogenous levels of IGF-1 in the injured heart are emerging and while the optimal delivery methods, doses, time spans, and frequencies may not be established, it is known that IGF-1 is a necessary component in sustaining cardiomyocyte function in the diseased and failing heart. In this review, I provide background on heart failure and the pathophysiological injurious processes that up and coming therapeutics, targeting IGF-1, can potentially modulate, with IGF-1 as a regulator of cardiac repair and regeneration.

Heart Failure Background:

About 5.7 million adults in the United States suffer from chronic heart failure (CHF), with an estimated 50% of those diagnosed surviving 5 years.[1] Heart disease continues to be the leading cause of death in the United States with 610,000 deaths per year. It is also the leading cause of hospitalizations in patients 65 years of age and above.[2] Heart failure is a chronic condition in which the heart cannot efficiently pump blood to the body, due to low cardiac output, limiting the body's systemic oxygen availability. Cardiac output is the amount of blood pumped per minute, which is the product of the heart rate (beats per minute) times the stroke volume (amount of blood pumped per beat). While most cases of heart failure are associated with low cardiac output, there is a small minority of patients who experience high output heart failure.[3] Heart failure can manifest as a result of coronary artery disease, previous myocardial infarction, hypertension, cardiomyopathy, arrhythmias, and valve disorders amongst other risk factors. Regardless of the etiology or change in cardiac output, the issue lies in the heart's irregular contractility. Left sided heart failure occurs when the left ventricle (LV) cannot work sufficiently hard to pump blood to the body. The left ventricle is larger than the other chambers as it is responsible for the systemic delivery of oxygenated blood and needs sufficient force to push blood to the body through the Aorta; whereas the right ventricle is only responsible for delivering blood to the lungs. The left ventricle can undergo compensatory remodeling in response to hemodynamic changes or cardiac injury leading to neurohormonal activation. Remodeling can be physiological, as a result of exercise, or it can be a pathological maladaptive response to cardiac injury. Systolic heart failure is a form of left sided heart failure in which the left ventricle has decreased function, leading to a reduced ejection fraction (EF), the amount of blood ejected as a fraction of the amount of blood in the left ventricular chamber. In contrast, diastolic heart failure, also a form of left sided heart failure, has preserved ejection fraction. The left ventricular muscle stiffens and is unable to completely fill with blood during diastole. Right sided heart failure usually occurs as a consequence of left sided heart failure. As the left ventricular function deteriorates, blood accumulates in the lungs and the right ventricle must compensate, for the left ventricular decreased function, by working harder. When the left ventricle fails, there is an accumulation of blood in pulmonary circulation, increasing the pulmonary capillary pressure. This increased fluid pressure in the pulmonary circulation causes a backup of blood in the lungs, ultimately damaging the right side of the heart. When the right ventricle fails, the blood backs up into the body's veins. This can lead to pulmonary edema and swelling in the feet, legs, and abdomen. When heart failure causes the buildup of fluid, it is known as congestive heart failure.

Heart failure can result from various pathophysiological injurious processes. The most common cause of heart failure in the United States is coronary artery disease (CAD). Lipoproteins accumulate in the intima of the blood vessel, where low density lipoprotein is oxidized by reactive oxygen species released by macrophages. These macrophages subsequently take up the oxidized low-density lipoprotein creating foam cells that integrate into the plaque forming in the arteries. The plaque impedes blood flow through the vessel. The complete blockage of the artery can lead to ischemia and myocardial infarct (MI). Myocardial infarction is the necrosis of heart tissue as a result of hypoxia due to ischemia. Hypertension is another cause of heart failure. Hypertension is a small vessel disease in which the body's arterioles narrow, leading to excessive force on the vessel walls from blood flow. This narrowing of the blood vessels leads to an increase in vascular resistance to blood flow. To maintain the circulation, the

arterial pressure upstream of the arterioles increases, thus leading to an increase in afterload, or the pressure the left ventricle must overcome to pump blood during systole. This increase in work load strains the heart and can lead to weakening of the heart muscle and less efficient function, as an increase in afterload can lead to a decrease in cardiac output. Another cause of heart failure is cardiomyopathy, a disease of the heart muscle. Cardiomyopathy is an enlargement, thickening, stiffening, or, in rare cases, fibrosis of the heart muscle affecting the function of the heart. Also, some viral infections can induce inflammation of the heart, myocarditis, which can progress to heart failure. Viruses such as the enterovirus Coxsackie B or retrovirus HIV can cause viral cardiomyopathy. Valve disorders are another pathophysiological injurious process that can weaken the heart and impair its function. The tricuspid, pulmonary, mitral, and aortic valves are meant to direct the flow of blood in the heart. When the direction of flow is affected either by a congenital defect or valvular stenosis, the heart must compensate by working harder to pump the blood properly through each chamber and out to the lungs or body. The rate at which the heart beats is another factor that can lead to heart failure. If an arrhythmia arises and is not controlled, whether a tachyarrhythmia or bradycardia, the abnormal rhythm can weaken the heart. Despite the etiology, each of these pathophysiological injury processes leads to the impairment of cardiomyocyte function or tissue necrosis.[4,5]

To compensate for such cellular loss and loss of function, neurohormonal signaling is activated, promoting increased sympathetic activity and decreased parasympathetic activity.[6] This neurohormonal compensation increases heart rate by releasing catecholamines (epinephrine and norepinephrine), and triggers vasoconstriction thus increasing blood pressure. This neurohormonal system also activates the renin-angiotensin-aldosterone signaling pathway, leading to an increase in Na+ reabsorption and water retention. Although this constitutive activation of the sympathetic nervous system and the renin-angiotensin system are activated as a protective and beneficial response; it can be harmful to the patient over a prolonged period as it results in maladaptive remodeling of the left ventricle and impaired function.

In this review the underlying pathology of heart failure and emerging therapeutics are discussed. I also highlight the significance of IGF-1 as a cardioprotective cytokine and its impact on cell proliferation, metabolism, survival, and regeneration. IGF-1 signaling is also discussed as a potential target of up and coming therapies aiming to address the pathophysiology of the diseased and failing heart.

Classifications of Heart Failure:

Heart failure is classified by physicians based on the New York Heart Association Functional Capacity Assessment as well as by the American College of Cardiology Foundation/American Heart Association Classification systems. Heart failure grading by the ACCF/AHA goes by four stages, A-D, rating a patient's stage of disease or progression. The NYHA grading system has four classes, I-IV, to rate each patient's operative capacity. The canonical heart failure symptoms are dyspnea, fatigue, low exercise tolerance, palpitations, peripheral and pulmonary edema, and angina. As the disease progresses, both scoring systems show the factors considered when determining the stage of HF and treatment options available for each patient.[7,8]

Table 1. Comparison of heart failure grading systems by the ACCF/AHA and NYHA.

ACCF/AHA Stages of HF	NYHA Functional Capacity Classification
A. At high risk for HF but without structural heart disease or symptoms of HF	None
B. Structural heart disease but without signs or symptoms of HF	I. Patients with Cardiac disease, but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitations, dyspnea, or anginal pain.
C. Structural heart disease with prior or current symptoms of HF	II. Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitations, dyspnea, or anginal pain.
	III. Patients with marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity caused fatigue, palpitations, dyspnea, or anginal pain.
D. Refractory HF requiring specialized interventions	IV. Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or of the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

Current Treatments:

Current treatment options for heart failure include life style changes, medications, implantable devices, and heart transplant. People with mild to moderate disease can alleviate symptoms and lead normal lives by tracking their fluid intake and limiting salt intake, being physically active, losing weight, eating a healthy diet, limiting caffeine, avoiding alcohol, and ceasing tobacco use. There are several research studies that show the combined use of different classes of drugs for treatment of heart failure. Although there is no cure for heart failure, symptoms and factors that can worsen the condition can be managed and improved through medications.

Some common drugs used for heart failure patients are diuretics, angiotensin converting enzyme (ACE) inhibitors, sinus node inhibitors, beta-adrenergic blockers, and digitalis cardiac glycosides, among other drug classes.[9] There are three types of diuretics, thiazides, potassium sparring, and loop. They all act on different sections of the nephron to inhibit reabsorption of sodium or both sodium and chloride. ACE inhibitors, work to inhibit the conversion of angiotensin I to angiotensin II by blocking the activity of ACE. By blocking the activity of angiotensin II, ACE inhibitors promote vasodilation, decrease blood pressure and improve blood flow. Sinus node inhibitors block the I_f or funny current. The I_f is a hyperpolarization-activated current, or flow of positively charged ions that passes through the hyperpolarization-activated cyclic nucleotide-gated channels, in the sinus node pacemaker cells, to initiate depolarization of the cells. This inhibition of the I_f reduces the heart rate by slowing diastolic depolarization and leads to an increase in stroke volume. Beta-blockers are competitive agonists of the catecholamines', epinephrine and norepinephrine, binding sites on the beta-adrenergic receptors of the sympathetic nervous system. By inhibiting the activity of the two catecholamines, flight or fight responses such as increased heart rate, vasoconstriction and increased blood pressure, are reduced. As the disease progresses, Inotropic agents, which are intravenous drugs, can help those with severe heart failure by improving cardiac output. There are negative and positive inotropes. Negative inotropes weaken the muscular contractile force while positive inotropes strengthen muscular contractile force and enhance cardiac output. The glycosides inhibit the Na^+/K^+

ATPase exchange, decreasing Na^+ concentrations that result in the inability of Na^+/Ca^+ exchange and secretion of Ca^+ from the cell. This leads to an increase in cardiac function. However, their use is limited, or they are used as short-term treatments for patients as they can cause severe cardiovascular adverse effects.[10]

Another treatment option is the use of implantable devices such as pacemakers, implantable cardiac defibrillators (ICDs), and left ventricular assist devices (LVAD). ICDs, are used in people with serious arrhythmias and for people with severe heart failure. The device functions as both a pacemaker and defibrillator. If the heart rate is below the pacing threshold, it will function as a pacemaker and upon recognition of a life-threatening arrhythmia, it can deliver an electric defibrillation shock to normalize the rhythm. Cardiac resynchronization therapy (CRT), or biventricular pacing, is another treatment option for those with heart failure. For CRT, both ventricles must have a lead implanted and connected to the Pacemaker or ICD. The device is set to synchronize the right ventricular and left ventricular contractions. Another therapy device used for the treatment or management of heart failure is the LVAD. The LVAD was first designed as a "Bridge to Transplant," but has more frequently been used as a long-term final therapy (destination therapy), as most people on the heart transplant list are waiting for long periods of time. Its use is indicated for patients with irreversible end stage heart failure at risk of death. The LVAD is surgically implanted and functions as a mechanical pump that pulls blood from the LV and ejects it through the aorta, essentially functioning as the left ventricle. Some of the complications implicated with LVAD implantation are bleeding, thrombosis, stroke, right heart dysfunction, and infection.[11] It has been shown that LVAD therapy slightly increases ejection fraction in patients when used as a destination therapy. Yet, the ideal treatment for heart failure is a heart transplant.[12] When a patient's disease is so severe that medications, lifestyle changes, and implantable devices are not enough to improve the condition or quality of life, a heart transplant is the only option. The damaged heart is replaced with a heart when it becomes available. The issue lies in the availability of healthy hearts. It can take several months to match donor MHC Class I and II molecules. If the donor heart is tolerated by the recipient's immune system, the outlook for patients with heart transplants is positive. About 90 percent of patients live longer than a year post transplant, but the number of patients who receive a heart transplant is relatively low with close to 2,500 transplants per year. Despite the advances in treatment of heart failure, it remains incurable.[13]

Treating heart failure with lifestyle changes, various drugs and surgical procedures has shown success in the management of symptoms and slowing progression of the disease. The combination of medical devices and medications has been shown to improve left ventricular ejection fraction (LVEF) in patients with heart failure. However, it is important to realize that these medications are targeting the neurohormonal aberrations resulting from heart failure and not the underlying pathology of the condition.[14,15] The decrease in cardiomyocyte function or necrosis of these cells is the underlying cause of cardiac functional deficiency. Emerging therapies in cardiac repair and regeneration offer hope for addressing the underlying pathogenesis of heart failure.

Emerging Therapeutics:

Stem cells therapies have shown to be promising in the study of regenerative medicine. Stem cells are undifferentiated cells that are capable of self-renewal and differentiation giving rise to specialized cells of various lineages. The effects of cells derived from embryonic stem

cells, induced pluripotent stem cells (iPSC's), skeletal myoblasts derived from satellite cells (SM's), and bone marrow derived stem cells such as, hematopoietic stem cells, mesenchymal stem cells (MSC's), and endothelial progenitor cells have been assessed in heart failure animal studies showing improvement in cardiac function.[16-19] New approaches for CHF treatment have studied the effects and benefits of directly injecting cardiac stem cells and/or progenitor cells into the heart, implanting multicellular and electromechanical patches; as well as directly injecting growth factors or using cardiac patches that release growth factors onto the heart.[17,20-22] Studies have used both acute MI models and chronic MI (as a model for heart failure) to study the effects of these treatment approaches. Stem cell therapies are being used as an approach to treat cardiac disease by promoting the repair and regeneration of cardiac tissue. As cardiomyocytes have limited regenerative and repair capacity, stem cell treatments have recently shown the potential to repair ischemic cardiac tissue.

One of the issues that stem cell therapies run into, is the high death rate the cells exhibit when transplanted.[23] Many studies have shown various cell types that can be transformed into cardiomyocytes both in vivo and in vitro such as, embryonic stem cells, induced pluripotent stem cells, hematopoietic stem cells, endothelial progenitor cells, and bone marrow derived mesenchymal stem cells. The route by which the cells are delivered to the cardiac tissue plays a role in the survival rates of these cells. When cells are injected into the myocardium, coronary arteries, or intravenously; there is an activation of inflammation, poor cell retention in myocardial tissue, and lack of stem cell recruitment to the heart, respectively.[24,25] Methods involving the implantation of cell grafts onto the epicardium have shown success in cell engraftment, the integration of the cells into the cardiac tissue. It has been found that when human embryonic stem cell cardiac progenitors are placed on the epicardium via a scaffold, cell engraftment is more successful than when delivered by intramyocardial injection.[17,26] It has also been shown that epicardial placement of mesenchymal stromal cell sheets enhances mesenchymal cell survival, and compared to intramyocardial injection, improves treatment response in an acute MI model.[27] The use of stem cells, CRISPR/CAS9 genome editing, administration of exosomes, and identification of new therapeutic targets in heart failure is opening doors for novel approaches to treat heart failure.

Genome editing through the CRISPR/CAS9 system has revolutionized gene therapy. The CRISPR/CAS9 system is a genome editing technology that allows scientists to add, remove or alter sections of DNA. In the context of cardiac repair, CRISPR has not only been used for editing potential therapeutic targets via gene deletions and insertions, but also for the reprogramming of somatic fibroblasts and their differentiation into induced cardiac progenitor cells (iCPCs). One of the limitations that has been implicated in CRISPR/CAS9 delivery, by adeno associated virus, is the limited packing potential of the virus.[28] To overcome this limitation, a transgenic mouse line that expresses Cas9 in cardiomyocytes was generated. Once the cells expressed Cas9, single guide RNA was delivered by Adeno Associated Virus 9 (AAV9), to then guide the Cas9 for rapid and specific genomic modifications. This system was used to knockdown the Myh6 gene, which encodes the cardiac myosin heavy chain alpha protein inducing cardiac dilation and heart failure three weeks post single guide RNA delivery. There was no evident toxicity due to the constitutive Cas9 expression during this short period. This Cas9 expression thus eliminates one component of CRISPR that must be introduced and allows for more packaging area for the single guide RNA in the AAV9.[29] The CRISPR system has also been used to target ten progenitor genes found on mice tail tip fibroblasts used to reprogram the

cells. The fibroblasts were reprogrammed and generated iCPCs that were injected into the myocardium of infarcted mice. Heart function was measured using echocardiography which showed significant improvement of ejection fraction and fractional shortening (the reduction of the left ventricular diameter between end-diastole and end-systole), in mice injected with the iCPC's compared to the control mice injected with the tail tip fibroblasts. Immunostaining of harvested heart tissue sections also showed a reduction in infarct area in the iCPC treated mice. It was also confirmed by immunofluorescence that the induced cardiac progenitor cells, engrafted onto the infarcted heart, can differentiate into cardiomyocytes, smooth muscle cells and endothelial cells through CRISPR/CAS9 reprogramming.[30]

Another potential therapeutic target for heart failure is seen in the effects of exosomes. Exosomes are small lipid vesicles that are formed as a result of endosomes, or multi-vesicular bodies, budding into their lumen forming intralumenal vesicles as shown in fig. 1. Upon the fusion of the multi-vesicular body and the lipid bilayer, the exosomes are released into the extracellular space via exocytosis.[31] For years exosomes were thought to be part of a mechanism for cells to shed debris or excess proteins. It was discovered in 2007 that exosomes contained mRNA and miRNA and were also involved in a mechanism of exchange between cells; preserving the cargo from degradation while en route to the recipient cell to induce paracrine signaling effects.[32] Exosomes are released from most cell types and it is now known that it is via a controlled ceramide sphingomyelinase dependent pathway. When sphingomyelinase is inhibited, exosome release is reduced.[33] Aside from retaining its cell of origin's mRNA and miRNAs, exosomes also contain various cytosolic and transmembrane proteins, lipids and DNA that can be transferred between cells. Some proteins that have been found in exosomes, and are commonly used as exosomal specific markers, are transmembrane proteins known as tetraspanins that associate with integrins and receptors.[34]

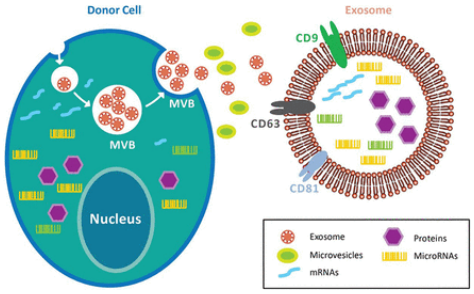

Figure 1. Biogenesis of the exosome adapted from Jung et al., 2017, illustrates the structure of exosomes and their extracellular release. An endosome buds into the cell and forms a multi-vesicular body (MVB) by budding into its own lumen. The MVB then fuses with the plasma membrane to release its contents. The exosome contains contents from its cell of origin and is identified by exosomal markers such as the tetraspanin proteins CD9, CD63 and CD81.

Exosomes have also been shown to play a role in cardiovascular disease. The release of exosomes from cardiac fibroblasts and their involvement in cross talk with cardiomyocytes via secreted miRNAs has been reported. It was demonstrated that fibroblast exosomes are rich in miRNA-21, a microRNA that has been shown to play a role in heart disease. Fibroblast derived exosome transport and uptake by cardiomyocytes was studied to determine the crosstalk between the cells mediated by exosomes. Cardiomyocytes were incubated in medium rich in fibroblast derived exosomes or depleted of exosomes through ultracentrifugation. The cardiomyocytes

treated with the exosomal-rich media exhibited hypertrophic effects while those incubated with media depleted of exosomes did not. They also performed tests to confirm the uptake of exosomes by cardiomyocytes. The fibroblast derived exosomes were labeled with a green fluorescent marker, PKH67. Cardiomyocytes were incubated with the fluorescently labeled exosomes and analyzed by confocal microscopy, which revealed uptake of the labeled exosomes by the cardiomyocytes. They confirmed that this cross talk between cardiac fibroblasts and cardiomyocytes is indeed via an intracellular uptake mechanism, rather than an extracellular binding of surface receptors, leading to cytoplasmic localization. Once the exosome contents are received by the target cardiomyocyte, the miRNA binds its target mRNA at the 3' untranslated region (UTR), silencing specific genes in that region. In this case the miR-21 bound the 3' UTR of the sarcoplasmic protein sorbin and SH3 domain-containing protein 2 (SORBS2). This SORBS2 silencing led to the pathological hypertrophy of cardiomyocytes showing the influence that exosomes and their contents can have on their target cells.[35]

The cardioprotective effects of exosomes may potentially be responsible for some of the paracrine effects from stem cells when delivered to the injured heart. Transplantation of mesenchymal stem cells into ischemic myocardium contributes to cardiomyocyte protection, decreasing apoptosis and inducing angiogenesis by activating paracrine factors.[36,37] It is currently understood that cells mediate repair through extracellular signaling regulated by the secretion of cytokines, chemokines and growth factors. A study demonstrated that MSCs injected into murine hearts also induced cardioprotective paracrine effects via the release of exosomes. The mechanism by which exosomes may confer this protection appears to be through the activation of signaling pathways. There was a reduction in infarct size mediated by the MSC derived exosomes when mesenchymal stem cells were transplanted in the infarcted hearts. It had been previously found that MSC culture medium contained more than 200 proteins.[38] It was also determined that the secretions had repair potential in myocardial ischemic tissue injury. Intravenous and intracoronary delivery of MSC conditioned medium, immediately post-acute MI, reduced infarct size. Fractionation studies showed that only the fraction of the conditioned medium that contained products greater than 1000 kDa (100-220 nm) provided these protective effects in the murine heart model of ischemia and reperfusion injury. This indicated that the component responsible for the MSCs' protective paracrine effects, is a large complex secreted by the MSCs.[39] Further studies identified the protective component of MSC secretions were exosomes derived from the stem cells. Exosomes represent ideal vehicles for delivery methods as they are lipid vesicles that can induce immediate responses in their target cells once the cargo is delivered upon up taking the exosomes. This data shows the use of stem cell secretions as another applicable therapeutic potential of stem cells and exosomes on paracrine cell function.

While heart failure is correlated with the hyperactivation of neurohormonal signaling, it has also been shown that progression of heart failure with reduced ejection fraction, is associated with the down regulation or decreased expression of certain molecules.[40] MicroRNA 1, or miR-1 is one of the most abundant cardiac miRNAs. Its association with cardiac hypertrophy has been correlated with Insulin like growth factor-1 (IGF-1) expression levels. Decreased levels of miR-1 are inversely correlated with IGF-1 and cardiac hypertrophy.[41,42] IGF-1 mRNA is a target of miR-1 as it binds IGF-1's 3'UTR, modulating protein expression levels. It is known that in the heart, the IGF-1 pathway plays a role in regulation of heart disease. IGF-1 is essential for cardiomyocyte growth, survival and proliferation.[43] It was demonstrated that myocardial infarction, by ligation of the left anterior descending coronary artery, increased miR-1 expression

levels and reduced IGF-1 protein expression at the transcription level.[44] Many studies have demonstrated the association between decreased circulation of Insulin-like growth factor-1 and the increased risk for cardiovascular disease.[45-47] This IGF-1 deficiency has also played a negative role in post-acute myocardial infarction recovery prognosis.[48] These findings show that IGF-1 is involved in cardiovascular disease progression and determining the factors involved in its regulation may lead to the finding of mechanisms that modulate cardiac dysfunction mediated by IGF-1.

Insulin like growth factor-1 Signaling:

Insulin-like growth factor-1, also known as somatomedin C, is a protein-peptide hormone with pleiotropic effects on its targets upon binding the IGF-1 receptor (IGF-1R), such as growth and differentiation. In 1978, it was found that human IGF-1 is a single chain polypeptide of 70 amino acids, crosslinked by 3 disulfide bridges.[49] IGF-1 is encoded by the IGF-1 gene found on chromosome 12 in humans and chromosome 10 in mice. The gene has six exons and two promoters positioned on exon 1 and exon 2.[50] The primary production site of IGF-1 is in the liver, from which it is then released into the blood stream as a systemic growth factor. Growth Hormone (GH) is made in the anterior pituitary gland, secreted into the bloodstream and triggers production of IGF-1 by the liver. IGF-1 is also produced in tissues such as the heart and skeletal muscle in which it acts as a local growth factor in an autocrine or paracrine manner.[51] The IGF-1 gene can give rise to multiple splice variants that differ in their terminal ends. The IGF-1 isoforms are classified based on their differing N-terminal signal peptides and C-terminal E peptides.[52] Human IGF-1 undergoes post transcriptional modifications such as alternative splicing in the E peptide sequence of the C-terminus to generate the 3 different isoforms that are locally secreted, Ea, Eb and Ec.[53] The IGF-1 isoforms are termed Class I if they contain exon I and Class II if they contain exon II in their gene sequence. Class I isoforms are predominantly found in the extrahepatic tissues, usually containing the carboxy terminal Ea domain and acting in a paracrine or autocrine manner. Class II isoforms containing the carboxyterminal Eb domain are predominantly expressed in the liver and are more responsive to GH regulation, thus acting in an endocrine manner.[54,55] IGF-1Ec is also known as mechano-growth factor, or MGF, as it is expressed after muscle damage induced by mechanical stress.[56,57] In humans the Ea peptide is composed of the exons 4 and 6, Eb contains exons 4 and 5, and exons 4, 5, and 6 are found in the Ec peptide. Each of the isoforms gives rise to the mature IGF-1 protein with variation only at the C-terminus. IGF-1Ea and MGF have both been reported to induce cardioprotective effects.[58-61] IGF-1 is transiently produced by tissues in response to GH during pre-natal and post-natal development, as well as in response to exercise and injury.[62-66]

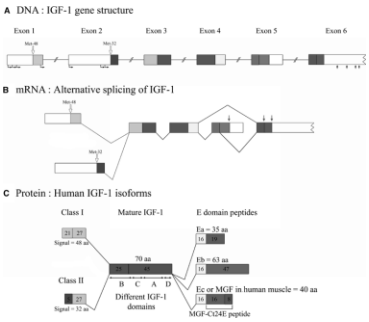

Figure 2. Adapted from Mills et al., 2007. Figure A.) The IGF-1 gene structure is represented with its six exons. Exon 1 and 2 contain various transcription initiation sites as denoted by the

black horizontal arrows. The translation initiation sites are represented by the vertical white arrows at the AUG initiation codons M-48 and M-32. B.) Alternative splicing of the IGF-1 mRNA can occur between exons 1 or 2, and exons 4, 5, and 6. C.) The human IGF-1 isoforms Ea, Eb, and Ec or MGF are represented and the result of alternative splicing for diversity. The number of amino acids (AA) is also denoted for each of the polypeptides. The domains, B, C, A, and D of the pro-IGF-1 are also shown. The B domain is associated with IGF-1R activation and interaction with IGF-1 binding proteins. The signal peptide and E peptide undergo pre and post-translational modifications converting the pre-mRNA to the 70 AA mature IGF-1 peptide. The mature IGF-1 isoform is represented in this figure by the 25 AA from exon 3 and 45 AA from exon 4.

IGF-1 in rodents has some differences from human IGF-1. Both IGF-1 genes are comprised of 6 exons, but while three E peptides have been found in humans, only two have been detected in rodents, Ea and Eb. In rodents, IGF-1Eb is 70% homologous to the human pro-IGF-1Ec peptide. In both humans and rodents, IGF-1Ea transcripts are generated by splicing exon 4 and exon 6, excluding exon 5. Human IGF-1Ec and rodent IGF-1Eb include exon 5. Exon 5 in rodents is 52 nucleotides while in humans it contains 515 nucleotides.[67] The inclusion of exon 5 produces a premature stop codon in both rodent Eb and human Ec peptides in exon 6. In humans, exon 5 is included in the Eb and Ec peptides, but not in the Ea peptide.[68,69] A homolog to human IGF-1Eb has not been observed in rodents, only in nonhuman primates.[70] It is important to understand the differences and similarities in human and rodent IGF-1 when considering the IGF-1 isoform used in animal studies. It is also important to understand the IGF-1 signaling pathways in the heart and which specific residues are modified by the upstream regulators for signal transduction.

The IGF signaling system is comprised of IGF 1 and 2, their respective cell surface receptors IGF-1R and IGF-2R, IGF-binding proteins (IGFBPs), and IGFBP proteases that work together to regulate cell growth, differentiation, and survival.[71,72] There are six IGFBPs in humans that bind IGF with high affinity and serve the functions of transporting IGF in circulation, transporting it out of the vasculature, localizing it to the target cells, and regulating its subsequent binging to IGF-R. This also increases the half-life of IGF-1.[73,74] About 85-95% of IGF-1 in the body is bound to the IGFBP3 regulating its transport and binding to receptors. IGFBPs 1, 3, 4, and 6 promote IGF-1 induced growth. IGFBPs 2 and 5 however, limit the interaction of IGF-1 with IGF-1R, thus inhibiting IGF-1's function.[75] To achieve its functions, IGF-1 must bind its receptor IGF-1R. IGF-1R is a receptor tyrosine kinase with two extracellular alpha subunits and two intracellular beta subunits that are linked by disulfide bonds. Its gene is found on chromosome 15 in humans and it is ubiquitously expressed. The ligand binds to the receptor at the alpha subunit, which triggers autophosphorylation, or cross phosphorylation, of the tyrosine residues in the beta subunit, changing its conformation to an active conformation.[76] This cross phosphorylation leads to the phosphorylation and activation of Insulin receptor substrate-1 (IRS-1, also IRS-2) from the IRS family, or Src homology 2 domain containing protein (Shc), which are intracellular adaptor proteins that interact with other SH2 domain containing proteins like Grb2 and PI3K.[77] IRS-1 and Shc thus couple IGF-1R to its cytosolic effector adaptor proteins such as PI3K or Grb-2, which bind the adaptor proteins that are docked at the receptor's phosphorylated tyrosine residues. Grb-2 contains an SH2 domain and two SH3 domains, one at the N-terminus and one at the C-terminus. The SH2 domain binds to the phosphorylated tyrosine residues on Shc while the SH3 domains bind proline rich sequences of

the SOS C-terminus; SOS (Son of Sevenless) is a guanine nucleotide exchange factor (GEF). PI3K contains two subunits, p85 and p110. The p85 subunit is the regulatory subunit of the protein and the p110 is the catalytic subunit. P85 contains two SH2 domains that can bind the phosphor-tyrosine on IRS, and one SH3 domain. Knowing which domains, specific amino acid residues or binding interactions, to target allows researchers to inhibit or constitutively activate a specific protein to determine its effect in the pathway of interest. It also allows researchers to determine what adaptive mechanisms or pathways the body activates to compensate for loss of another, when inhibiting or knocking out certain activation sites or entire proteins. IGF-1R activation can lead to the initiation of different signaling pathways such as the Phosphatidyl inositol 3-kinase (PI3K)/protein kinase B (AKT), and the small GTPase Ras/mitogen-activated protein kinase (MAPK) pathways inducing growth and survival, or proliferation, respectively.[78-79]

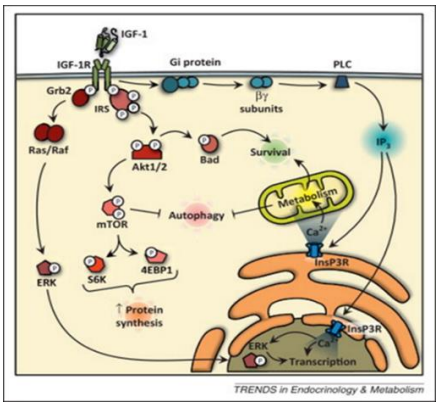

Figure 3. Adapted from Troncoso et al., 2014, illustrates the canonical PI3K/AKT and MAPK pathways as well as the non-canonical pathways that are known to be activated in cardiomyocytes.

In the survival pathway shown above, IGF-1 binds IGF-1R which triggers the receptor dimerization and autophosphorylation of the activation lip near the catalytic site in the cytosol. This leads to a conformation change that subsequently allows for the phosphorylation of additional tyrosine residues that serve as docking sites for scaffold proteins containing SH2 domains. IRS 1 binds the phospho-tyrosine on the receptor via its SH2 domain, then binds the PI3K regulatory subunit and activates PI3K. PI3K phosphorylates PIP_2 converting it to PIP_3. PIP_3 then recruits and localizes the Serine/Threonine (Ser/Thr) kinase Akt, also known as protein kinase B, to the plasma membrane where it can be activated. There are 3 members in the Akt family, but Akt 1 and Akt 2 are the most abundant isoforms expressed in cardiac tissue. PDK 1, 3-phosphoinositide-dependent kinase 1, phosphorylates Akt's kinase domain Thr-308, while mTORC2, mechanistic (or mammalian) target of rapamycin complex 2, phosphorylates Ser-473 on Akt's C-terminal hydrophobic motif.[80] Akt phosphorylates Bad, a pro-apoptotic BCl-2 protein. Akt phosphorylates at motifs with the sequence RXRXXS. Bad contains two motifs with that sequence, RSRHSS in which Ser-112 can be phosphorylated and RGRSRS in which Ser-136 is phosphorylated. However, it is known that Akt-induced Bad phosphorylation preferentially phosphorylates at the Ser-136 allowing Bad to interact with a 14-3-3 protein to preserve its phosphorylation and promote survival.[81]

Bad is a BCL-2 protein. BCL-2 family members are regulators of apoptosis, some are antiapoptotic proteins while others are proapoptotic proteins. Bad is a proapoptotic protein that inhibits pro-survival proteins such as BCL-2 and BCL-$X_{L,}$ which are anti-apoptotic proteins and inhibit cytochrome C release from the mitochondria mediated by Bax and Bak. Bak is constitutively inserted in the mitochondrial membrane. Bax is shuttled to the mitochondria and inserted into the membrane to creates pores allowing for the release of cytochrome C. After cytochrome C has been released it binds Apaf-1 and caspase 9 to form the apoptosome. This leads to the activation of effector caspase 3 and apoptosis. Upon phosphorylation, Bad is rendered "inactive" and can no longer inhibit BCL-2 or BCL-$X_{L.}$ The transport of Bax and its localization to the mitochondria is inhibited, as well as Bak activation. This inhibition does not provide a release mechanism for cytochrome C and the cell evades apoptosis. It has also been demonstrated that Akt can prevent cell death by phosphorylating Ser-196 on caspase 9, which prevents cytochrome C from activating it. Akt regulates these downstream signaling events leading to cell survival, but also regulates cell growth and proliferation signals.[82]

Akt can also activate mTOR, a Ser/Thr kinase that belongs to the phosphatidylinositol kinase-related (PIKK) family involved in suppression of autophagy and stimulating protein synthesis.[83] There are two complexes formed by mTOR in cells, mTORC1 and mTORC2. The mTORC1 is sensitive to rapamycin inhibition while mTORC2 is not. The mTORC2 complex is involved in cytoskeletal organization and cell survival and is upstream of Akt. The mTORC1 complex is downstream of Akt and modulates cell growth and increased protein synthesis. There are two major substrates that mTORC1 regulates, eIF4E binding protein (4EBP1) and the p70 ribosomal protein S6 kinase (S6K). They are normally bound to eIF3, the eukaryotic initiation factor 3, which maintains their inactive state. Upon mTORC1 activation, it binds eIF3 and phosphorylates 4EBP1 and S6K releasing them from eIF3. The phosphorylation of 4EBP1 however is complex and occurs in an ordered manner of 8 phosphorylation sites found in the human and rodent sequence. The sites are identified as Thr-37, Thr-46, Ser-65, Thr-70, Ser-83, Ser-101, and Ser-112 (in rodents each number is lowered by one). Phosphorylation of Thr-37 and Thr-46 appears to be the priming event for subsequent phosphorylation of Ser-65 and Thr-70 allowing for the release of 4EBP1 from eIF4E.[84,85] The activation of 4EBP1 allows for the release of eIF4E, the eukaryotic initiation factor, that is required for cell cycle progression, initiating cap-dependent translation and inducing cell growth.[83] Phosphorylation of S6K by mTORC1 occurs on the Thr-389 of the hydrophobic motif, but is essential for S6K activity. The activation loop in the kinase domain is phosphorylated by PDK1 at Thr-229 and is the best understood phosphorylation site on S6K. The activation of S6K leads to mRNA translation of components needed for increasing protein synthesis.[86] IGF-1 signaling through Akt/mTORC1 and S6K has also been shown to inhibit autophagy by increasing mitochondrial Ca^{2+} uptake, rescuing ATP levels, increasing S6K phosphorylation, and rescuing mitochondrial respiration in cells under nutrient starvation conditions. This highly conserved catabolic process, initiated as a protective mechanism for the cell during periods of stress or nutrient deficiency, is shown to be protective in the heart during ischemia induced cell starvation. However, it can be maladaptive when it results from severe afterload stress induced heart failure.[87,88] The canonical pathways of IGF-1 and IGF-1R, PI3K/Akt and MAPK, have also displayed cross talk between Akt and ERK, compensating for each other's activation.[89]

The MAPK signaling pathway shown in fig. 3, is initiated by the IGF-1 ligand binding IGF-1R. The adaptor protein Shc can dock at the phospho-tyrosine of the receptor's cytoplasmic

tail and recruit Grb2. Grb2 docks at the tyrosine residues on the Shc protein via its SH2 domain. Grb2 also exists as a complex with SOS. It binds via its SH3 domain to SOS's proline rich C-terminus sequence. SOS is a GEF that can then catalyze Ras-GTP to Ras-GDP. SOS binds to the Ras-GDP complex and triggers the dissociation of the GDP allowing spontaneous binding of GTP to Ras. Upon the activation of Ras by GTP binding, Raf is recruited to the plasma membrane and can bind Ras-GTP for activation. There are 3 Raf proteins in mammals, A-Raf, B-Raf, and C-Raf (also known as Raf-1). IGF-1 signaling activates Raf-1. Phosphorylation of five sites within the kinase domain are required for Raf-1 activation, Tyr-341, S-338, S-494, Thr-491, and S-621. Raf-1 however can also be inactivated through phosphorylation at sites that are found outside of the kinase domain. PKA, the cyclic AMP (cAMP) dependent kinase A, can inactivate Raf when cAMP levels are elevated in the cell by phosphorylating it and stimulating the 14-3-3 protein to bind and block Raf-Ras interactions at the plasma membrane.[90,91] Activated Raf phosphorylates and activates MEK at Ser-218 and Ser-222,[92] which then activates MAPK, or ERK. The dimeric form of ERK, ERK1/2 is phosphorylated by MEK at Thr-202/185 and Tyr-204/187. Both the Thr and Tyr phosphorylation are required for full activation of ERK. ERK1/2 phosphorylates substrates that contain proline directed motifs, Pro-XXX-Ser/Thr-Pro. Activated ERK1/2, can catalyze the phosphorylation of cytoplasmic regulatory molecules and it can also translocate to the nucleus where it can activate various transcription factors.[93] In IGF-1 signaling, it has been demonstrated that the MAPK signal cascade leads to the phosphorylation and activation of ERK1/2 which then activates genes involved in pro-survival pathways or progression through the cell cycle. Another IGF-1 regulated MAPK pathway is mediated by 14-3-3 proteins interacting with the IGF-1R. Through this pathway, activated Raf-1is translocated to the mitochondria where it can phosphorylate Bad preventing its pro-apoptotic effects.[94]

IGF-1R activation by IGF-1 also initiates a non-canonical pathway involved in phospholipase C, PLC, activation. IGF-1 signaling increases Ca^{2+} levels in cardiomyocytes through a GB γ subunit-PI3K-PLC dependent pathway that involves IP3. G proteins are heterotrimeric proteins that act as molecular switches in cell signaling. It has been shown that IGF-1R interacts with a G protein formed by the beta and gamma complexes, Gβγ. The Gβγ subunit of the G protein has been shown to activate PI3K-γ through its catalytic p110 subunit.[95] PI3K-γ activation then leads to the activation of PLC-γ which then catalyzes the hydrolysis of PIP_2 at the plasma membrane to generate diacylglycerol, DAG, and the second messenger inositol triphosphate, IP_3.[96] IP_3 diffuses into the cytosol and binds its receptor InsP3, located at the endoplasmic reticulum. The activation of the InsP3 receptors induces the release of Ca^{2+} and increase of cytoplasmic and nuclear Ca^{2+} levels.[97,98] Cytoplasmic Ca^{2+} levels promote uptake of Ca^{2+} by the mitochondria increasing cell respiration and mitochondrial metabolism. Nuclear Ca^{2+} level increases are involved in regulating specific target proteins, such as ERK activation, or regulating gene transcription. These Ca^{2+} mediated effects promote survival by regulating autophagy and preventing apoptosis.[78,99]

The IGF-1 signaling pathways play a role in regulation of heart disease. IGF-1 has growth promoting benefits, anti-apoptotic effects, promotes vasodilation, and increases LVEF and function.[40] It has been shown that when IGF-1 is expressed locally in cardiomyocytes, it provides protection to the heart from oxidative stress and promotes restoration of function post myocardial infarction.[51] This gain-of-function evidence indicates that IGF-1 plays an essential role in sustaining cardiomyocyte function post injury. It is known that IGF-1 serum levels are reduced in heart failure studies. It has also been demonstrated that partial deficiencies in IGF-1

are associated with reductions in heart function, angiotensin II sensitivity, and increased interstitial and perivascular fibrosis.[45] This loss-of-function evidence also shows IGF-1 is a necessary component in sustaining heart function. Although, IGF-1 has a positive effect on cardiac function in animal models, it has yet to be translated successfully to clinical trials. Even though it is known that there is a temporary and slight increase in endogenous IGF-1R and IGF-1 post MI in cardiomyocytes, the IGF-1 increase is not significant enough to induce regenerative effects.[20,100] It may be a result of IGF-1's relatively short half-life, about 15 minutes, but nevertheless, it has led researchers to investigate different ways to administer IGF-1 locally and increase endogenous IGF-1 in the heart.

Previous Work:

There have been many efforts to identify cell types and molecular pathways that promote repair and regeneration of cardiac tissue. The emergence of regenerative therapeutic approaches in the heart is exciting as the heart was previously thought to foster no regenerative capacity; but recent evidence has suggested that the heart does have an innate, but limited, capacity for cardiomyocyte regeneration.[101,102] IGF-1 has established a significant role in possible regeneration treatment of many tissues, not just the heart, such as the brain, liver, periodontal tissue, and in skeletal muscle.[103-106] Even though IGF-1's potential has yet to be fully elucidated, it has shown promise in the future of therapeutics, especially for heart disease as a cardioprotective cytokine. Although many studies have begun administering IGF-1, optimal doses and time of administration have not been identified. Yet, IGF-1 doses varying from 1 ng to 100 ng have shown cardio protective and regenerative benefits not only in cell cultures, but in animal studies as well.[20,21,107] Animal models of acute myocardial infarction and chronic myocardial infarction, used as heart failure model, are studied to understand the effects of emerging therapeutic approaches on the diseased and failing heart.

-Supporting studies:

Recent studies have demonstrated improvements in left ventricular function with an electromechanical engineered cardiac tissue. This cardiac patch is composed of a bio-absorbable vicryl (polygalactin 910) mesh embedded by human dermal fibroblast co-cultured with human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs). The patch demonstrates synchronous and spontaneous contractions within 48 hours of culture. The effects of the patch were studied on adult male Sprague-Dawley rats that underwent left coronary artery ligation or were assigned to a sham control group. Three weeks post ligation, the treatment group underwent patch implantation while a CHF control group did not receive the patch. The placement of this bioengineered patch on infarcted epicardium resulted in improved systolic and diastolic function and displayed electromechanical coupling between the patch and rat myocardium through electrophysiologic mapping studies. Left ventricular ejection fraction increased by 14%, left ventricular end diastolic pressure decreased 45% and the time constant of relaxation (Tau) decreased 29%. The rats treated with the hiPSC-CM patch also exhibited an increase in gene expression of IGF-1, vascular endothelial growth factor (VEGF), angiopoietin 1 which is involved in vascular development, gap junction alpha-1 protein CX43 which is a connexin and component of gap junctions, and βMYH7 which encodes for the myosin heavy chain beta (slow twitch isoform) predominantly expressed in the heart.[108] This cardiac patch demonstrated effective surgical implantation, electromechanical coupling, and improvements in heart function of rats with CHF. The implantation of the patch was also effective in increasing the gene

expression of IGF-1 when compared to the controls. Although the direct effects of this increase in IGF-1 (22.9-fold change in gene expression compared to control) were not addressed, this study demonstrates a possible mechanism of increasing endogenous IGF-1 through the implantation of stem cell derived cardiomyocytes and fibroblasts via a bio-absorbable human engineered cardiac patch.

In attempts to understand the stimulatory effects of IGF-1 in vivo, 3D cardiac tissue was engineered from human embryonic stem cell (hESC) derived cardiomyocytes and rat tail collagen type I gel. These engineered cardiac tissue constructs were treated daily with 100 nM of IGF-1 and/or 100 ng/mL of NRG-1, neuregulin-1β, an essential growth factor necessary for complete cardiac conduction development and structural development. The engineered cardiac tissue was also electrically stimulated, and the construct was contractile within 48 hours of formation. IGF-1 increased the hESC derived cardiomyocyte proliferation 3 times when compared to the untreated controls in the engineered tissues. However, when cells were treated both with IGF-1 and NRG-1 they exhibited lower proliferative rates than that of the control. There was an increase in yellowing of media from the engineered cardiac tissue cells treated with NRG1 or NRG-1+IGF-1. Because NRG-1 has previously been implicated in cellular respiration changes, it was investigated whether NRG-1 had any effects on mitochondrial content and metabolic rates. It was found that the oxygen consumption rate and extracellular acidification rate ratio was increased in cells treated with NRG-1, slightly lower for NRG-1+IGF-1 and the ratio decreased in cells treated with IGF-1. The ratio is indicative of relative levels of mitochondrial respiration and glycolysis. An increased ratio reflects increases in mitochondrial respiration, while a decreased ratio reflects increases in glycolysis. It is known that proliferating cells have increased rates of glycolysis and this may explain why cells treated with IGF-1+NRG-1 exhibited decreased proliferative rates.[107] This study demonstrates the proliferative effects IGF-1 confers in cells.

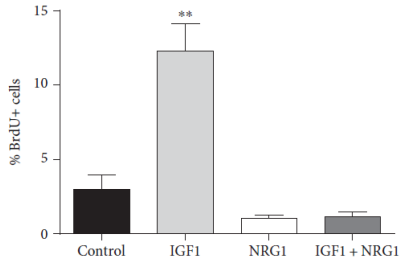

Figure 4. Adapted from Rupert et al., 2017, shows the percent of bromodeoxyuridine, BrdU, positive cells. The percent was calculated by counting the BrdU, brown stained nuclei of the total cardiac nuclei per group. The IGF-1 treated engineered cardiac tissue cells expressed a 3-fold increase in proliferation rates over the control and when combined with NRG-1. NRG-1 negated proliferative increases and its effects was dominant when combined with IGF-1 treatment in cells. IGF-1 only treated cells exhibited the most significant proliferation, demonstrating its potential in inducing cell proliferation in a cardiac tissue model. n = 3 biological replicates with ≥100 cardiomyocytes counted per group. $**p < 0.01$.

The delivery of growth factors as a potential therapeutic has indeed flourished more recently; not just of one, but multiple factors delivered at once. The role that the sustained delivery of growth factors plays was investigated in a study in the context of ischemia. The sustained delivery of IGF-1 and VEGF to ischemic rodent hindlimbs was investigated to

determine the effects of the growth factors in tissue loss and decreased or complete loss of function. Both growth factors, IGF-1 and VEGF, were mixed into biodegradable alginate hydrogels either in combination or alone for sustained delivery on the ischemic muscle. After inducing ischemic injury to the rat hind limbs, immediate treatment with injectable alginates containing 3 μg of VEGF and/or IGF-1 was administered. VEGF is known to promote angiogenesis and IGF-1 has been found to promote cell proliferation and survival. This study demonstrates the protective and restorative effects of IGF-1, however, instead of in cardiomyocytes, IGF-1 function was demonstrated in skeletal muscle cells. The sustained delivery of IGF-1 enhanced muscle fiber regeneration and cell protection from apoptosis when compared to the non-treated control, and VEGF only group. The IGF-1+VEGF treated group also displayed protection from apoptosis. There was a significant vessel density increase in the muscle with VEGF delivery from the gel when compared to the IGF-1 only treatment and blank control. The sustained delivery of both growth factors also resulted in an increase in vessel density when compared to the IGF-1 only, blank alginate control, and to bolus delivery of IGF-1+VEGF. This new blood vessel growth also led to hypoxic and necrotic tissue protection. Bolus delivery, delivery of substance all at once, did not confer any neoangiogenic or perfusion benefits and had minimal regenerative effects. Even though the optimal IGF-1 delivery dose or expression level may not be identified, it has been shown that sustained delivery of growth factors is more effective than the bolus administration.[106] Thus, IGF-1's protective effects are conferred more effectively when delivered over time than when administered all at once.

In the ischemic heart, studies have also demonstrated the cardioprotective effects conferred by delivering or overexpressing IGF-1. A cardiac patch has been developed that is composed of collagen-based scaffolds with alginate microparticles that release both hepatocyte growth factor (HGF) and IGF-1. HGF's effect on cell motility and IGF-1's cytoprotective and proliferative effects together presented a powerful combination for myocardial repair. Due to previous studies, in which the endogenous growth factor levels are not sufficient to induce a proliferative, pro-angiogenic or motogenic response (cell motility), after ischemic insult, it was postulated that extended release of these growth factors has the potential to promote recruitment and expansion of cardiac stem cells. The growth factors were encapsulated in alginate microparticles and added to a collagen slurry that is a component of their scaffold. The growth factors were both added at concentrations of 1μg/mg of polymer. This growth factor release mechanism was tested in vitro to determine the effects it had on rat cardiac stem cells. There was an initial burst release of IGF-1 and HGF within the first 24 hours of incubation with the cells. Over five days, a total of 178 ng of IGF-1 were released, 18% of the initial amount. After day 5, the release level dropped, and by day 15 there was little to no IGF-1 released. HGF release also decreased after day 5, but more of it was released, in comparison to IGF-1, with 37% of its initial amount. To test the motogenic response induced by HGF, a migratory assay was performed. Cells were added to hanging well cell culture inserts and supplemented with HGF scaffold release media, fresh HGF (positive control), or solely serum free media (negative control). Cells exposed to fresh HGF exhibited an increased level of migration, while serum free media only cells had minimal migratory responses. The cells exposed to the HGF scaffold release media also demonstrated an increase in migration, however it was to a lesser extent in comparison to the fresh HGF. IGF-1 activity was assessed by incubating cells with free IGF-1 only, IGF-1 scaffold release media, or IGF-1 from scaffolds without micro-alginate release particles where IGF-1 was free-loaded onto the scaffold (IGF free scaffold). Controls were represented by cells incubated without IGF-1. Cell proliferation was measured via a picogreen dsDNA assay. It showed a cell

growth increase in cells that were exposed to the IGF-1 scaffold release media and free IGF-1. This was indicative of IGF-1's growth promoting activity and its sustained effect even after release from the patch as shown in fig. 5.[20] This study supports the notion that endogenous IGF-1 levels are not optimal for cytoprotective effects in cells after injury. The delivery of external IGF-1 displays an increase in cell proliferation and mediates other processes that are needed in cardiac repair.

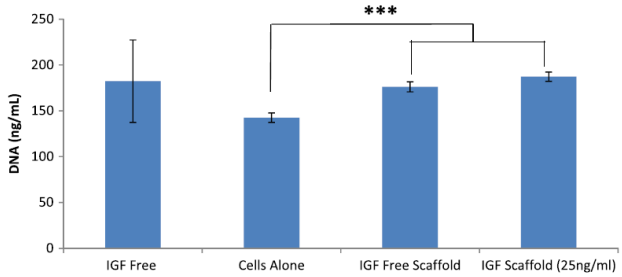

Figure 5. Adapted from O'Neill et al., 2017, shows dsDNA levels of free IGF-1 and IGF-1 scaffold release media (25ng/ml) in IGF-1 treated rat cardiac stem cells compared to an untreated control. The IGF-1 treated cultures show an increase in DNA levels, indicating that, IGF-1 remains active post scaffold release and has effects on cellular proliferation and reduction of apoptosis. Data are represented as mean ±SD, n = 3. *** $p < 0.0001$.

Another study investigated whether intramyocardial injections of IGF-1 and HGF in pigs one-month post MI, increased activation of endogenous cardiac stem cells. It had been previously shown that intracoronary codelivery of IGF-1 and HGF activated endogenous cardiac stem/progenitor cells, reduced pathological remodeling and improved ventricular function in a model of acute MI in pigs.[109] To investigate the role of IGF-1 and HGF therapy in post MI heart failure, a pig model of chronic MI was used in which adverse remodeling and fibrosis are already taking place. A pH sensitive hydrogel was used as a carrier for sustained release of IGF-1 and HGF to test the effectiveness of growth factor sustained delivery as a treatment for chronic MI. The delivery system used was a UPy, ureido-pyrimidinone, based hydrogel scaffold that was mixed with IGF-1 and HGF. The hydrogel is in a fluid state at a basic pH and a gel in a neutral pH environment. The hydrogel can be delivered using a catheter delivery system that serves as a guide and delivers trans-endocardial injections through transfemoral catheterization. Infarcts were induced in the pigs by 75-minute intracoronary balloon occlusion of the left circumflex artery followed by reperfusion. A month later, the pigs were administered ten intramyocardial injections in the infarct border zone, with 0.2 mL containing 0.5 µg/mL of either IGF-1 or HGF in a 0.9% saline solution; IGF-1+HGF, same concentrations, in the UPy hydrogel; or UPy hydrogel only as a negative control. The growth factor only treatment with IGF-1+HGF and UPy-growth factor treatment showed a trend in reducing cardiac adverse remodeling as well as promoting angiogenesis and improving cardiac systolic and diastolic function.[110] It has again been demonstrated that IGF-1 plays an essential role in cardiac repair mechanisms post MI, specifically in cardio-protection during heart failure progression post MI.

	CTRL	IGF-1/HGF	UPy-IGF-1/HGF
Cardiac adverse remodeling			
CM Hypertrophy (µm)	21.2±2.8	18.4±2.6	16.0±1.9*
Fibrosis (gray value per mm^2)	40.7±18.1	26.5±13.7	25.8±22.1

Figure 6. Adapted from Koudstaal et al., 2014. There was a decrease in cardiomyocyte hypertrophy as revealed by histological analysis when compared to the growth factor treated

groups. However, the delivery of IGF-1 and HGF via the UPy hydrogel system showed a significant decrease in hypertrophy 16.0 ± 1.9 μm, when compared to the control group with cardiomyocyte diameter measured at 21.2 ± 2.8 μm. Fibrosis visualized by histological staining of collagen was also decreased in the UPy-IGF-1/HGF treated group, 25.8 ± 22.1, when compared to the control group, 40.7 ± 18.1. Data are represented as the mean ±SD. *p <0.05 (vs. CTRL); p <0.05 (vs. IGF-1/HGF).

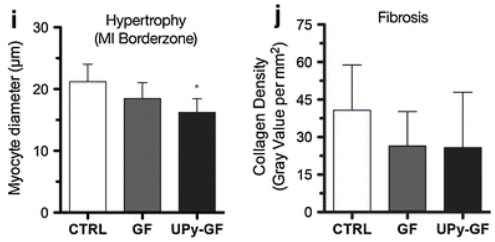

Figure 7. Adapted from Koudstaal et al., 2014. This data supplements the table presented in fig. 6 showing the decrease in cardiomyocyte diameter and collagen density in the groups treated with growth factors, IGF-1 and HGF. This demonstrated the effects of both IGF-1 and HGF on cardiomyocytes, attenuating hypertrophy and fibrosis. It shows a significant decrease in cardiomyocyte pathological hypertrophy when animals are treated with UPy-IGF-1+HGF hydrogel. All data are represented as the mean ±SD; n=3, 4, and 5 for CTRL, GF, and UPy-GF, respectively. *p <0.05 (vs. CTRL).

In another study, a hybrid hydrogel system that contained gelatin nanoparticles encapsulated with both 6-Bromoindirubin-3-oxime (BIO) and IGF-1 for sustained co-delivery was created. BIO is a reversible competitive inhibitor of ATP, on glycogen synthetase kinase-3, that maintains self-renewal of human and mouse embryonic stem cells and induces dedifferentiation and proliferation of cardiomyocytes and endothelial cells. Delivery of cells or growth factors either by injection or sustained release systems placed on the epicardium remains a challenge. With growth factors, the half-life, frequency and time span of delivery, and the possibility of toxicity cause issues when developing these delivery systems. In this study however, a novel pH sensitive hydrogel with encapsulated gelatin nanoparticles (NPs) containing BIO and IGF-1, was injected into the hearts of male Sprague-Dawley rats one-week post MI, at the site of injury. When preparing the gelatin NPs, 10 μL of 5 μM BIO, dissolved in 500 μL of dimethyl sulfoxide (DMSO), was added either individually or in combination with 10 μL of 10 mM IGF-1 into 10 mL of 5% gelatin solution. The solution was kept in a dark environment and 30 mL acetone were subsequently added to form the NPs. The release of the two factors is thought to occur through the breaking of chemical bonds holding the gelatin NPs and the oxide alginate hydrogel together. Six weeks after treatment, the delivery of both BIO and IGF-1 induced the proliferation of resident cardiac cells and promoted angiogenesis. There were also improvements in cardiac function. It was determined by echocardiography that the left ventricular ejection fraction in the group treated with BIO and IGF-1 was increased, in comparison to the control group six weeks post injection, demonstrating IGF-1's role in restoring LVEF.[21] IGF-1 only treatment showed a significant increase in LVEF, and its effect was greater when combined with BIO. However, IGF-1 only treatments display a protective effect that is promising when compared to controls not treated with IGF-1. IGF-1's pleiotropic effects show potential in repair of the failing heart.

Table 2 Functional evaluation of ischemic heart

	LVEF	FS	LVDD (cm)	LVSD (cm)
Sham				
Baseline	56.0±8.7	30.5±2.0	0.66±0.02	0.48±0.01
Week 6	53.5±7.8	31.3±2.3	0.67±0.02	0.50±0.02
Control				
Baseline	28.2±5.8	12.3±1.9	0.78±0.08	0.42±0.03
Week 6	18.8±3.5	10.3±2.1	0.95±0.09	0.65±0.06
IGF-1				
Baseline	29.1±5.5	12.9±1.8	0.76±0.05	0.44±0.03
Week 6	42.8±6.2*	17.7±2.3*	0.81±0.09*	0.38±0.02*
BIO				
Baseline	27.5±5.9	12.6±2.0	0.77±0.09	0.40±0.03
Week 6	32.1±4.3*	14.3±2.2*	0.75±0.04*	0.39±0.02*
BIO and IGF-1				
Baseline	28.6±4.9	11.8±1.7	0.79±0.08	0.45±0.04
Week 6	55.6±8.7*	27.4±3.8*	0.82±0.09*	0.39±0.02*

Figure 8. Adapted from Fang et al., 2015, shows cardiac function measured six weeks post treatment by echocardiography. Rats in the control group showed a significant decrease in LVEF from 28.2 ±5.8 to 18.8±3.5. Rats injected with BIO only had a slight increase from their baseline of 27.5±5.9 to 32.1±4.3. The IGF-1 only group had a greater increase from 29.1±5.5 to 42.8±6.2. Rats treated with codelivery of BIO and IGF-1 had the most significant increase in LVEF, from 28.6±4.9 to 55.6±8.7. Data are represented as the mean ±SEM. *$p<0.05$.

While most studies involve the delivery of stem cells or cytokines to the heart, there are limited studies that look at the effects of the delivery of both cells and growth factors such as IGF-1. A study was done to determine the effects of intramyocardial injection of mesenchymal stem cells (MSCs) overexpressing IGF-1. Mesenchymal stem cells were transduced with human IGF-1 by an adeno-virus vector encoding the IGF-1 gene. Controls were MSCs without any transduction (normal) and MSCs with an empty vector (null). Female rats underwent coronary artery ligation to induce acute MI. Immediately after, the treated MSCs, null, or normal were injected in and around the infarct zone. One-week post MI, western blot on the rat heart tissue showed an increase in myocardial IGF-1. The overexpression of IGF-1 revealed, by Real Time PCR, the induced cellular release of growth factors such as HGF, VEGF, and SDF-1α (a potent chemoattractant for stem cells). The $^{IGF\text{-}1}$MSCs secreted IGF-1 for about twelve days and activated PI3K/Akt signaling. Western blot showed an increase in expression levels of PI3K, phosphorylated Akt, BCL-X_L, and SDF-1α in the $^{IGF\text{-}1}$MSCs, as compared to the null and normal groups. Even though the ^{null}MSCs and ^{normal}MSCs did have some IGF-1 expression post MI, the levels were negligible as seen in fig. 9.[111]

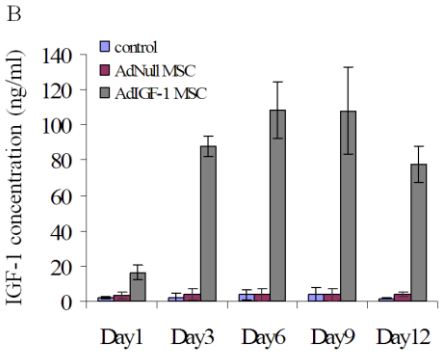

Figure 9. Adapted from Haider et al., 2008. Secreted IGF-1 was measured by ELISA on conditioned medium from the $^{IGF\text{-}1}$MSCs, ^{null}MSCs, and ^{normal}MSCs collected on days 0-14 at two-day intervals. Analysis showed that the peak levels of secretion were between day 6 and 9 at about 110 ng/ml. Even though there is a slight increase in IGF-1 concentration in the null and

control groups, it is not a significant increase as that shown by the $^{IGF\text{-}1}$MSCs. Expression of IGF-1 in the conditioned medium from the $^{IGF\text{-}1}$MSCs was significantly higher as compared with the control cells on all time points from day 1 to day 12 of observation. $p<0.05$, significance not denoted by author.

The study demonstrated that the MSCs overexpressing IGF-1 had increased survival under anoxic conditions when compared to the ^{null}MSCs. Angiogenesis was also observed after treatment with the $^{IGF\text{-}1}$MSCs, and this local overexpression of IGF-1 also showed preservation of the LV wall thickness and function. It was also found that IGF-1 promoted paracrine activation of SDF-1α, which transduces chemotaxis, survival, proliferation, cell adhesion, and transcription signals upon binding its receptor, CXCR4.[111,112] The enhanced expression of other cytokines was significantly lower in the ^{null}MSCs, compared to the $^{IGF\text{-}1}$MSCs, suggesting a dependence on IGF-1. Not only does IGF-1 induce cytoprotective effects via its own downstream signaling, but it can also have stimulatory effects on cells to activate other signaling pathways associated with different signaling proteins.

-Limitations:

While IGF-1 stimulation has demonstrated protective and regenerative effects promoting survival of cardiomyocytes and skeletal muscle cells among others, there are some limitations and concerns implicated with IGF-1 therapy. There are still questions regarding the most effective dose, half-life, and an effective delivery system. Also, a recent study found an association with IGF-1 signaling and the formation of epicardial adipose tissue in the murine heart. It was shown that epicardial cells originating from a cell lineage expressing the transcription factor Wt1, encoded by the Wilms tumor 1 gene, differentiate into epicardial adipose tissue after myocardial infarction, when stimulated by IGF-1. Mice underwent ligation of the left anterior descending artery. Mesenchymal stem cells were obtained from rodent femurs while the Wt1 positive epicardial derived cells were obtained from explanted hearts two days post MI. Cells cultured with IGF-1 exhibited an augmentation in adipogenesis, when hypoxic conditions were present. When IGF-1R was inhibited or Wt1 was inactivated, the cells did not differentiate into adipocytes post MI. IGF-1 also did not stimulate adipogenesis in normal hearts without the induced MI stress. The volume of epicardial adipose tissue is strongly associated with coronary artery disease. [113,114] Thus, it may be an issue if IGF-1R activation post MI leads to an increase in epicardial adipose tissue. IGF-1 signaling in the heart, although cardioprotective and promising in myocyte functional recovery, may encourage the formation of epicardial adipose tissue.[113] This interaction between myocardial infarct induced stress and IGF-1 signal activation presents a potential negative consequence of IGF-1 signaling. The issue may lie in the dose response, frequency or time of delivery of IGF-1, or specifically in cell lineages expressing genes such as Wt1. Still, this potential negative outcome will need to be taken into consideration when exploring therapeutics that modulate the IGF-1 pathway; as well as possible methods that mitigate any associated negative effects.

Conclusion:

The progression of heart failure and the role that cardiovascular signaling pathways play is complex and not fully elucidated. The IGF-1/IGF-1R signaling pathway in cardiomyocytes is essential in the role of CHF and its progression. Most IGF-1 studies, modeling ischemic heart injury and its progression to heart failure, demonstrated that locally expressed IGF-1 can

promote angiogenesis, increase left ventricular ejection fraction and function, mediate cardiomyocyte hypertrophy and fibrosis, protect from oxidative stress, promote survival, induce proliferation and enhance stem cell survival in the heart.[40] Its deficiency has been implicated in dysregulation of the IGF-1/GH axis, oxidative damage, reduction of left ventricular function, and alteration of gene expression that is important for functional cardiac proteins.[45] Even though there have been promising results with the use of IGF-1 on cardiac function, both in vitro and in vivo in animal models, it has not yet successfully been translated to humans. Despite all the recent advances in medicine, heart disease is still incurable. IGF-1 has established a role as an inducer of cardiomyocyte growth, proliferation, survival, and regeneration. It has shown promise in the future of cardiac disease therapeutics and the reversing or repairing of functional and structural deficiencies of the failing heart. The ideal therapy for cardiac disease remains elusive. However, there are many key components, such as growth factors, cell types, and other cytokines, contributing to repair and regeneration in the heart, that have been found and are currently and continuously being investigated. The optimization of the delivery, doses/amounts, in-vivo engraftment, binding, and persistence of these key components will be pivotal in the efficiency of treatments directed towards the underlying pathogenesis of heart failure.

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