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(54) Title: MEDICAMENTS FOR THE TREATMENT OF CORONARY SYNDROMES

(57) Abstract: Methods and compositions are provided for the treatment of coronary syndromes such as acute or decompensated coronary syndromes. For example, use of the methods and medicaments result in the production of myocardial-derived growth factors following heart attacks or other events associated with acute or decompensated coronary syndromes.

MEDICAMENTS FOR THE TREATMENT OF CORONARY SYNDROMES

FIELD OF INVENTION

[0001] The present invention relates to medicaments and associated methods for the treatment of coronary syndromes, more specifically to medicaments and methods resulting in the production of myocardial-derived growth factors following heart attacks or other events associated with acute or decompensated coronary syndromes.

BACKGROUND

[0002] According to the World Health Organization (WHO), the prevalence of cardiovascular diseases (CVD) now contributes to approximately one in three deaths globally (The World Health Report 2002). CVD is already the leading cause of death in Europe and by 2010 is expected to be the leading cause of death in developing countries (European Cardiovascular Disease Statistics, 2000 Edition). In the United States, coronary heart disease (CHD) considered alone is already the single leading cause of death, with more than one million cases of new and recurrent coronary attack each year (American Heart Association (AHA), 2000 Statistics).

[0003] A heart attack (myocardial infarction, MI) generally occurs when the blood supply to the heart muscle (myocardium) is severely reduced or stopped because one or more of the coronary arteries supplying blood to the myocardium becomes constricted or blocked. Typically this is caused by the buildup of plaque (depositions of fat-like substances) within the interior layers of the coronary arteries, a process referred to as coronary atherosclerosis or coronary artery disease. When plaque eventually tears, bursts or ruptures it tends to create a focus for blood clot formation, which can block the coronary artery and cause a heart attack, which is sometimes referred to as a coronary thrombosis or coronary occlusion. However, even coronary blood vessels that do not appear to be atherosclerotic can sometimes temporarily contract or go into spasm for reasons that are poorly understood. When this occurs the artery narrows and may reduce or block blood flow to the “downstream” portion of the myocardium (i.e. the portion served by the affected artery), potentially causing a heart attack.

[0004] Clinically, the cluster of symptoms and/or conditions compatible with acute myocardial ischemia (acutely insufficient blood flow to the myocardium) is typically referred to as acute coronary syndrome (ACS). Patients experiencing ACS may or may not have coincident ST segment elevation (referring to a rise in a particular portion, the ST segment, of an electrocardiogram (ECG or EKG)). Most patients who have ST segment elevation also develop a Q-wave acute myocardial infarction or heart attack. Patients who exhibit acute ischemic discomfort or pain without an associated ST segment elevation are generally experiencing either unstable angina or a non-ST segment elevation myocardial infarction that usually leads to a non-Q-wave myocardial infarction.

[0005] Under any of the foregoing or related situations, when the blood supply to a portion of the myocardium is cut off for more than a few minutes, the affected heart muscle cells (cardiomyocytes) begin to die or suffer injuries that can also kill or disable the individual, depending on how much of the heart muscle ultimately dies or becomes damaged. Timing is critical in saving such a patient's life for, as has been suggested in the case of a heart attack, "time is muscle." However, even when blood flow has been restored to the affected heart muscle by reperfusion, typically using balloon angioplasty (PTCA) and/or the administration of thrombolytic agents to dissolve clots, the unavoided death or ongoing injury of cardiomyocytes often results in a situation in which the myocardium becomes increasingly compromised in its ability to function.

[0006] Continued deterioration of heart function generally leads to heart failure, or congestive heart failure (CHF), a condition in which the heart can no longer pump enough blood to the body's other organs to satisfy demands. As blood flow out of the heart declines (particularly when left ventricular ejection fractions drop below 40%), blood returning to the heart through the veins tends to back up, causing congestion in the tissues and typically swelling (edema). Fluid can also collect in the lungs which interferes with breathing, especially when an individual is lying down. Heart failure also frequently interferes with the ability of the kidneys to efficiently dispose of sodium, causing additional water retention and edema. In the U.S. alone, it was recently estimated that heart failure affects approximately 5 million individuals and that 400,000 to 700,000 new cases are diagnosed each year (Heart Failure Society of America, 2000 Statistics). The resulting impact on the healthcare system

and on patients' quality of life is even more substantial when one considers the fact that heart failure and its complications in the elderly are generally the leading cause of hospitalization.

[0007] Current therapies for acute coronary syndromes focus on reperfusion of the affected myocardium, typically using PTCA and/or thrombolytics. Although restoring blood flow is absolutely essential, it is typically not accomplished quickly enough to avoid death or injury to at least some portion of the cardiomyocytes. Death of cardiomyocytes is generally an ongoing process involving necrotic cell death as well as apoptotic or programmed cell death. The resulting death and injury of cells in the myocardium can ultimately lead to heart failure and its complications as noted above. There remains a need for effectively treating acute coronary syndromes.

[0008] Insulin-like growth factors have been shown to be multifunctional proteins implicated in a number of biological mechanisms and relevant to a number of disease states. In addition to playing a role in angiogenesis, IGF-1 has been shown to have anti-apoptotic properties, positive inotropy activity, and myocyte proliferation properties. Circulating IGF-1 is largely bound to one of six specific IGF-binding proteins (IGFBPs), which modulate the bioactivity by limiting or potentiating the access of IGF-1 to specific tissues. For example, IGFBP-3 binds more than 95% of the IGF-1 in serum, extending its half-life and forming a circulating reservoir. Once released from this complex, IGF-1 can leave the circulation and enter target tissues with the aid of other IGFBPs.

[0009] Despite early suggestions of the multiple roles of IGF-1 in human physiology, efforts to develop IGF-1 as a therapeutic have met with little success. One explanation for these difficulties is that because IGF-1 is a relatively ubiquitous protein in the human body, found not only in association with particular cells in particular tissues but also circulating in the blood (primarily complexed with IGFBPs), it has been difficult to effectively target a particular tissue to produce a therapeutically effective amount (i.e., an appropriate level (sufficient but not excessive) and of sufficient duration) while at the same time limit the effects of such a therapeutic to amelioration of the disease being treated and not result in unintended and/or undesirable side effects. Ensuring that the IGF-1 remains at the site requiring treatment in sufficient concentration and for sufficient time to have effect has been difficult, particularly, for example, where the IGF-1 therapeutic is delivered systemically. In addition, a clinically-relevant disease setting or model is important (but often not used) in

efforts to develop IGF-1 as a therapeutic. These issues with use of IGF-1 as a therapeutic become particularly relevant in the cardiovascular disease setting, where treatment of a particular organ, the heart, and/or particular cells within the organ, such as cardiomyocytes, is critical for eliciting a physiologically-relevant response and therapeutically-relevant effects.

[0010] All patents, patent applications, and publications cited herein are hereby incorporated by reference in their entirety.

SUMMARY OF INVENTION

[0011] The present invention relates to medicaments and techniques for the treatment of coronary syndromes such as those associated with a heart attack (myocardial infarction). The medicaments and techniques of the present invention are preferably employed relatively soon after diagnosis of a probable or possible myocardial infarction. In that aspect, the medicaments and techniques can be applied in association with or following the application of acute procedures such as acute reperfusion and/or administration of thrombolytics. While the foregoing acute interventions can be essential to restoring flow, and therefore saving the life of a heart attack victim, it is believed that they are often not sufficient to actually protect against coincident or ongoing damage to cells within the myocardium – which damage can ultimately progress to heart failure. As such, while most therapeutic interventions in this disease progression have focused on either the acute situation (e.g. restoring flow after infarct) or on later treating the chronic symptoms and conditions associated with heart failure (e.g. diuretics, digoxin, ACE inhibitors and/or beta blockers), few if any effective therapies are focused on the intermediate stage events following acute reperfusion, a stage that is the principal focus of the present approach and which is referred to herein as decompensated coronary syndrome (DCS). In addition, few if any effective medicaments or techniques are focused directly on protecting or improving function of cardiomyocytes per se, which is one important aspect of the present application.

[0012] Applicants have developed means of treating this resulting "intermediate" state, DCS, by triggering the production of certain growth factors (especially insulin-like growth factor 1 (IGF-1)) within the heart itself at the time of DCS. By treating DCS (and/or slowing its progression), the medicaments and techniques of the present application can be used to

prevent the deadly progression of these disease states, thereby potentially improving the quality of life for these patients and preventing or delaying the onset of heart failure.

[0013] Accordingly, in one aspect of the present invention, the medicaments and techniques are used to treat a post-acute heart condition referred to as decompensated coronary syndrome. A preferred embodiment of the present invention is based on increasing the production of certain growth factors, especially insulin-like growth factor 1 (IGF-1) alone or in combination with other factors, within the heart following the onset of an acute coronary syndrome. Without wishing to be bound by theory, it is believed (and experimental results appear to confirm) that the increased production of growth factors such as IGF-1, within the heart at the time of DCS, promotes the protection and/or improved function of heart cells such as cardiomyocytes. It is further believed that the positive effects observed, and the lack of apparently deleterious effects, may in part be due to certain preferred technical features of this approach. For example, in preferred embodiments of the present invention, production is triggered in situ within the heart. Preferably there is significantly less production of growth factors triggered at sites outside of the heart. In the case of initiating the production of IGF-1, the resulting growth factor molecules (termed myocardial-derived IGF-1 or mdIGF-1) are preferably secreted and available locally within the myocardium. Also, in preferred embodiments, the growth factors are produced and secreted locally within the heart over a relatively sustained period of time. Preferably the myocardial-derived growth factors are produced over a period of at least several days to several weeks. Also, without wishing to be bound by theory, it is believed that treating the heart using the medicaments and techniques of the present invention when the heart is at the same time in a state of compromised function as associated with DCS may be important to the development of the effects observed.

[0014] As described and illustrated in more detail below, initiating or triggering the production of such growth factors in situ within the heart can be accomplished using one or more of several different approaches. In preferred embodiments of the present invention, the growth factor production that is initiated is both relatively local to the heart (preferably the majority of the protein production initiated is located within the heart, more preferably at least 70%, even more preferably at least 90%, of the protein production initiated) and relatively sustained (preferably the protein production occurs over a period of at least several (2-3) days, even more preferably at least several weeks). A number of different approaches

can be used to achieve such endpoints. For example, in preferred embodiments, a gene (i.e. a nucleic acid capable of encoding a protein, preferably operably linked to a promoter that is expressed in the heart) is introduced into the heart, whereby expression of the encoded protein results in the production of growth factor molecules such as IGF-1 within the heart. As will be appreciated by those of skill in the art, the gene may either encode the growth factor directly or it may encode a factor that triggers production of the growth factor. An example of the former would be a gene encoding IGF-1. An example of the latter would be a gene encoding a transcription factor (such as a zinc-finger DNA binding protein) that induces expression of the endogenous IGF-1 gene which is already located within the target cell. The gene is preferably encompassed within a gene delivery vector that can be used to facilitate packaging, delivery and expression of the gene within the heart. Preferred vectors include adenoviral and other viral or other sub-cellular vectors as described below and in the art. Cells, including stem cells (such as mesenchymal stem cells) or cells derived from stem cells, can also be used as a carrier or vector to deliver the gene to the heart, in which case such cells can also play complementary and/or additional roles in improving cardiac function, as further described below and in the art.

[0015] The vector can be introduced into the heart using any number of different approaches, as also described below and in the art. For example, the vector can be introduced to the heart using the associated circulation, e.g. the coronary arteries supplying the myocardium or the coronary venous circulation receiving blood from the myocardium. By way of illustration, the vector may be released from a catheter and infused into one or more of the coronary arteries (or a saphenous vein graft and/or an internal mammary artery graft) by which it can be delivered to the myocardium by anterograde diffusion; or it may be delivered to the myocardium by retrograde perfusion by placing the catheter into a coronary sinus or other site receiving blood from the myocardium (e.g. by applying positive pressure to drive retrograde flow). The vector can also be introduced into the myocardium by direct injection, such as from a needle (e.g. during surgery or percutaneously using a catheter that can be introduced into a ventricle and guide a needle or other injection device that can then inject the vector directly into one or more preferably multiple sites in the myocardium). The vector can also be introduced into the heart on a stent which may also serve to maintain patency of a previously occluded vessel (e.g. by employing a stent comprising the vector on its surface or within reservoirs from which it can be released over time). In the case of stent-

mediated delivery, the vector may for example be released from the luminal face of the stent, in which case it can be introduced to the myocardium by anterograde perfusion over time. Vector can also be released from the exterior surface of the stent, in which case it may be used to transfect cells in or adjacent to the coronary arteries. Where it is apparent from diagnostic imaging or other means that one or more particular areas of the myocardium were most significantly impacted by the events associated with the acute coronary syndrome or myocardial infarction (e.g. a portion of the myocardium normally served by a blocked artery), the vector may be delivered in such a way as to direct more of it to the region at greatest risk. This may be done, for example, by infusing relatively more vector into a particularly affected coronary artery, or by injecting the vector into particular regions of the myocardium.

[0016] In one preferred aspect, the present invention provides a method for ameliorating cardiac decompensation or improving cardiac function following acute coronary syndrome in a patient, comprising delivering to the heart of the patient a nucleic acid for inducing the synthesis of insulin-like growth factor (IGF) in the heart of the patient (i.e. a nucleic acid capable of inducing the synthesis of IGF in the heart of a patient). Preferably the nucleic acid contains a sequence encoding insulin-like growth factor-1 (IGF-1) or a factor which promotes transcription of an endogenous IGF-1 gene. Preferably the nucleic acid is contained in a vector that is delivered to the myocardium. Preferably the nucleic acid is operably linked to a promoter that is expressed in the heart. The promoter may be, for example, a cardiac-specific promoter as described below or in the art, or it may be a promoter that is expressed in multiple tissues. The promoter may be a constitutive promoter, such as a cytomegalovirus promoter, or it may be an inducible promoter as described below or in the art.

[0017] In preferred embodiments, the nucleic acid employed in the present methods encodes human IGF-1. In further preferred embodiments, the transgene is a modified polynucleotide encoding human IGF-1, the modified polynucleotide comprising alternate codons in place of native codons, as described herein.

[0018] In one aspect, the IGF-1 transgene employed in the present methods is comprised within a vector, preferably a viral vector, more preferably a replication-defective viral vector, such as an adenovirus or adeno-associated virus (AAV). Preferably, the IGF-1 transgene is operably linked to one or more regulatory sequences such as, for example, promoter sequences, enhancers, modifiers, polyadenylation sequences and/or the like. Even more

preferably, the transgene encoding IGF-1 is associated with additional polynucleotide sequences that enhance the efficiency of its transcription and/or translation. By way of illustration, a preferred promoter for use herein is an enhanced CMV promoter ("eCMV" promoter).

[0019] Also provided herein are kits for ameliorating cardiac decompensation or improving cardiac function following acute coronary syndrome in a patient, comprising a device for delivering an agent to the heart of the patient and a nucleic acid for inducing the synthesis of IGF in the heart of the patient. Such kits may comprise, for example, a catheter. Exemplary catheters include infusion catheters by which fluid may be released into a vessel carrying blood to the heart, such as a coronary artery or from the heart such a coronary sinus, or an injection catheter by which fluid or other material may be injected directly into a tissue, such as from a needle or other injection device passed through or incorporated into said catheter. Such kits may also comprise stents by which the nucleic acid may be delivered to the heart, in which case the nucleic acid may be incorporated within or on the surface of the stent, from which it may be released over a period of time. Various compositions and combinations as may be employed in methods of the present invention (as summarized above and elsewhere herein) may be incorporated into such kits of the present invention.

[0020] Also provided herein is a disease relevant animal model for testing the effectiveness of various methods or compositions of the present invention on amelioration of cardiac decompensation following acute coronary syndrome or myocardial infarction and for evaluating the effectiveness and safety of various techniques and compositions of the present invention. Other models known in the art will also be useful in evaluating various techniques and compositions of the present invention, as will be appreciated by those of skill in the art.

BRIEF DESCRIPTION OF DRAWINGS

[0021] Fig. 1 is a diagram of a timeline for the acute myocardial infarction and treatment protocol described in Example 1.

[0022] Fig. 2 shows graphs depicting the effect of Ad5.IGF-1 on the risk regions and infarct sizes in the acute myocardial infarction model (hatched bars, Ad5.IGF-1 treated animals; open bars, saline treated animals). Fig. 2A is a graph depicting left circumflex

(LCx) risk region as a percentage of left ventricle. Fig. 2B is a graph depicting infarct size as a percentage of LCx region at risk. Fig. 2C is a graph depicting infarct size as a percentage of left ventricle. In Fig. 2B and 2C, * indicates infarct extended at day 9 and # indicates incomplete LCx occlusion.

[0023] Fig. 3 shows graphs depicting regional and global myocardial function in Ad5.IGF-1 treated animals (filled symbols; pigs 1289, 1292, 1303) and saline treated animals (open symbols; pigs 1291, 1301, 1302). The measurements obtained at the papillary level in the center of the left circumflex region.

[0024] Fig. 4 is a graph depicting visual scores for myocardial function (1 = normal, 2 = hypokinesis, 3 = akinesis, 4 = dyskinesis) in the left circumflex region in Ad5.IGF-1 treated animals (filled symbols; pigs 1289, 1292, 1303) and saline treated animals (open symbols; pigs 1291, 1301, 1302). Day 0 refers to early reperfusion after the 60 min LCx occlusion.

[0025] Fig. 5 shows graphs depicting systolic excursion of the lateral wall during dobutamine infusion in Ad5.IGF-1 treated animals (filled symbols; pigs 1289, 1292, 1303) and saline treated animals (open symbols; pigs 1291, 1301, 1302). Fig. 5A is a graph depicting systolic excursion and Fig. 5B is a graph depicting systolic excursion expressed as a percentage of the pre-myocardial infarction value observed on Day -1.

[0026] Fig. 6A depicts cardiomyocytes cultured with serum under normoxic conditions. Fig. 6B depicts cardiomyocytes cultured under serum deprivation and hypoxic conditions. Fig. 6C depicts cardiomyocytes transduced with AdX.IGF-1 and cultured under serum deprivation and hypoxic conditions. Fig. 6D depicts genomic DNA fragmentation in the cardiomyocyte cultures from panels A, B, and C of Fig. 6.

[0027] Fig. 7 depicts genomic DNA fragmentation assay for the cardiomyocytes cultured as indicated.

[0028] Fig. 8 is a graph depicting ANF production by cardiomyocytes transduced with Ad.Null (control vector) or Ad5.IGF-1 or treated with phenylephrine.

[0029] Fig. 9 is a graph depicting proliferation of human coronary artery endothelial cells in control medium, transduced with Ad5.IGF-1, and in supplemented growth medium as measured by the MTT tetrazolium method.

DETAILED DESCRIPTION OF INVENTION

Definitions

[0030] "Acute coronary syndrome" or "ACS" is a cluster of symptoms and/or conditions well known to those of skill in the art. Generally, the term refers to a cluster of symptoms and/or conditions compatible with acute myocardial ischemia (acutely insufficient blood flow to the myocardium). Acute myocardial ischemia is frequently, but not always, associated with chest pains (angina pectoris) that may be triggered by insufficient blood supply to the myocardium. Patients experiencing ACS may or may not have coincident ST segment elevation (referring to a rise in a particular portion, the ST segment, of an electrocardiogram (ECG or EKG)). Most patients who have ST segment elevation also develop a Q-wave acute myocardial infarction or heart attack. Patients who exhibit acute ischemic discomfort or pain without an associated ST segment elevation are generally experiencing either unstable angina or a non-ST segment elevation myocardial infarction that usually leads to a non-Q-wave myocardial infarction. Typically, such acute coronary syndromes result in some degree of cardiac decompensation, even more typically progressive cardiac decompensation which can ultimately lead to heart failure.

[0031] "Cardiac decompensation" refers to a condition in which the heart, particularly the myocardium, has been negatively impacted or injured. Cardiac decompensation is generally associated with a deterioration of ventricular function leading to inadequate systemic circulation. Cardiac decompensation can arise acutely, for example as the result of an acute myocardial infarction, or it can arise over time, for example as a result of increasing scarring or cardiac cell death following multiple cumulative ischemic events. Cardiac decompensation may develop after an acute coronary syndrome even after acute therapies such as reperfusion have been employed, since cell death or injury within the myocardium can continue to progress when blood flow has been restored to ischemic tissue.

[0032] "Heart failure" is clinically defined as a condition in which the heart does not provide adequate blood flow to the body to meet metabolic demands. Thus, heart failure is a form of cardiac decompensation. Symptoms include breathlessness, fatigue, weakness, leg swelling, and exercise intolerance. Heart failure is typically associated with declines in left ventricular ejection fractions, which may drop to below 40%. In addition, as blood flow out of the heart declines, blood returning to the heart through the veins tends to back up, causing congestion in the tissues and typically swelling (edema). Fluid can also collect in the lungs which interferes with breathing, especially when an individual is lying down. On physical examination, patients with heart failure tend to have elevations in heart and respiratory rates, rales (an indication of fluid in the lungs), edema, jugular venous distension, and, in many cases, enlarged hearts. Patients with severe heart failure suffer a high mortality; typically 50% of the patients die within two years of developing the condition. In some cases, heart failure is associated with severe coronary artery disease ("CAD"), typically resulting in myocardial infarction and either progressive chronic heart failure or an acute low output state, as described herein and in the art. In other cases, heart failure is associated with dilated cardiomyopathy without associated severe coronary artery disease.

[0033] "Myocardial ischemia" ("MI") is a condition in which the heart muscle does not receive adequate levels of oxygen and nutrients, which is typically due to inadequate blood supply to the myocardium (e.g., as a result of coronary artery disease).

[0034] As used herein, the expression "having therapeutic effect" refers to ameliorating symptoms of a disease or disorder following administration of an IGF-1 transgene composition in accordance with the present invention. In particular, a patient who has suffered acute coronary syndrome or myocardial infarction is successfully "treated" for the condition if the patient shows observable and/or measurable reduction in or absence of one or more of the symptoms that typically arise following such acute coronary syndrome or myocardial infarction (collectively, "cardiac event") and/or improvement in short and/or long term clinical outcome after receiving a therapeutic composition in accordance with the present invention. By way of example, a patient in whom development of cardiac decompensation is delayed, reduced and/or prevented following administration of a nucleic acid inducing the synthesis of IGF-1 in accordance with the present invention is said to have been successfully treated. A symptom, disease or disorder is "ameliorated" if it is improved

or made more tolerable. Thus, indicators of successful treatment of acute coronary syndrome or myocardial infarction or other relative improvement or retention of cardiac function, in accordance herewith, include the patient showing or feeling a reduction in any one of the symptoms of acute angina or myocardial infarction and/or delay and/or prevention of one of more of the symptoms of cardiac decompensation following acute coronary syndrome or myocardial infarction, such as for example, a delay in the occurrence of or lessening of the expected extent of conditions or symptoms associated with myocardial deterioration (including, e.g., increased end diastolic volume or other measures of chamber dilation, increased size or extent of infarct areas, increased extent of scar formation, ventricular remodeling, decreased left ventricular ejection fractions). Other indicators include a delay and/or prevention of one of more of the symptoms of heart failure (including, e.g., fatigue, weakness, breathlessness, leg swelling or peripheral edema, rales or pulmonary edema, increased heart or respiratory rates, edema or jugular venous distension). In the days, weeks or months following treatment, the patient may also show greater exercise tolerance, have or retain a relatively normal heart size, have or retain a relatively normal cardiac physiology and function, and in general, require fewer hospital visits related to the sequelae of the acute coronary syndrome or myocardial infarction than a similarly situated untreated patient. The improvement in cardiovascular function may be adequate to meet the metabolic needs of the patient and the patient may not exhibit symptoms under mild exertion or at rest. Many of these signs and symptoms are readily observable by physical examination and/or measurable by routine procedures familiar to a physician. Indicators of improved cardiovascular function include relatively maintained contractile function in the treated tissues and/or relatively limited infarct zones and/or cell death. Various methods well known to those of skill in the art may be employed to measure infarct size, follow ventricular geometry, assess serial cardiac function and/or measure cardiomyocyte viability, including for example, cardiac imaging modalities such as echocardiography, magnetic resonance imaging (MRI) and positron emission tomography (PET). Cardiac function before and after IGF-1 transgene transfer can be compared using these methods. Improved heart function is associated with decreased signs and symptoms, as noted above. In addition to echocardiography, one can measure ejection fraction (LV) by nuclear (non-invasive) techniques as is known in the art. One can also measure a reduction in major adverse cardiac events (MACE), typically as a

composite endpoint including factors such as a one-year incidence of death or re-infarction for patients treated.

[0035] "Treatment" or "therapy" as used herein refers to administering agents to an individual patient that are capable of eliciting a prophylactic, ameliorative, curative or other beneficial effect on the individual.

[0036] As used herein, "polynucleotide" and "nucleic acid" are used interchangeably to refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides, or analogs thereof. This term refers to the primary structure of the molecule, and thus includes double- and single-stranded DNA, as well as double- and single-stranded RNA. It also includes altered polynucleotides such as methylated and/or capped polynucleotides.

[0037] "Gene" and "transgene" (i.e. a transferred gene or a gene to be transferred) each refer to a polynucleotide or portion of a polynucleotide comprising a nucleotide sequence that encodes a protein or peptide. For most situations, it is desirable for a gene to further comprise a promoter operably linked to the coding sequence or sequences in order to be effectively expressed. Enhancers, repressors and other regulatory sequences may also be included within the gene in order to modulate activity of the gene, as is well known in the art. (See, e.g., the references cited below). Genes or transgenes need not and in preferred embodiments do not include introns within the coding sequence.

[0038] As used herein, a "transgene encoding IGF-1" and an "IGF-1 transgene" are used interchangeably to refer to a transgene encoding pre-pro insulin-like growth factor (ppIGF-1), mature IGF, or a biologically active fragment of IGF-1. In preferred embodiments, the transgene employed in the present methods encodes ppIGF-1 which is then processed by the cell into mature IGF-1.

[0039] "Codon" refers to a three nucleotide sequence encoding an amino acid or stop signal. "Native" codon refers to the naturally occurring codon that encodes an amino acid located at a specific location within a polypeptide amino acid sequence. See, for example, the human derived nucleotide sequence encoding pre-pro IGF-1 listed in Table 1. As used herein,

"alternate" codons are codons that are different from the native codon but encode the same amino acid (in the same position) as a native codon.

[0040] "Recombinant," as applied to a polynucleotide means that the polynucleotide is the product of various combinations of cloning, restriction and/or ligation steps, and other procedures that result in a polynucleotide construct that is distinct from a polynucleotide found in nature. A recombinant virus or viral vector is a virus (or vector) comprising recombinant polynucleotides.

[0041] A "heterologous" component refers to a component that is introduced into or produced within a different entity from that in which it is naturally located. For example, a polynucleotide derived from one organism and introduced by genetic engineering techniques into a different organism is a heterologous polynucleotide which, if expressed, can encode a heterologous polypeptide. Similarly, a promoter or enhancer that is removed from its native coding sequence and operably linked to a different coding sequence is a heterologous promoter or enhancer.

[0042] A "promoter," as used herein, refers to a polynucleotide sequence that controls transcription of a coding sequence to which it is operably linked. A large number of promoters, including constitutive, inducible and repressible promoters, from a variety of different sources, are well known in the art (and identified in databases such as GenBank) and are available as or within cloned polynucleotide sequences (from, e.g., depositories such as the ATCC as well as other commercial or individual sources).

[0043] An "enhancer," as used herein, refers to a polynucleotide sequence that enhances transcription of a coding sequence to which it is operably linked. A large number of enhancers, from a variety of different sources are well known in the art (and identified in databases such as GenBank) and available as or within cloned polynucleotide sequences (from, e.g., depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoter sequences (such as the commonly-used CMV promoter) also comprise enhancer sequences. Thus, as used herein, the term "promoter" also includes any enhancers associated therewith.

[0044] A "transcription regulator" or "regulatory sequence" refers to a polynucleotide sequence that controls, enhances or otherwise effects transcription of a gene (or transgene) to which it is operably linked. Examples of such regulatory sequences include, without limitation, promoters, enhancers, polyadenylation sequences and the like.

[0045] "Operably linked" refers to a juxtaposition of two or more components, wherein the components so described are in a relationship permitting them to function in their intended manner. A promoter is operably linked to a coding sequence if the promoter controls transcription of the coding sequence. Although an operably linked promoter is generally located upstream of the coding sequence, it is not necessarily contiguous with it. An enhancer is operably linked to a coding sequence if the enhancer increases transcription of the coding sequence. Operably linked enhancers can be located upstream, within, or downstream of coding sequences. A polyadenylation sequence is operably linked to a coding sequence if it is located at the downstream end of the coding sequence such that transcription proceeds through the coding sequence into the polyadenylation sequence.

[0046] A "vector" (sometimes referred to as a delivery or gene delivery or gene transfer vector or "vehicle") refers to a macromolecule or complex of molecules comprising a polynucleotide (or transgene) to be delivered to a host cell, whether *in vitro*, *ex vivo*, or *in vivo*. The transgene to be delivered may comprise one or more coding sequences of interest as well as additional polynucleotide sequences that may, for example, enhance transfer, integration, or expression.

[0047] A "replicon" refers to a polynucleotide comprising an origin of replication which allows for replication of the polynucleotide in an appropriate host cell. Examples include chromosomes of a target cell into which a heterologous nucleic acid might be integrated (e.g., nuclear and mitochondrial chromosomes), as well as extrachromosomal replicons (such as replicating plasmids and episomes).

[0048] "Gene delivery," "gene transfer," and the like as used herein, are terms referring to the introduction of an exogenous polynucleotide into a host cell, irrespective of the method used for the introduction. Such methods include a variety of well-known techniques such as vector-mediated gene transfer (by, e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes) as well as techniques facilitating the delivery

of "naked" polynucleotides (such as electroporation, "gene gun" delivery and various other techniques used for the introduction of polynucleotides). The introduced polynucleotide may be stable or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are known to be capable of mediating transfer of genes to mammalian cells, as is known in the art and described herein.

[0049] "*In vivo*" gene delivery, gene transfer, gene therapy and the like, as used herein, are terms referring to the introduction of an exogenous polynucleotide (which may or may not be a heterologous polynucleotide), for example, contained in a gene delivery vector, directly into the body of an organism, such as a human or non-human mammal, whereby the exogenous polynucleotide is introduced into a cell of such organism *in vivo.*

[0050] As used herein "gene transfer" means the process of introducing a nucleic acid molecule, particularly a transgene, into a cell. Gene transfer is commonly performed to enable the expression of a particular product encoded by the gene. The product may include a protein, polypeptide, anti-sense DNA or RNA, or enzymatically active RNA. Gene transfer can be performed in cultured cells or by direct administration into animals. Generally gene transfer involves the process of nucleic acid molecule contact with a target cell by non-specific or receptor-mediated interactions, uptake of nucleic acid molecules into the cell through the membrane or by endocytosis, and release of nucleic acid molecule into the cytoplasm from the plasma membrane or endosome. Expression may require, in addition, movement of the nucleic acid molecule into the nucleus of the cell and binding to appropriate nuclear factors for transcription.

[0051] As used herein "gene therapy" is a form of gene transfer and is included within the definition of gene transfer as used herein and specifically refers to gene transfer to express a therapeutic product from a cell *in vivo* or *in vitro*. Gene transfer can be performed ex vivo on cells which are then transplanted into a patient, or can be performed by direct administration of the nucleic acid molecule (or associated complex) into the patient.

References

[0052] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology and the like, which are within the skill of the art. Such techniques are explained in the literature. See e.g., Molecular Cloning: A Laboratory Manual (Sambrook et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989); Current Protocols in Molecular Biology (Ausubel et al., 1987 and updated 2002); Essential Molecular Biology (Brown, IRL Press 2000); Gene Expression Technology (Goeddel, Academic Press 1991); Methods for Cloning and Analysis of Eukaryotic Genes (Bothwell et al., Bartlett Publ. 1990); Gene Transfer and Expression (Kriegler, Stockton Press 1990); Recombinant DNA Methodology (Wu et al., Academic Press 1989); PCR: A Practical Approach (McPherson, IRL Press at Oxford University Press 1995); Cell Culture for Biochemists (Adams, Elsevier Science Publishers 1990); Gene Transfer Vectors for Mammalian Cells (Miller et al., 1987); Mammalian Cell Biotechnology (Butler, 1991); Animal Cell Culture (Pollard et al., Humana Press 1990); Culture of Animal Cells, 4th Ed. (Freshney et al., Alan R. Liss, 2000); Flow Cytometry and Sorting (Melamed et al., Wiley-Liss 1990); the series Methods in Enzymology (Academic Press, Inc.); Techniques in Immunocytochemistry (Bullock et al., Academic Press 1982, 1983, 1985, 1989); Handbook of Experimental Immunology (Weir, Blackwell, 5th ed., 1996); Cellular and Molecular Immunology (Abbas et al., W.B. Saunders Co. 5th ed., 2003); Current Protocols in Immunology (Coligan et al., 1996); the series Annual Review of Immunology; the series Advances in Immunology; Oligonucleotide Synthesis (Gait, 1984); and Animal Cell Culture (Freshney, IRL Press 1992, 2nd ed.).

[0053] Additional references describing delivery and logistics of surgery which may be used in the methods of the present invention include the following: The Textbook of Interventional Cardiology, 4th ed. (Topol, W.B. Saunders Co. 2002); Vascular Surgery, 5th ed. (Rutherford, W.B. Saunders Co. 2000); The Cecil Textbook of Medicine, 22nd Ed. (Elsevier, Inc. 2004); and Sabiston Textbook of Surgery, 17th Ed. (Elsevier, Inc. 2004). Additional references describing cell types found in the blood vessels, and those of the vasculature which may be useful in the methods of the present invention include, for example, A Textbook of Histology (Bloom & Fawcett, W.B. Saunders Co. 1975).

[0054] Various publications have postulated on the uses of gene transfer for the prevention of disease, including heart disease, and are referenced herein. Additionally, see, for example, Methods in Virology, Vol. 7: Gene Transfer and Expression Protocols, Murray (ed.), Weiss, Clifton, N.J., 1991; Mazur et al. (1994) *Mol. Cell. Biol.* 21:104-111; French (1993) *Herz.* 18:222-229; Williams (1993) *Am. J. Med. Sci.* 306:129-136; and Schneider et al. (1993) *Circulation* 88:1937-1942. Similarly, various publications describe various vectors that may be useful in gene therapy methods. See, e.g., Hammond et al., WO 96/26742; Hammond, WO 02/089856, hereby incorporated by reference in its entirety; Flotte et al., WO 95/13365; Trempe et al., WO 95/13392; Gnatenko (1997) *J. of Invest. Med.* 45:87-97; and other references cited herein.

Detailed Description

[0055] Various preferred aspects of the present invention are summarized below and further described and illustrated in the subsequent detailed descriptions and Figures.

[0056] The present invention relates to methods of treatment to ameliorate deleterious effects of acute coronary syndrome and/or myocardial infarction.

[0057] In methods provided herein, a composition comprising a transgene encoding IGF-1 is delivered to the heart of a patient wherein the transgene is locally expressed to provide local effect from the expressed protein. Preferably the transgene composition is first delivered shortly after the patient presents with acute coronary syndrome or myocardial infarction in order that the therapeutic effects of the composition may be realized as quickly as possible. In one aspect, the transgene composition provides cardioprotective effects following acute coronary syndrome or myocardial infarction which can prevent, delay or ameliorate subsequent cardiac decompensation.

[0058] In a preferred embodiment, the transgene composition is delivered to the effected region of myocardium by intracoronary injection into a coronary artery supplying blood to that region. In alternative embodiments, the transgene composition is delivered locally by direct injection into the effect region of myocardium; or by ex vivo transfection of cells which are then introduced (or re-introduced) into the effected myocardium; or with use of a medical device, such as a stent, located in a vessel in the region of effected myocardium.

[0059] In another aspect, the methods of the present invention employ both a transgene encoding IGF-1 and IGF-1 protein. The transgene and protein may be comprised within a single composition or may be separately comprised. If separate, the IGF-1 protein composition is preferably administered simultaneously with or just prior to administration of the IGF-1 transgene composition. In the case of ex vivo treatment, the cells to be introduced into the myocardium are preferably prepared and introduced immediately or shortly following administration of the IGF-1 protein composition.

[0060] In particularly preferred embodiments, the transgene is comprised within a gene delivery vector. The gene delivery vector may be viral or non-viral. Preferably a viral vector is employed, more preferably a replication-deficient viral vector, most preferably an adenovirus or adeno-associated virus (AAV) is employed. Preferably, regulatory sequences are selected to optimize transcription, translation and/or expression of the IGF-1 transgene as described elsewhere herein.

[0061] The methods of the present invention allow for highly efficient gene transfer *in vivo* without significant necrosis or inflammation at the site of delivery and without significant adverse effects remote or peripheral to the organ of treatment, the heart. This is primarily due to the local nature of expression and effect of the IGF-1 transgene/protein. Based on these results, some of which are described in detail in the Examples below, it is seen that a sufficient degree of *in vivo* gene transfer to effect *in vivo* functional changes is achieved. The transfer of an IGF-1 transgene, either alone or in combination with IGF-1 protein, will delay, lessen the severity of or prevent cardiac decompensation and will enhance muscle function in the treated myocardium. Since IGF-1 protein can be used to initiate cardioprotection during the time before gene expression has begun, the inclusion of the protein can be employed to further enhance therapeutic effect.

[0062] In one aspect, the methods (and vectors employed therein) are provided to treat (including prevent) cardiac decompensation following myocardial infarction or acute coronary syndrome. Methods of assessing improvement in heart function and reduction of symptoms are described herein and well-known in the art. Prevention or alleviation of cardiac decompensation can include reduced infarct size, improved cardiomyocyte viability, and/or improved cardiac function within days to weeks after *in vivo* IGF-1 transgene transfer in the patient relative to not receiving the IGF-1 transgene.

[0063] In an example provided below, the present methods of *in vivo* transfer of a transgene encoding IGF-1 are used to demonstrate that such gene transfer is effective in substantially preventing cardiac decompensation following myocardial infarction or acute coronary syndrome. As the data below suggest, expression of an exogenously-provided IGF-1 transgene results in smaller infarct size, improved cardiomyocyte viability and improved cardiac function.

[0064] Because such cardiac events deprive the heart muscle of adequate blood resulting in cardiomyocyte cell death and scar formation, and because cell death (or apoptosis) accelerates as it progresses, early intervention to protect the heart from such damage is advantageous. However, cardiac decompensation can develop and/or worsen over time following acute coronary syndrome or myocardial infarction and thus even delayed treatment with the IGF-1 transgene composition can be advantageous.

[0065] In treating acute coronary syndrome or myocardial infarction in accordance herewith, the transgene composition is preferably administered to the patient as soon after diagnosis of the disease or condition as possible. In some embodiments, the IGF-1 transgene composition is administered within 24 hours of diagnosis of acute coronary syndrome or following a heart attack. In some embodiments, the IGF-1 transgene composition is administered within 1, 2, 3, 4, 5, 6, or 7 days after diagnosis of acute coronary syndrome or heart attack. In some embodiments, the IGF-1 transgene composition is administered within 1 week of a diagnosis of acute coronary syndrome or heart attack. In some embodiments, the IGF-1 transgene composition is administered within 1-7 days of a diagnosis of acute coronary syndrome or heart attack. In some embodiments, in patients with a coronary vessel occlusion, the IGF-1 transgene composition is administered shortly after reperfusion is begun or introduction of a stent.

[0066] As described herein, a number of different vectors can be employed to deliver the IGF-1 transgene *in vivo* according to the methods of the present invention. By way of illustration, the replication-deficient recombinant adenovirus vectors exemplified herein achieved highly efficient gene transfer *in vivo* without cytopathic effect or inflammation in the areas of gene expression.

[0067] The means and compositions used to deliver vectors carrying IGF-1 transgenes depend on the particular vector employed, as is well known in the art. Typically, however, a vector can be in the form of an injectable preparation containing a pharmaceutically acceptable carrier/diluent such as phosphate buffered saline, for example. Other pharmaceutical carriers, formulations and dosages are described herein.

[0068] Compositions or products for use in the methods of the present invention may conveniently be provided in the form of formulations suitable for administration to a patient, into the blood stream, e.g., by intra-arterial injection. A suitable administration format may best be determined by a medical practitioner. Suitable pharmaceutically acceptable carriers and their formulation are described in standard formulations treatises, *e.g., Remington's Pharmaceuticals Sciences* by E.W. Martin. *See also* Wang et al. (1998) "Parental Formulations of Proteins and Peptides: Stability and Stabilizers", *Journals of Parental Sciences and Technology,* Technical Report No. 10, Supp. 42:2S. Vectors of the present invention should preferably be formulated in solution at neutral pH, for example, about pH 6.5 to about pH 8.5, more preferably from about pH 7 to 8, with an excipient to bring the solution to about isotonicity, for example, 4.5% mannitol or 0.9% sodium chloride, pH buffered with art-known buffer solutions, such as sodium phosphate, that are generally regarded as safe, together with an accepted preservative such as metacresol 0.1% to 0.75%, more preferably from 0.15% to 0.4% metacresol. The desired isotonicity may be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol), or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions. If desired, solutions of the above compositions also can be prepared to enhance shelf life and stability. The therapeutically useful compositions of the invention are prepared by mixing the ingredients following generally accepted procedures. For example, the selected components may be mixed to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water and/or a buffer to control pH or an additional solute to control tonicity.

[0069] For use by the physician, the compositions will be provided in dosage form containing an amount of a vector of the invention which will be effective in one or multiple doses in order to provide a therapeutic effect. As will be recognized by those in the field, an

effective amount of therapeutic agent will vary with many factors including the age and weight of the patient, the patient's physical condition, and the level of effect to be obtained, and other factors.

[0070] The effective dose of the viral vectors of this invention will typically be in the range of about 10^7 - 10^{13} viral particles, preferably about 10^9 - 10^{11} viral particles. As noted, the exact dose to be administered is determined by the attending clinician, but is preferably in 5 ml or less of physiologically buffered solution (such as phosphate buffered saline), more preferably in 1-3 ml.

[0071] The preferred mode of administration is by injection into one or more localized sites (e.g., one or both coronary arteries or by direct injection into the myocardium) using a suitable catheter or other *in vivo* delivery device.

[0072] The presently preferred means of *in vivo* delivery for acute coronary syndrome or myocardial infarction in accordance herewith (especially for vector constructs that are not otherwise targeted for delivery and/or expression that is restricted to the myocardium), is by injection of the vector into a blood vessel or other conduit directly supplying the myocardium or tissue, preferably by injection into one or both coronary arteries. By way of illustration, for delivery to the myocardium, such injection is preferably achieved by catheter introduced substantially (typically at least about 1 cm) within the lumen of one or both coronary arteries or one or more saphenous veins or internal mammary artery grafts or other conduits delivering blood to the myocardium. Preferably the injection is made in both left and right coronary arteries to provide general distribution to all areas of the heart (e.g., Left Anterior Descending (LAD), LCx and Right (RCA)). In some instances, the injection may be made in either the left or the right coronary arteries (e.g., RCA, LAD, LCx, or LAD + LCx). By injecting an adenoviral vector preparation in accordance herewith, optionally in combination with a vasoactive agent to enhance gene delivery or in combination with IGF-1 protein to provide cardioprotection as the transgene is being taken up within the myocardium and expressed, it is possible to perform effective adenovirus-mediated IGF-1 gene transfer for the treatment of cardiac decompensation following acute coronary syndrome or myocardial infarction without any undesirable effects.

[0073] The vectors are delivered in an amount sufficient for the transgene to be expressed and to provide a therapeutic benefit. For viral vectors (such as adenovirus), the final titer of the virus in the injectable preparation is preferably in the range of about 10^7-10^{13} viral particles, which allows for effective gene transfer. An adenovirus vector stock preferably free of wild-type virus can be injected deeply into the lumen of one or both coronary arteries (or grafts), preferably into both right and left coronary arteries (or grafts), and preferably in an amount of about 10^9 - 10^{11} viral particles as determined by optical densitometry. Preferably the vector is delivered in a single injection into each conduit (e.g. into each coronary artery).

[0074] To further augment the localized delivery of the gene therapy vector, and to enhance gene delivery efficiency, in accordance with the present invention, one can infuse a vasoactive agent, such as histamine or a histamine agonist or a vascular endothelial growth factor (VEGF) protein or a nitric oxide donor (e.g. sodium nitroprusside), into the tissue to be treated, either coincidently with or, preferably, within several minutes before, introduction of the angiogenic gene therapy vector.

[0075] By injecting the vector composition directly into the lumen of the coronary artery by coronary catheters, it is possible to target the gene rather effectively, and to minimize loss of the recombinant vectors to the proximal aorta during injection. This type of injection enables local transfection of a desired number of cells, especially cardiac myocytes, in the affected myocardium with IGF-1-encoding genes, thereby maximizing therapeutic efficacy of gene transfer, and minimizing undesirable secondary effects of IGF-1 at extracardiac sites.

[0076] Vector constructs that are specifically targeted to the myocardium, such as vectors incorporating myocardial-specific binding or uptake components, and/or which incorporate IGF-1 transgenes that are under the control of myocardial-specific transcriptional regulatory sequences (e.g., cardiomyocyte-specific promoters) can be used in place of or, preferably, in conjunction with such directed injection techniques as a means of further restricting expression to the myocardium, (e.g. the ventricular myocytes). For vectors that can elicit an immune response, it is preferable to inject the vector directly into a blood vessel supplying the myocardium as described above, although the additional techniques for restricting the extracardiac delivery or otherwise reducing the potential for an immune response can also be employed. Vectors targeted to tissues supplied by the peripheral vasculature, such as vectors

targeted to skeletal muscle or promoters specifically expressed in skeletal muscle, can likewise be employed.

[0077] As described in detail below, it was demonstrated that using techniques of the present invention for *in vivo* delivery of a viral vector containing an IGF-1 transgene, transgene expression did not occur in hepatocytes and viral RNA could not be found in the urine at any time after intracoronary injection. In addition, no evidence of extracardiac gene expression in the eye, liver, or skeletal muscle could be detected by PCR two weeks after intracoronary delivery of transgenes in this manner.

[0078] A variety of catheters and delivery routes can be used to achieve intracoronary delivery, as is known in the art (see, e.g., the references cited above, including: The Textbook of Interventional Cardiology, 4th ed. (Topol, W.B. Saunders Co. 2002); Vascular Surgery, 5th ed. (Rutherford, W.B. Saunders Co. 2000); The Cecil Textbook of Medicine, 22nd Ed. (Elsevier, Inc. 2004); and Sabiston Textbook of Surgery, 17th Ed. (Elsevier, Inc. 2004)). Direct intracoronary (or graft vessel) injection can be performed using standard percutaneous catheter based methods under fluoroscopic guidance. Any variety of coronary catheter, or a Stack perfusion catheter, for example, can be used in the present invention. For example, a variety of general purpose catheters, as well as modified catheters, suitable for use in the present invention are available from commercial suppliers such as Abbott Laboratories, Cordis Corp. and Boston Scientific. Also, where delivery to the myocardium is achieved by injection directly into a coronary artery (which is presently most preferred), a number of approaches can be used to introduce a catheter into the coronary artery, as is known in the art. By way of illustration, a catheter can be conveniently introduced into a femoral artery and threaded retrograde through the iliac artery and abdominal aorta and into a coronary artery. Alternatively, a catheter can be first introduced into a brachial or carotid artery and threaded retrograde to a coronary artery. The capillary bed of the myocardium can also be reached by retrograde perfusion, e.g., from a catheter placed in the coronary sinus. Such a catheter may also employ a proximal balloon to prevent or reduce anterograde flow as a means of facilitating retrograde perfusion. For delivery to tissues supplied by the peripheral vasculature, catheters can be introduced into arteries supplying such tissues (e.g., femoral arteries in the case of the leg) or may be introduced, by example, as a bolus injection or infusion into the affected tissue.

[0079] Various combinations of vectors comprising IGF-1 genes and catheters or other *in vivo* delivery devices (e.g., other devices capable of introducing a pharmaceutical composition, generally in buffered solution, into a blood vessel or into muscle) can be incorporated into kits for use in accordance with the present invention. Such kits may also incorporate one or more vasoactive agents to enhance gene delivery, and may further include instructions describing their use in accordance with any of the methods described herein.

[0080] In another aspect, a member of the fibroblast growth factor (FGF) family may be exchanged for IGF-1 in the methods and compositions throughout this teaching. Accordingly, a polynucleotide encoding an FGF may be used in the methods and compositions provided herein as an alternative to a polynucleotide encoding IGF-1. Members of the FGF family for use in the present invention include, but are not limited to, acidic FGF (aFGF, also known as FGF-1), basic FGF (bFGF, also known as FGF-2), FGF-4, FGF-5, and FGF-6. For example, a polynucleotide encoding FGF-2 may be used in the methods and compositions provided herein in place of a polynucleotide encoding IGF-1.

Nucleic Acids Encoding Polypeptides

[0081] The amino acid sequence of mature human IGF-1 (hIGF-1) as well as nucleic acid sequences encoding human pre-pro IGF-1 (ppIGF-1) and modified human ppIGF-1 have been described. See, *e.g.*, Rinderknecht et al. (1978) *J. Biol. Chem.* 253:2769-2776; Jansen *et al.* (1983) *Nature* 306:609-611; U.S. Pat. No. 4,963,665 (Rotwein et al.); and Banks et al., WO 85/00831. Such native and modified IGF-1 polypeptides and nucleic acids are of use in accordance with the present invention. Also, in some embodiments, modified polynucleotides encoding hIGF-1 which nucleic acid sequences are designed to provide improved transgene transcription and/or translation are of use in the present invention. In these modified polynucleotides, particular codons are changed to alternate codons that code for the same amino acid. Accordingly, in these embodiments, the IGF-1 polypeptide encoded by the modified polynucleotide has the same amino acid sequence as the native IGF-1 polypeptide.

[0082] In some embodiments, the polynucleotides encoding IGF-l, comprise one or more of the alternate codon specified in Table 1. Table 1 illustrates the 153 native codons of human pre-pro insulin-like growth factor-1 (hppIGF-1) ("native codon" in Table 1, SEQ ID

NO:1) and the corresponding amino acids encoded thereby ("IGF-1 aa" in Table 1, SEQ ID NO:2). Certain codons have been identified (indicated with an asterisk (*) in Table 1) that when changed to alternate codons encoding the same amino acid can result in improved transcription and/or translation of the encoded IGF-1 polypeptide. Also shown in Table 1 are alternate codons for those amino acid positions that preferably are modified to construct polynucleotides of use in accordance with some embodiments of the present invention. While the codons in Table 1 are represented as DNA, it will be appreciated by those of skill in the art that other modified and unmodified nucleic acids, including, for example RNA, may likewise be used to construct polynucleotides in accordance herewith.

[0083] Due to redundancy in the genetic code, generally several different codons can specify a particular amino acid. The frequency of use of a particular codon in a coding sequence varies with some codons used more often ("major codons") and other codons used less often ("rare codons"). Frequency of codon use can be depicted as the number of occurrences in 1000 codons or as the fraction of occurrences of the codon in its synonymous codon family. For example, in a particular codon frequency database of highly expressed human genes, the codons for isoleucine, ATA, ATT and ATC, occur 5%, 18% and 77%, respectively, in the synonymous isoleucine family.

[0084] In some embodiments, modified IGF-1 polynucleotides have alternate codons replacing native codons that occur less than 8% in its synonymous codon family. In some embodiments, modified IGF-1 polynucleotides have alternate codons replacing native codons that occur less than 15% in its synonymous codon family. In some embodiments, modified IGF-1 polynucleotides have one or more rare codons replaced with a more common alternate codon. In some embodiments, modified IGF-1 polynucleotides have 5 or more rare codons replaced with a more common alternate codon. In some embodiments, modified IGF-1 polynucleotides have 10 or more rare codons replaced with a more common alternate codon. In some embodiments, modified IGF-1 polynucleotides have 15 or more rare codons replaced with a more common alternate codon.

[0085] In some embodiments using alternative codons, the modified polynucleotides of the present invention comprise at least 5, preferably at least 10, more preferably at least 20, still more preferably at least 30 and most preferably more than 50 of the alternate codons selected from Table 1. As denoted in Table 1, certain alternate codons are preferred at certain

locations within the polynucleotide. By way of example, the second codon in the native coding sequence for hppIGF-1, which encodes glycine, is GGA. In some embodiments of the present invention, if that codon is to be modified, it is preferably modified to be GGC which also encodes glycine. Alternatively, however, this codon may be selected from alternate codons GGT or GGG. In particularly preferred embodiments, a multiplicity of preferred codon alterations, that is those marked with an asterisk (*) in Table 1, are made to the polynucleotides of the present invention.

Table 1. hppIGF-1 and exemplary alternate codons

IGF-1	1				5					10					15			
aa	M	G	K	I	S	S	L	P	T	Q	L	F	K	C	C	F	C	D
native		*	*		*	*	*	*		*	*	*			*	*	*	*
codon	atg	gga	aaa	atc	agc	agt	ctt	cca	acc	caa	tta	ttt	aag	tgc	tgc	ttt	tgt	gat
alt.		ggc	aag		tcc	agc	ctg	ccc		cag	ctg	ttc			tgt	ttc	tgc	gac
codon							ttg											

IGF-1		20					25					30					35	
aa	F	L	K	V	K	M	H	T	M	S	S	S	H	L	F	Y	L	A
native		*	*							*	*	*	*	*	*			*
codon	ttc	ttg	aag	gtg	aag	atg	cac	acc	atg	tcc	tcc	tcg	cat	ctc	ttc	tac	ctg	gcg
alt.		ctg	aaa							agc	agc	agc	cac	ctg	ttt			gcc
codon										tct								

IGF-1				40					45					50				
aa	L	C	L	L	T	F	T	S	S	A	T	A	G	P	E	T	L	C
native				*	*				*		*	*	*	*		*	*	*
codon	ctg	tgc	ctg	ctc	acc	ttc	acc	agc	tct	gcc	acg	gct	gga	ccg	gag	acg	ctc	tgc
alt.				ctg	act				agc		acc	gcc	ggc	ccc		acc	ctg	tgt
codon									tcc		aca		ggg	cct			ctt	

IGF-1	55					60					65					70		
aa	G	A	E	L	V	D	A	L	Q	F	V	C	G	D	R	G	F	Y
native	*	*	*		*	*	*	*	*			*	*		*		*	*
codon	ggg	gct	gag	ctg	gtg	gat	gct	ctt	cag	ttc	gtg	tgt	gga	gac	agg	ggc	ttt	tat
alt.	ggc	gcc	gaa		gtc	gac	gcc	ctg	caa			tgc	ggc		cgc		ttc	tac
codon	gtt	gca																

IGF-1			75					80					85					90
aa	F	N	K	P	T	G	Y	G	S	S	S	R	R	A	P	Q	T	G
native					*	*	*		*	*	*			*	*		*	*
codon	ttc	aac	aag	ccc	aca	ggg	tat	ggc	tcc	agc	agt	cgg	agg	gcg	cct	cag	aca	ggc
alt.					acc	ggc	tac		agc	tcc	agc			gcc	ccc		acc	ggg
codon					act				tct					gct	cca			

IGF-1				95				100				105						
aa	I	V	D	E	C	C	F	R	S	C	D	L	R	R	L	E	M	Y
native			*			*	*		*	*	*	*		*	*			*
codon	atc	gtg	gat	gag	tgc	tgc	ttc	cgg	agc	tgt	gat	cta	agg	agg	ctg	gag	atg	tat
alt.			gac			tgt	ttt		tca	tgc	gac	ctg		aga	ctt			tac
codon																		

IGF-1		110				115				120				125				
aa	C	A	P	L	K	P	A	K	S	A	R	S	V	R	A	Q	R	H
native		*		*	*	*			*	*		*	*	*				
codon	tgc	gca	ccc	ctc	aag	cct	gcc	aag	tca	gct	cgc	tct	gtc	cgt	gcc	cag	cgc	cac
alt.		gcc		ctg	aaa	ccc			agc	gcc		agc	gtg	cgg				
codon									tct									

IGF-1			130				135				140							
aa	T	D	M	P	K	T	Q	K	E	V	H	L	K	N	A	S	R	G
native								*	*	*	*	*			*	*		*
codon	acc	gac	atg	ccc	aag	acc	cag	aag	gaa	gta	cat	ttg	aag	aac	gca	agt	aga	ggg
alt.								aaa	gag	gtg	cac	ctg			gcc	agc		ggc
codon										gtc					gct			gga

IGF-1	145				150			153		
aa	S	A	G	N	K	N	Y	R	M	
native	*	*	*	*						
codon	agt	gca	gga	aac	aag	aac	tac	agg	atg	tag
alt.	agc	gcc	ggc	aat						
codon	tca									

[0086] The compositions of the present invention may be constructed using standard molecular biology techniques with reference to the teachings herein, for example Table 1 and the Examples. In one aspect, the modified polynucleotides of the present invention have been designed to be translated at least as efficiently, preferably more efficiently than the native human IGF-1-encoding polynucleotide. In another aspect, the present polynucleotides are designed for efficient production of hIGF-1 protein, in particular, efficient production by human cells, whether in vitro or in vivo.

[0087] Accordingly, polynucleotides encoding IGF-1 for use in the present compositions and methods include the following (in modified polynucleotides, substituted nucleotides are bold, capital letters):

Native hppIGF-1 polynucleotide (SEQ ID NO:1):

```
atg gga aaa atc agc agt ctt cca acc caa tta ttt aag tgc tgc ttt tgt gat ttc
ttg aag gtg aag atg cac acc atg tcc tcc tcg cat ctc ttc tac ctg gcg ctg tgc
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ctg ctc acc ttc acc agc tct gcc acg gct gga ccg gag acg ctc tgc ggg gct gag
ctg gtg gat gct ctt cag ttc gtg tgt gga gac agg ggc ttt tat ttc aac aag ccc
aca ggg tat ggc tcc agc agt cgg agg gcg cct cag aca ggc atc gtg gat gag tgc
tgc ttc cgg agc tgt gat cta agg agg ctg gag atg tat tgc gca ccc ctc aag cct
gcc aag tca gct cgc tct gtc cgt gcc cag cgc cac acc gac atg ccc aag acc cag
aag gaa gta cat ttg aag aac gca agt aga ggg agt gca gga aac aag aac tac agg
atg tag

Modified hppIGF-1 polynucleotide 1 (SEQ ID NO:3):

atg ggC aaG atc agc agC ctG ccC acc caG CtG ttC aag tgc tgc ttC tgC gaC ttc
Ctg aag gtg aag atg cac acc atg AGc AGc AGC caC ctG ttc tac ctg gcC ctg tgc
ctg ctG acc ttc acc agc AGC gcc acC gcC ggC ccC gag acC ctG tgc ggC gcC gag
ctg gtg gaC gcC ctG cag ttc gtg tgC ggC gac agg ggc ttC taC ttc aac aag ccc
acC ggC taC ggc AGc agc agC cgg agg gcC ccC cag acC ggc atc gtg gaC gag tgc
tgc ttc cgg agc tgC gaC ctG agg agg ctg gag atg taC tgc gcC ccc ctG aag ccC
gcc aag AGC gcC cgc AGC gtG cgG gcc cag cgc cac acc gac atg ccc aag acc cag
aag gaG gtG caC Ctg aag aac gcC agC aga ggC agC gcC ggC aac aag aac tac agg
atg tag

Modified hppIGF-1 polynucleotide 2 (SEQ ID NO:4):

atg ggC aaG atc TCC agC ctG ccC acc caG ttG ttC aag tgc tgT ttt tgC gaC ttc
Ctg aaA gtg aag atg cac acc atg AGc tcc tcT cat ctc ttT tac ctg gcC ctg tgc
ctg ctc acT ttc acc agc tcC gcc acA gct ggG ccT gag acC ctT tgT ggT gcA gaA
ctg gtC gat gcC ctG caA ttc gtg tgt gga gac CgC ggc ttC taC ttc aac aag ccc
acT ggg tat ggc AGc TCc TCt cgg agg gcT ccA cag aca ggG atc gtg gaC gag tgc
tgT ttT cgg TCA tgC gat ctG agg agA ctT gag atg tat tgc gca ccc ctc aaA cct
gcc aag tcT gcC cgc AGC gtG cgG gcc cag cgc cac acc gac atg ccc aag acc cag
aaA gaa gtC caC ttg aag aac gcT agt aga ggA TCA gcC ggC aaT aag aac tac agg
atg tag

Modified hppIGF-1 polynucleotide 3 (SEQ ID NO:5):

atg ggC aaG atc TCc agC ctG ccC acc caG CtG ttC aag tgc tgc ttC tgC gaC ttc
Ctg aag gtg aag atg cac acc atg AGc tcc tcT caC ctG ttc tac ctg gcC ctg tgc
ctg ctc acc ttc acc agc tcC gcc acC gcC ggC ccC gag acC ctG tgc ggC gcC gag
ctg gtg gaC gcC ctG cag ttc gtg tgC ggC gac agg ggc ttC taC ttc aac aag ccc
acC ggg taC ggc AGc TCc TCt cgg agg gcC ccC cag acC ggc atc gtg gaC gag tgc
tgT ttc cgg agc tgC gaC ctG agg agA ctg gag atg taC tgc gcC ccc ctG aag ccC
gcc aag AGC gcC cgc AGC gtG cgG gcc cag cgc cac acc gac atg ccc aag acc cag
aag gaG gtG caC Ctg aag aac gcC agC aga ggC agC gcC ggC aac aag aac tac agg
atg tag

[0088] While transgenes encoding full-length, pre-pro IGF-1 are exemplified herein, it will be appreciated by those of skill in the art that transgenes encoding biologically active fragments and analogs of IGF-1, as well as transgenes encoding IGF-1 derived from other animals, particularly mammals, may likewise be employed in the present methods and are likewise contemplated herein. Such transgenes encoding biologically active fragments, analogs and nonhuman IGF-1 may also include alternate codons as described in Table 1 for efficient transcription and/or translation. Amino acids and regions of IGF-1 necessary for

IGF-1 receptor binding and biological activity are known in the art, as are biologically active fragments and analogs of IGF-1. Methods and assays for identifying IGF-1 analogs and fragments with biological activity are also known in the art. See, for example, Cara et al. (1988) *Endocrinology* 122:2881-2887; Cascieri et al. (1988) *Biochemistry* 27:3229-3233; Cascieri et al. (1988) *Endocrinology* 123:373-381; Bayne et al. (1988) *J. Biol. Chem.* 263:6233-6239; Bayne et al. (1989) *J. Biol. Chem.* 264:11004-11008; Carlsson-Skwirut et al. (1989) *Biochim. Biophys. Acta* 1011:192-197; Bayne et al. (1990) *J. Biol. Chem.* 265:15648-15652; Drakenberg et al. (1990) *Acta Endocrinol.* 123:43-50; Tomas et al. (1991) *J. Endocrinol.* 128:97-105; Tomas et al. (1992) *Biochem. J.* 282:91-97; Cascieri et al. (1993) *Adv. Exp. Med. Biol.* 343:33-40; Tomas et al. (1996) *J. Endocrinol.* 150:77-84.

[0089] In some embodiments, a promoter is responsible for driving transcription of the IGF-1 encoding gene. Selection of the appropriate promoter is based on published data as well as empirical evidence as illustrated herein. Preferably, the IGF-1 transgenes employed in the present invention are operably linked to one or more promoters that direct transcription of the transgene in a mammalian cell, such as a cell in the heart, skeletal muscle or other target tissue, including for example, a cardiomyocyte, a fibroblast, or an endothelial cell. Presently preferred promoters include generally constitutive promoters such as a cytomegalovirus immediate-early enhancer/promoter (“CMV promoter”), Rous sarcoma virus promoter (“RSV promoter”), Simian Virus 40 (“SV40 promoter”) or human elongation factor-1 alpha/HTLV enhancer (“Hef-1 alpha / HTLV promoter”).

[0090] In some embodiments, the IGF-1 transgene is operatively linked to a functional portion of the immediate-early enhancer/promoter regulatory region of human CMV (hCMV). In some embodiments, the IGF-1 transgene is operatively linked to a shortened immediate-early enhancer/promoter regulatory region of hCMV (a truncated CMV promoter or “tCMV promoter”), for example, a promoter of about 517 nucleotides in length, a promoter of about 742 nucleotides in length or a promoter of about 795 nucleotides in length. The sequence of the hCMV promoter regulatory region is known in the art, for example, as described in Bebbington, WO 89/01036 and U.S. Pat. Nos. 5,168,062 (Stinski) and 5,385,839 (Stinski). In some embodiments, the IGF-1 transgene is operatively linked to an enhanced CMV promoter (“eCMV promoter”) comprising a CMV immediate-early enhancer/promoter and 5’ untranslated region of the major immediate early gene of hCMV. In some

embodiments, the eCMV promoter is about 1.7 kb in length. As demonstrated herein, eCMV operatively linked to an IGF-1 encoding polynucleotide leads to increased IGF-1 production as compared to a 517 nucleotide tCMV promoter. An exemplary eCMV promoter sequence for use in the compositions and methods provided herein is as follows (SEQ ID NO:6):

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CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
ACGGACTCTG TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
TGGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
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[0091] Other promoter systems include inducible systems (e.g., tetracycline-inducible, ecdysone and others). Alternatively, a tissue-specific promoter, such as a cardiac-specific promoter (e.g., a cardiomyocyte-specific promoter) or skeletal muscle-specific promoter may be employed. Exemplary tissue specific promoters include cardiomyocyte-specific myosin light chain and the cardiomyocyte-specific myosin heavy chain. Many promoters and promoter systems are commercially available through vendors such as Stratagene, Invitrogen

Corp., Promega Corp., Invivogen and others. Preferably, the transgene is also operably linked to a polyadenylation signal and may likewise be linked to transcription and/or translation enhancers or similar regulatory sequences. Enhancers exemplified herein are the heat shock protein 70 transcription enhancer (HSP70) and QBI SP163 transcription enhancer (Invitrogen Corp.). An exemplary translation enhancer used herein is the Kozak enhancer element. As will be appreciated by those of skill in the art, post-transcription and/or post-translation signaling sequences may likewise be included in the IGF-1-encoding transgene in accordance with the present invention.

[0092] In some embodiments, the IGF-1 encoding transgene can be used in conjunction with at least one other protein factor. Thus, in some embodiments, the nucleic acid encoding the IGF-1 also comprises a sequence encoding a second protein factor such that both polypeptides are expressed in the heart. In other embodiments, the IGF-1 transgene is administered in combination with at least one other protein factor (other than IGF-1). Exemplary second protein factors include factors which induce angiogenesis, promote cardiac cell survival, promote cardiac cell function, and/or recruit cells within the heart. Such factors include, but are not limited to, a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), a platelet-derived growth factor (PDGF), a hypoxia-inducible factor (HIF), and angiogenic polypeptide regulator. In some embodiments, the second protein factor is an anti-apoptotic factor, a hepatocyte growth factor, a beta-adrenergic signaling protein (beta-ASP), an endothelial PAS domain 1 (EPAS-1), or a granulocyte colony-stimulating factor (G-CSF).

[0093] Such combinations can exhibit additive and/or synergistic effects. Numerous other combinations will be apparent to those of skill in the art based on these teachings. Combinations of nucleic acids that can be used in accordance with the present invention can be provided within a single vector (e.g., as separate coding seuences, each under the control of a promoter, or as a single transcriptional or translational fusion gene). Combinations of genes can also be provided as a combination of vectors (which may be derived from the same or different vectors, such as a combination of adenovirus (Ad) vectors, or an Ad vector and an adeno-associated virus (AAV) vector); which can be introduced to a patient coincidentally or in series. In the case of Ad and AAV, the presence of Ad, which is normally a helper virus for AAV, can enhance the ability of AAV to mediate gene transfer. An Ad vector may thus

be introduced coincident with or prior to introduction of an AAV vector according to the present invention. In addition to transfection efficiency, the choice of vector is also influenced by the desired longevity of transgene expression. By way of illustration, since IGF-1 can bring about long-term effects without requiring long-term expression, an IGF-1 transgene may be introduced using an adenovirus (or other vector that does not normally integrate into host DNA) which might be used prior to or in combination with the introduction of an AAV vector carrying a transgene for which longer-term expression is desired (e. g., a beta-ASP transgene). Other combinations of transgenes and/or vectors will be apparent to those of skill in the art based on the teachings and illustrations of the present invention.

Gene Delivery

[0094] As stated above, various types of gene delivery vectors are known and readily adaptable for use herein. Vectors useful in the present invention include, for example, viral vectors, lipid-based vectors (e.g. liposomes) and the like, capable of delivering a transgene into cells in vitro, ex vivo and/or in vivo thereby facilitating expression of said transgene within said cells. Presently preferred are viral vectors, particularly replication-deficient viral vectors including, for example, replication-deficient adenovirus and adeno-associated virus vectors. For ease of production and use in the present invention, replication-deficient adenovirus vectors are exemplified herein. In contrast to some other viral delivery systems, adenovirus generally does not require host cell replication for gene expression because integration is not normally a component of the adenoviral life cycle. Thus, adenovirus can infect non-dividing cells making it well suited for expressing recombinant genes in nonreplicative cells, such as cardiac myocytes.

[0095] A variety of other vectors, both viral and non-viral, can likewise readily be employed to deliver IGF1-encoding nucleic acids in accordance with the present invention. For in vivo use, the vectors are preferably suitable for such by their nature or modifiable for such use. With respect to viral vectors, adenovirus (Ad), adeno-associated virus (AAV), lentivirus (e.g. based on HIV, feline immunodeficiency virus), herpes virus vaccinia virus, various RNA viruses and bovine papillomavirus are exemplary. By way of illustration, AAV vectors useful in the gene therapy methods and compositions of the present invention are preferably replication-deficient in humans, for example, due to deletion of the *rep* and/or *cap*

genes, essential to AAV replication, and the transgene (including associated promoters and other regulatory sequences) inserted therein is preferably flanked by AAV inverted terminal repeat (ITR) sequences. The resulting recombinant AAV vector is then replicated in a packaging cell line supplying the missing AAV functions (i.e., the rep and/or cap genes) in trans. References describing these and other gene delivery vectors are known in the art, a number of which are cited herein.

[0096] As described above and in the scientific literature, a number of retrovirus-derived systems have also been developed to be used in gene delivery, particularly in vivo gene delivery. By way of illustration, the lentivirus genus of retroviruses (for example, human immunodeficiency virus, feline immunodeficiency virus and the like) can be modified so that they are able to transduce cells that are typically non-dividing (see, e.g., Naldini et al. (1996) *Science* 272:263-267; Miyoshi et al. (1998) *J. Virol.* 72:8150-8157; and Buchschacher et al. (2000) *Blood* 15:2499-2504; U.S. Pat. No. 6,013,516 (Verma et al.)). While HIV-based lentiviral vector systems have received some degree of focus in this regard, other lentiviral systems have recently been developed, such as feline immunodeficiency virus-based lentivirus vector systems, that offer potential advantages over the HIV-based systems (see e.g., Poeschla et al. (1998) *Nat. Med.* 4:354-357; Romano et al. (2000) *Stem Cells* 18:19-39 and references reviewed therein).

[0097] In addition to viral vectors, non-viral vectors that may be employed as a gene delivery means are likewise known and continue to be developed. For example, non-viral protein-based delivery platforms, such as macromolecular complexes comprising a DNA binding protein and a carrier or moiety capable of mediating gene delivery, as well as lipid-based vectors (such as liposomes, micelles, lipid-containing emulsions and others) have been described in the art (see e.g., Romano et al. (2000) *Stem Cells* 18:19-39 and references reviewed therein). Improvements in lipid-mediated in vivo gene delivery have been facilitated by the development of new cationic formulations and vector delivery co-factors (see e.g., Kollen et al. (1999) *Hum. Gene Ther.* 10:615-622; Roy et al. (1999) *Nat. Med.* 5:387-391; Fajac et al. (1999) *Hum. Gene Ther.* 10:395-406; Ochiya et al. (1999) *Nat. Med.* 5:707-710). Additionally, the development of systems which combine components of viral and non-viral mediated gene delivery systems have been described and may be employed herein (see e.g., Di Nicola et al. (1999) *Hum. Gene Ther.* 10:1875-1884).

[0098] Recombinant viral vectors comprise heterologous non-viral genes or sequences. Since many viral vectors exhibit size-constraints associated with packaging, and since replication-deficient viral vectors are generally preferred for in vivo delivery, the heterologous genes or sequences are typically introduced by replacing one or more portions of the viral genome. Such viruses may become replication-deficient as a result of the deletions, thereby requiring the deleted function(s) to be provided in trans during viral replication and encapsidation (by using, e.g., a helper virus or a packaging cell line carrying genes necessary for replication and/or encapsidation) (see, e.g., the references and illustrations of viral vectors herein). Modified viral vectors in which a polynucleotide to be delivered is carried on the outside of the viral particle have also been described (see, e.g., Curiel et al. (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88:8850-8854).

[0099] In a further aspect of the present invention, where the compositions are employed in gene therapy methods, the efficiency of gene delivery using a vector such as a viral vector (e.g. adenovirus or adeno-associated virus) may be enhanced by delivering the vector into a blood vessel or into a tissue that is co-infused or pre-infused with a vasoactive agent, for example histamine, a histamine agonist, a vascular endothelial growth factor (VEGF) protein or a nitric oxide donor, such as sodium nitroprusside. The use of such vasoactive agents has been described and illustrated by e.g., Hammond, WO 99/40945; Hammond, WO 02/089856, each of which are hereby incorporated by reference in their entirety. Most preferably the vasoactive agent is infused into the blood vessel or tissue coincidently with or within several minutes prior to introduction of the vector composition. Vasoactive agent, as used herein, refers to a natural or synthetic substance that induces increased vascular permeability and/or enhances transfer of macromolecules such as gene delivery vectors from blood vessels, e.g. across capillary endothelia. By augmenting vascular permeability to macromolecules or otherwise facilitating the transfer of macromolecules into the capillary bed perfused by an artery (or served by a vein), vasoactive agents can enhance delivery of these vectors to the targeted sites and thus effectively enhance overall expression of the transgene in the target tissue. By way of illustration, histamine has been used as a vasoactive agent and was found to substantially enhance delivery of a vector to an infused site such as the myocardium. See, e.g., Hammond, WO 99/40945. Histamine derivatives and agonists, such as related compounds that interact with histamine receptors, which can be employed include, for example, 2-methylhistamine, 2-pyridylethylamine, betahistine, and 2 thiazolylethylamine.

These and additional histamine agonists are described, for example, in Garrison JC., Goodman and Gilman's The Pharmacological Basis of Therapeutics (8th Ed: Gilman, Rall, Nies, Taylor, eds) Pergamon Press, 1990, pp 575-582 and in other pharmacological treatises. In addition or alternatively to histamine or histamine agonists, vascular endothelial growth factors (VEGFs), VEGF agonists (as described herein and in the cited references) or a nitric oxide donor (e.g., sodium nitroprusside) can be used to induce increased vascular permeability and can therefore be used as a vasoactive agent to enhance gene delivery in the context of the compositions and methods described herein. As with histamine, the vasoactive agent is preferably infused into a blood vessel supplying the target site over several minutes prior to infusion of vector.

[0100] The present invention contemplates the use of targeting not only by physical means such as delivery of vector directly into the cardiac muscle, or delivery into a vessel supplying blood thereto or transporting blood therefrom, but also by use of targeted vector constructs having features that tend to target gene delivery and/or gene expression to particular host cells or host cell types (e.g. cardiomyocytes). Such targeted vector constructs would thus include targeted delivery vectors and/or targeted vectors, as described in more detail below and in the published art. Restricting delivery and/or expression is beneficial as a means of further focusing the potential effects of the gene therapeutic and of minimizing any undesirable secondary effects that may be realized by systemic delivery and/or expression. The potential usefulness of further restricting delivery/expression depends in large part on the type of vector being used and the method of introduction of such vector. By way of example, where the vector is delivered to cells *ex vivo*, further targeting of the vector is not critical. Similarly, as described herein, delivery of viral vectors via intracoronary injection to the myocardium has been observed to provide, in itself, highly targeted gene delivery. However, other means of limiting delivery and/or expression can also be employed, in addition to or in place of the illustrated delivery methods, as described herein.

[0101] Targeted delivery vectors include, for example, vectors (such as viruses, non-viral protein-based vectors and lipid-based vectors) having surface components (such as a member of a ligand-receptor pair, the other half of which is found on a host cell to be targeted) or other features that mediate preferential binding and/or gene delivery to particular host cells or host cell types. As is known in the art, a number of vectors of both viral and non-viral origin

have inherent properties facilitating such preferential binding and/or have been modified to effect preferential targeting (see, e.g., Douglas et al. (1996) *Nat. Biotech.* 14:1574-1578; Kasahara et al. (1994) *Science* 266:1373-1376; Miller et al. (1995) *FASEB J.* 9:190-199; Chonn et al. (1995) *Curr. Opin. in Biotech.* 6:698-708; Schofield et al. (1995) *British Med. Bull.* 51:56-71; Schreier (1994) *Pharmaceutica Acta Helvetiae* 68:145-159; Ledley (1995) *Hum. Gene Ther.* 6:1129-1144; Conary et al., WO 95/34647; Overell et al., WO 95/28494; and Truong et al., WO 96/00295).

[0102] As stated above and in the cited references, vectors can also comprise components or functionalities that further modulate gene delivery and/or gene expression, or that otherwise provide beneficial properties to the targeted cells. Such other components include, for example, components that influence binding or targeting to cells (including components that mediate cell-type or tissue-specific binding); components that influence uptake of the vector by the cell; components that influence processing and/or localization of the vector and its nucleic acid within the cell after uptake (such as agents mediating intracellular processing and/or nuclear localization); and components that influence expression of the polynucleotide. Such components can also include markers, such as detectable and/or selectable markers that can be used to detect or select for cells that have taken up and are expressing the nucleic acid delivered by the vector. Such components can be provided as a natural feature of the vector (such as the use of certain viral vectors which have components or functionalities mediating binding and uptake), or vectors can be modified to provide such functionalities (such as viruses which have been modified to include a cell binding or targeting protein on the exterior surface of their envelope or capsid). A detectable marker gene allows cells carrying the gene to be specifically detected (e.g., distinguished from cells which do not carry the marker gene). One example of such a detectable marker gene is the lacZ gene, encoding beta-galactosidase, which allows cells transfected with a vector carrying the lacZ gene to be detected by staining, as described below. Other such detectable marker genes include a gene which encodes green fluorescent protein and a gene which encodes a luciferase enzyme, both of which are widely used detectable marker systems. Selectable markers can be positive, negative or bifunctional. Positive selectable markers allow selection for cells carrying the marker, whereas negative selectable markers allow cells carrying the marker to be selectively eliminated. A variety of such marker genes have been described, including bifunctional (i.e. positive/negative) markers (see, e.g., Lupton, WO 92/08796; Lupton, WO 94/28143). Such

marker genes can provide an added measure of control that can be advantageous in gene therapy contexts. A large variety of such vectors is known in the art and are generally available (see, e.g., the various references cited above).

[0103] References describing adenovirus vectors and other viral vectors which could be used in the compositions and methods of the present invention, in addition to those cited above, include the following: Horwitz, pp. 1679-1721, Adenoviridae and Their Replication, in Fields et al. (eds.) Virology, Vol. 2 (1990) Raven Press New York; Graham et al., pp. 109-128 in Methods in Molecular Biology, Vol. 7: Gene Transfer and Expression Protocols, Murray, E. (ed.), Humana Press, Clifton, N.J. (1991); Miller et al. (1995) *FASEB J.* 9:190-199; Schreier (1994) *Pharmaceutica Acta Helvetiae* 68:145-159; Curiel et al. (1992) *Hum. Gene Ther.* 3:147-154; Graham et al., WO 95/00655; Falck-Pedersen, WO 95/16772; Denefle et al., WO 95/23867; Haddada et al., WO 94/26914; Perricaudet et al., WO 95/02697; Zhang et al., WO 95/25071). A variety of adenovirus plasmids are also available from commercial sources, including, e.g., Microbix Biosystems of Toronto, Ontario (see, e.g., Microbix Product Information Sheet: Plasmids for Adenovirus Vector Construction, 1996).

[0104] Additional references describing AAV vectors which could be used in the compositions and methods of the present invention include the following: Carter, Handbook of Parvoviruses, vol. I, pp. 169-228, 1990; Berns, Virology, pp. 1743-1764 (Raven Press 1990); Carter (1992) *Curr. Opin. Biotechnol.* 3:533-539; Muzyczka (1992) *Curr. Top. Microbiol. Immunol.* 158:92-129; Flotte et al. (1992) *Am. J. Respir. Cell Mol. Biol.* 7:349-356; Chatterjee et al. (1995) *Ann. NY Acad. Sci.* 770:79-90; Kotin (1994) *Hum. Gene Ther.* 5:793-801; Flotte et al. (1995) *Gene Therapy* 2:357-362; WO 96/17947; Du et al. (1996) *Gene Therapy* 3:254-261; Kaplitt et al. (1996) *Ann. Thorac. Surg.* 62:1669-1676; Samulski et al. (1989) *J. Virol.* 63:3822-3828; Zolotukhin et al. (1999) *Gene Therapy* 6:973-985; Atkinson et al., WO 99/11764.

[0105] References describing non-viral vectors which could be used in the composition and methods of the present invention include the following: Ledley (1995) *Hum. Gene Ther.* 6:1129-1144; Miller et al. (1995) *FASEB J.* 9:190-199; Chonn et al. (1995) *Curr. Opin. in Biotech.* 6:698-708; Schofield et al. (1995) *British Med. Bull.* 51:56-71; Brigham et al. (1993) *J. Liposome Res.* 3:31-49; Philip et al. (1994) *Mol. Cell Biol.* 14:2411-2418; Perales et al. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91:4086-4090; Solodin et al. (1995) *Biochemistry*

34:13537-13544; Hanson et al., WO 95/25809; Gao et al., WO 96/22765; Brigham, WO 91/06309; Felgner et al., WO 91/17424; Szoka et al., WO 93/19768; Debs et al., WO 93/25673; Overell et al., WO 95/28494; Jessee,WO 95/02698; Haces et al., WO 95/17373; Lin et al., WO 96/01840 and U.S. Pat. Nos. 5,264,618 (Felgner et al.); 5,283,185 (Epand et al.); 5,334,761 (Gebeyehu et al.); 5,459,127 (Felgner et al.).

[0106] In some embodiments, introduced cells can be used as a carrier or vector to deliver the gene to the heart. Cells appropriate for this use include cells transfected ex vivo with an IGF-1 transgene or cells in which endogenous IGF-1 expression is activated. Of particular interest for this cell-based therapy are stem cells, such as stem cells differentiated or stimulated to express endogenous IGF-1, stem cells transfected with an IGF-1 encoding vector, and cells that express IGF-1 which were derived ex vivo from stem cells. Cells for expressing IGF-1 can be introduced into a patient by any of a variety of suitable methods described herein for administration of other vectors such as injection into one or more localized sites (e.g., one or both coronary arteries supplying the myocardium, the coronary venous circulation, or direct injection into the myocardium) or by any other suitable conventional method.

Construction of An Exemplary Recombinant Adenoviral Vector

[0107] Exemplary recombinant replication-deficient adenoviral vectors comprising a nucleic acid encoding IGF-1 have been constructed. In one example, a replication-deficient human adenovirus, serotype 5 (Ad5) vector comprising a nucleic acid encoding human IGF-1 (hIGF-1) of SEQ ID NO: 1 was constructed. Additionally, Ad5 vector comprising modified nucleic acid encoding hIGF-1 of SEQ ID NO: 3 has been constructed. Various methods are known to those of skill in the art for the construction of recombinant adenoviral vectors, including the rescue recombination technique (as described for example in Graham (1988) *Virology* 163:614-617).

[0108] In the rescue recombination method of vector construction, exemplified herein, the transgene of interest is cloned into a shuttle vector that contains a promoter (if not already present in the transgene), a polylinker and partial flanking adenoviral sequences from which ElA and ElB genes, which are essential for viral replication (see, e.g., Graham (1988) *Virology* 163: 614-617), have been deleted. By way of example, plasmid ACCMVpLpA

(Gomez-Foix et al. (1992) *J Biol Chem* 267:25129-25134) may be employed to construct a shuttle vector comprising a polynucleotide in accordance herewith. This plasmid contains a polylinker, a CMV promoter and an SV40 polyadenylation signal flanked by partial adenoviral sequences lacking E1A and E1B genes. Analogous plasmids may likewise may used, a number of which are commercially available. The use of plasmid pAC1 or ACCMVpLpA facilitates the cloning process.

[0109] Once constructed, the shuttle vector is co-transfected into HEK293 cells (which contain adenovirus E1 sequences capable of complimenting the missing adenoviral genes) with a plasmid containing the entire human adenoviral 5 genome plus additional sequences rendering the plasmid too large to be encapsidated. Co-transfection can be conducted, for example, by calcium phosphate precipitation or lipofection (Zhang et al. (1993) *Biotechniques* 15:868-872) or other techniques known to those skilled in the art. By way of example, plasmid JM17 (pJM17) encodes the entire human adenovirus 5 genome plus portions of the vector pBR322 including the gene for ampicillin resistance (4.3 kb). Although pJM17 encodes all of the adenoviral proteins necessary to make mature viral particles, the inclusion of the additional sequences (i.e., pBR322 sequences) renders it too large to be encapsidated (40 kb versus 36 kb for wild type). In a subset of co-transfected cells, rescue recombination between the transgene-containing shuttle vector and the plasmid having the entire adenoviral 5 genome occurs, resulting in a recombinant adenoviral genome that is deficient in the ElA/ElB sequences, contains the transgene of interest, but lacks the additional sequences, such as the pBR322 sequences, and thus is small enough to be encapsidated. Such rescue recombination then provides recombinant, replication-deficient adenovirus containing the transgene of interest.

[0110] After identification and purification of successful recombinants, viral stocks are propagated in HEK293 cells to titers typically ranging between 10^8 and 10^{13} viral particles. The adenoviral constructs are then purified to provide high titer, high purity stocks preferably with less than about one (1) replication competent adenovirus (RCA) particle per million, more preferably with fewer than 1 per 10^9 and most preferably with fewer than 1 per 10^{12}.

[0111] Alternatively to the rescue recombination method of viral production, a single plasmid to adenoviral vector (SPA™) method can be and preferably is employed, as described in Zhu et al., WO 2005/001133. This method of construction of recombinant

adenovirus does not require the use of shuttle plasmids or homologous recombination. For example, a replication-defective adenoviral vector plasmid is provided which vector comprises a heterologous promoter sequence linked via a multiple-cloning site (MCS) to a polyadenylation signal which promoter/MCS/ polyadenylation signal sequence is preferably located with the adenoviral vector in place of the E1A/E1B adenoviral sequences. The transgene of interest, for example encoding IGF-1, is then provided with a first linking region (LR1) at its 5' end and a second linking region (LR2) at its 3'end, which linking regions comprise restriction endonuclease recognition sites RE1 and RE2, respectively and correspond to different restriction endonuclease sites of the multiple-cloning site. The replication-defective adenoviral vector plasmid and IGF-1-encoding polynucleotide are then digested with the restriction endonucleases recognizing the RE1 and RE2 sites and ligation reactions carried out to ligate the IGF1-encoding polynucleotide into the multiple-cloning site of the replication-defective adenoviral vector plasmid. The recombinant vector is then transfected into adenoviral complementing cells, such as HEK293 cells under conditions suitable for production of adenovirus resulting in production of recombinant, replication-defective adenovirus comprising the polynucleotide of interest.

[0112] In yet another alternative, also described in Zhu et al., WO 2005/001133, a recombinant adenovirus comprising a nucleic acid encoding IGF-1 in accordance herewith is constructed not only without homologous recombination but without the need for plasmid construction and/or amplification. In this method, standard molecular biology techniques such as PCR, restriction endonuclease digestion and ligation reactions are employed to generate a linear adenoviral vector comprising the IGF-1-encoding nucleic acid. By way of example, in one embodiment, the following components are constructed:

i. LA(V)-TPR-LR1
ii. LR2-TTM-RA(V); and
iii. LR1-TP-LR2

wherein, LA(V) comprises adenovirus left arm sequences; TPR is a transcription promoting region comprising at least one promoter sequence; LR1 is a linking region 1 comprising at least one restriction endonuclease site; LR2 is a linking region 2 comprising at least one restriction endonuclease site that is different from the at least one restriction endonuclease site of LR1; TTM is a transcription termination mediating element comprising at least one

polynucleotide sequence that, when operably linked to a modified polynucleotide of the present invention, is capable of facilitating termination of the transcription of said modified polynucleotide; RA(V) comprises adenovirus right arm sequences and TP is a nucleic acid encoding IGF-1 according to the present invention. Together the LA(V) and RA(V) form a complete replication defective adenoviral genome. Thus, the three components (i, ii, iii) are digested with appropriate restriction endonucleases and ligated to one another to form a linear recombinant adenoviral vector comprising a modified polynucleotide of the present invention. The adenoviral vector is then transfected into a permissive cell line, such as HEK293 cells, and recombinant adenovirus generated.

Propagation and Purification of Adenovirus Vectors

[0113] Recombinant viral vectors, such as adenoviral vectors, can be plaque purified according to standard methods. By way of illustration, the resulting recombinant adenoviral viral vectors can be propagated in human 293 cells (which provide E1A and E1B functions in trans) to titers in the preferred range of about 10^{10}-10^{12} viral particles/ml. Propagation and purification techniques have been described for a variety of viral vectors that can be used in conjunction with the present invention. Adenoviral vectors are exemplified herein but other viral vectors such as AAV can also be employed. For adenovirus, cells can be infected at 80% confluence and harvested 48 hours later. After 3 freeze-thaw cycles of the infected cells, the cellular debris is pelleted by centrifugation and the virus purified by CsCl gradient ultracentrifugation (double CsCl gradient ultracentrifugation is preferred). Prior to *in vivo* injection, the viral stocks can be desalted (e.g., by gel filtration through Sepharose columns such as Sephadex G25). The desalted viral stock can also be filtered through a 0.3 micron filter if desired. We typically concentrate and purify the viral stock by double CsCl ultracentrifugation, followed by chromatography on Sephadex G25 equilibrated with phosphate buffered saline (PBS). The resulting viral stock typically has a final viral titer that is at least about 10^{10}-10^{12} viral particles/ml.

[0114] Preferably, the recombinant adenovirus is highly purified and is substantially free of wild-type (potentially replicative) virus. For these reasons, propagation and purification can be conducted to exclude contaminants and wild-type virus by, for example, identifying successful recombinant virus with PCR using appropriate primers, conducting two rounds of plaque purification, and double CsCl gradient ultracentrifugation.

Animal Models

[0115] Important prerequisites for successful studies of cardiovascular gene therapy are (1) constitution of an animal model that is applicable to clinical cardiovascular disease, particularly the disease(s) of interest, that can provide useful data regarding variations in ventricular and/or cardiac function and/or cardiomyocyte viability, and (2) accurate evaluation of the effects of gene transfer. From this point of view, earlier techniques, such as for example employing rats in an infarct model or employing pigs in a transient ischemia model, are less than satisfactory. Thus, we have made use of a porcine model that fulfills these prerequisites. The pig is a particularly suitable model for studying heart diseases of humans because of its relevance to human physiology. The pig heart closely resembles the human heart in the following ways. The pig has a native coronary circulation very similar to that of humans, including the relative lack of native coronary collateral vessels. Secondly, the size of the pig heart, as a percentage of total body weight, is similar to that of the human heart. Additionally, the pig is a large animal model, therefore allowing more accurate extrapolation of various parameters such as effective vector dosages, toxicity, etc.

[0116] An animal model described herein in Example 1 is exemplary of myocardial infarction. Since the cardiac decompensation that can result from myocardial infarction is similar to, and in fact typically more severe than, that resulting from acute coronary syndrome, this particular model is likewise relevant to that situation. Using this model, it was demonstrated that vector-mediated delivery of a gene encoding an IGF-1 protein alleviated cardiac deterioration.

[0117] In such a model, which mimics clinical myocardial infarction, a catheter is advanced (through a carotid artery or a femoral artery) into the coronary artery of interest, for example, the LCx or the LAD, and a balloon inflated until the artery is completely occluded. After approximately 45-120 minutes of occlusion, the balloon is deflated and the downstream region reperfused. To measure serum indicators of myocardial damage 5-10 ml blood samples are obtained before occlusion and then at intervals following the occlusion/reperfusion. Such serum indicators include, for example, troponin I, myoglobin, creatine kinase-MB, TNF-alpha, sphingolipids, C-reactive protein and IL-6. Additionally, hemodynamic variables and cardiodynamic function are assessed at various intervals and timepoints (e.g., before occlusion, during occlusion, post-occlusion during reperfusion,

and/or post-procedure during recovery). For example, to assess cardiodynamic function, echocardiographic (echo) images are taken of the region at risk, for example the LCx or LAD region, before occlusion of the artery, after administration of treatment and at various intervals (*e.g.*, 3 ,7 and 14 days) following the procedure. Exemplary echo measurements taken are left ventricular end diastolic dimension (LVEDD), left ventricular end systolic dimension (LVESD), % systolic wall thickening (% WTh), systolic excursion of the wall (Excursion), % fractional shortening (%FS) and % fractional area change (%FAC). Cardiodynamic function may also be assessed using ventriculograms (for example to measure ejection fraction and anterior segmental wall motion), by performing pressure-volume analyses and by measuring myocardial blood flow. These assessments of cardiodynamic function can also be performed following a dobutamine challenge.

[0118] Additional data are obtained when animals are sacrificed. For example, the hearts are dissected, cold perfused and stained with vital dyes; selected fresh tissue is removed and flash frozen in liquid nitrogen; small core samples of tissue, for example from the infarct and border zones as well as the interventricular septum and right ventricle, are obtained for infarct sizing and histological analysis; and tissue sections are stained for relevant markers, such as markers of hypertrophy (ANF), apoptosis (TUNEL and caspase-3) and proliferation (cdc2 and Ki67).

[0119] The strategy for therapeutic studies included the timing of transgene delivery, the means and the routes of administration of the transgene. As described herein, administration of the transgene may be in both left and right coronary arteries with various proportions of the total dose of transgene administered in a particular branch (e.g., 50% of dose into the LCx, 30% of dose into the LAD and 20% of dose into the RCA). Alternatively, the transgene may be administered in either the left or right coronary artery, with various proportions of the total dose of transgene administered to a particular branch (e.g., 100% of dose into the LAD; 70% of dose into the LAD and 30% of dose into the LCx; 60% of dose into the LAD and 40% of dose into the LCx; or 50% of dose into the LAD and 50% of dose into the LCx). In the occlusion/reperfusion model of myocardial infarction described herein, gene transfer was initiated approximately 15 minutes after reperfusion. In contrast, a recent study was reported wherein the authors, apparently intending to evaluate the cardioprotective effects of a transgene encoding IGF-1, administered the transgene to the hearts of rats 48 hours *prior* to

LAD ligation (30 min) and reperfusion (24 hr) (Chao *et al.* (2003) *J. Gene Med.* 5:277-286). While the authors report reduced infarction in the treated versus control rats, the correlation of the results to human clinical use or outcome is questionable, in part because this study does not closely duplicate the conditions that would be present in the treatment of clinical myocardial infarction in which gene transfer occurs *after* injury; it being unfeasible to treat human patients 48 hours (or realistically, any time) *before* infarction. Linked to the decision to design a more appropriate animal model of myocardial infarction was the desire to achieve efficient transgene delivery. The constraint that the technique should be applicable for the subsequent treatment of patients with myocardial infarction or acute coronary syndrome made several approaches untenable or less than ideal (continuous infusion of a peptide into the coronary artery, coating the heart with a resin containing the peptide to provide long-term slow release). Finally, the pig model provides an excellent means to follow ventricular and overall cardiac function as well as cardiomyocyte viability before and after gene delivery. The use of control animals that received the same vector (e.g., a recombinant adenovirus), but without the transgene, provided a control for these studies. Additionally, the pig has a native coronary circulation very similar of that of humans, including the relative lack of native coronary collateral vessels and a heart size, as a percentage of total body weight, that is similar to that of the human heart. Thus, based upon the foregoing and previous published studies, those of skill in the art will appreciate that the porcine occlusion/reperfusion model exemplified herein is particularly well suited for predicting whether delivery of a potential therapeutic, such as a vector construct coding for IGF-1, is effective to alleviate (i.e., relieve or delay or lessen) cardiac decompensation associated with acute coronary syndrome or myocardial infarction in humans.

[0120] The following Examples are provided to illustrate, but not limit, the invention.

EXAMPLES

[0121] To assist in understanding the present invention, the following Examples are included which describe preferred embodiments of the methods and compositions for use therein as disclosed and claimed herein. The examples relating to this invention should not be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the ability of one skilled in the art, after reviewing the teachings of the present invention in combination with the technical skills

known in the art, are considered to fall within the scope of the invention as described herein and hereinafter claimed.

Example 1: Porcine Model of Acute Myocardial Infarction – Occlusion/Reperfusion

[0122] A porcine model of acute myocardial infarction (MI) was developed to provide a diseased animal model for testing compositions for and/or methods of treatment that would be relevant to treatment in human beings. As described above, the model generally involves occlusion of a coronary artery, using for example an embolization coil or balloon. Contrast dye is used to confirm occlusion and after a period of about 45-120 minutes, the balloon is deflated or embolization coil removed and the artery allowed to reperfuse. Following is an example of a preferred procedure for creation of such a porcine model of MI, in accordance with the present invention.

Angiographic Procedures for Balloon Occlusion/Reperfusion

[0123] Eight Yorkshire pigs (*Sus scrofa*) weighing 40±6 kg were fasted overnight and weighed before individual transport to the gene injection room. Surgical anesthesia was induced with ketamine (33 mg/kg/IM) plus atropine (0.05 mg/kg/IM) and intubation achieved after mask inhalation of 3-5% isoflurane and oxygen. The animal was mechanically ventilated with 100% oxygen at a tidal volume 10-15 ml/kg and rate of 10-16 breaths per minute to maintain an End Tidal CO_2 ($EtCO_2$) of 35-45 mm Hg. Anesthesia was maintained with either 1-2% isoflurane plus oxygen or a cocktail of ketamine (0.125 mg/kg/min) plus propofol (0.0075 mg/kg/min) infused into a peripheral or jugular vein to maintain a surgical plane of anesthesia. Normal saline or lactated Ringers was administered IV at a rate 10-15 ml/kg/hr. The animal was placed on a surgical table with a heating pad and the incision site over the ventral neck shaved, aseptically cleaned and infiltrated with 1% lidocaine. A three to four inch incision was made over the midline of the ventral neck. An external jugular vein was isolated and cannulated with a silastic catheter. This catheter was also used for fluids and drug delivery during the occlusion/reperfusion procedure. The right or left carotid artery was isolated and cannulated with a 5-7 F Cordis sheath access catheter. A dose of 10,000 units of heparin was administered following introduction of the sheath and then 3000 units every 60 minutes to prevent coagulation and catheter related thrombogenesis. Using fluoroscopy, a 7 F coronary guide catheter (Cook, Inc.) was advanced into the left circumflex

artery (LCx). Echo images of the left circumflex region at risk were obtained by injections of LEVOVIST® (Bayer Schering Pharma AG) or DEFINITY® (Bristol Myers Squibb Medical Imaging, Inc.) contrast solutions through the guide catheter. Subsequently, a 4 F balloon wedge pressure catheter (Arrow International, Inc.) was advanced through the guide catheter and the balloon positioned at the occlusion site in the proximal LCx coronary artery. After stable physiological measurements were obtained the balloon was inflated to totally occlude the coronary artery. Occlusion was documented throughout the procedure by injecting radiopaque contrast agent through the guide catheter. The occlusion was maintained for 45-120 minutes before deflating the balloon and reperfusing the region downstream of the occlusion site. The electrocardiogram and arterial pressure (via the guide catheter or sheath access catheter) were carefully monitored through the procedure. Arrhythmias were treated with bolus injections of lidocaine (75-100 mg). Standard code procedures were followed to treat ventricular fibrillation. Intracoronary administration of the test article was initiated after 15 minutes of reperfusion through the central lumen of the catheter. Test article was delivered first into the LCx (50% of total dose), and then into the left anterior descending (30% of total dose) and right coronary artery (20% of total dose). Once the animal was stable throughout at least 30 minutes of reperfusion, the jugular catheter was exteriorized on the posterior neck for vascular access in subsequent echo studies. The neck incision was closed in two layers and the animal permitted to recover.

Blood Sampling for Plasma Enzymes

[0124] To measure plasma indicators of myocardial damage, 5-10 ml blood samples were obtained before balloon occlusion, during occlusion and then at intervals following reperfusion. Various time points for sampling were: pre-occlusion, during occlusion at 30 and 60 minutes, and 2, 15, 30, 60, 120, 180, 240, 300 and 360 minutes post-occlusion during reperfusion and 1, 2, 3, 7 and 14 post-procedure during recovery. The spectrum of plasma analytes that can be measured include: troponin I, myoglobin, creatine kinase-MB, TNF-alpha, sphingolipids, and C-reactive protein. The total blood removed was less than 10% of the animal's blood volume over the two-week period. Peak concentrations and area under the concentration vs. time curves for the various analytes were compared between the treatment groups. In some studies, plasma levels of IL-6 are also determined using an anti-IL6 antibody based assay.

Hemodynamic and Cardiodynamic Studies

[0125] Standard hemodynamic variables including heart rate, left ventricular pressure, and arterial pressure were assessed at baseline, during occlusion, 15-30 minutes of reperfusion and 14 days after reperfusion.

[0126] Cardiodynamic function was assessed using echocardiographic methods. Two-dimensional and M-mode echocardiographic images were obtained from a right parasternal approach. (Hewlett Packard Agilent 5500). Conscious animals were studied suspended in a comfortable sling to minimize body movement, and diazepam and/or midazolam was administered to alleviate agitation when necessary. Images were recorded in a resting state (basal) and again during dobutamine infusion (2.5, 5, 10, and 20 μg/kg/min). Echocardiographic measurements were made using standardized criteria (see, for example, Sahn *et al.* (1978) *Circulation* 58:1072-1083). Echocardiographic measurements were acquired by a single sonographer who was blinded to treatment.

[0127] The echo measurements included left ventricular end diastolic dimension (LVEDD), left ventricular end systolic dimension (LVESD), percent systolic wall thickening (%WTh) using the end systole lateral wall thickness (ESWTh) and the end diastole lateral wall thickness (EDWTh), systolic excursion of the wall, percent fractional shortening (%FS) and percent fractional area change (%FAC). The derivation of these measurements was as follows:

$$\% \text{WTh} = (\text{ESWTh} - \text{EDWTh}) \times 100 / \text{EDWTh}$$

$$\text{Excursion (cm)} = \text{ESWTh} - \text{EDWTh}$$

$$\%\text{FS} = (\text{LVEDD} - \text{LVESD})/\text{LVEDD} \times 100$$

$$\%\text{FAC} = (\text{LVED area} - \text{LVES area}) \times 100 / \text{LVED area}$$

[0128] Analyses of echo data were performed to compare all the treatment groups. Data was compared by repeated measures analysis of variance. Post hoc analysis was performed using Tukey's test.

Infarct Sizing and Histopathological Studies

[0129] Animals were sacrificed 14 days post occlusion/reperfusion. Deep anesthesia was achieved with 3-5% isoflurane and the heart rate and/or arterial blood pressure closely

monitored to confirm deep anesthesia. The beating heart was arrested in diastole with saturated KCl (20-50 ml) injected into the left atrium or left ventricle. The heart was excised, cold-perfused with saline to wash out all blood and processed for measurement of infarct size and collection of tissue for histological analysis.

[0130] Following cold-perfusion of the heart, the left anterior descending (LAD), left circumflex (LCx) and right (RCA) coronary arteries were perfused with papaverine (60 mg) to elicit vasodilation and then cannulated with PE tubing. To define the LCx region at risk, borders were delineated by perfusing the LAD and RCA with Evans Blue dye and the LCx with saline at a perfusion pressure of 100 mmHg. Small transmural samples were taken for histology and the left ventricular free wall plus septum were partially frozen for cutting. Transverse rings were sliced into 5 mm sections and separately weighed. Sections were then incubated with 2,3,5-Triphenyl Tetrazolium Chloride (TTC) for staining followed by fixation in 10% formalin. The ring sections were placed between plexiglass sheets and traces of each section were made on transparent sheets. Tracings of the hearts were scanned into digital format and manually traced using NIH Image. This analysis was used to calculate the LCx region at risk, infarct size and total left ventricular (LV) area. Ytrehus *et al.* (1994) *Am. J. Physiol.* 267:H2383-H2390. Region at risk and infarct size was compared by analysis of variance with a post hoc analysis by Tukey's test. The data is generally presented as region at risk (% LV), infarct size (% region at risk) and infarct size (%LV).

[0131] Following cold perfusion and staining the heart with vital dyes, fresh tissue was obtained from the right ventricle and left atrium and flash frozen in liquid N_2. Small core samples were obtained from the infarct and border zones as well as the interventricular septum and right ventricle for histological analysis. Tissue sections were stained for markers of hypertrophy (ANF), apoptosis (TUNEL and caspase-3) and proliferation (cdc2 and Ki67) using standard procedures known to those of skill in the art. Multiple fields from stained tissue sections were quantified for the specific markers and treatment groups were then compared for the relative level of each marker.

Example 2: Exemplary *In Vivo* Delivery in Porcine Model of Myocardial Infarction

[0132] Intracoronary treatment for acute myocardial infarction was evaluated in a blinded, randomized study comparing the IGF-1 encoding replication defective adenoviral vector

(Ad5.IGF-1) obtained in Example 4 with a placebo. Eight pigs (4 treated, 4 placebo) were used in the study, following the procedure of Example 1. The occlusion was maintained for 60 minutes before the balloon was deflated and reperfusion begun in the region downstream of the occlusion site.

[0133] Test article vials, containing Ad5.IGF-1 or a vehicle placebo, were labeled with a numerical blinding code and stored at –80° C until the time of administration. Ad5.IGF-1 vials contained a 1.1 ml dose at $1\text{x}10^{11}$ viral particles (vp)/ml in 20 mM Tris, 150 mM NaCl, 2 mM $MgCl_2$ and 2% sucrose (pH = 8). Vehicle placebo vials contained 1.1 ml of 20 mM Tris, 150 mM NaCl, 2 mM $MgCl_2$ and 2% sucrose (pH = 8). Intracoronary administration was initiated after 15 minutes of reperfusion and the LCx region at risk was treated first. The LCx region received 50% of the total dose ($5\text{x}10^{10}$ vp) whiles 30% was delivered into the left anterior descending coronary artery ($3\text{x}10^{10}$ vp) and 20% into the right coronary artery ($2\text{x}10^{10}$ vp). Echocardiographic data was collected pre-infarction (at day –1), occlusion/reperfusion and treatment (at day 0) and post-infarction (at days 3, 7 and 14). Infarct sizing and tissue harvesting occurred on day 15 post infarction. Fig. 1 illustrates the protocol timeline used.

[0134] Risk regions and infarct sizes are presented in Fig. 2. Test Pig 1273 had a very small risk region and a very large infarct, 74% of the LCx region at risk. Nine days following occlusion/reperfusion this animal became lethargic and exhibited a poor appetite. Sustained ventricular tachycardia (VT) was diagnosed and the animal was converted to normal sinus rhythm with a 200 joule shock. Based on behavioral observations, it is probable that the animal was in a sustained VT or abnormal rhythm for greater than 12 hours. Subsequently, this animal completed the 15 day protocol without further complications. Control Pig 1278 exhibited minimal myocardial infarction (0.7%). However, a review of angiographic films from the occlusion/reperfusion study revealed limited, but sustained residual flow distal to the inflated balloon. The remaining three control animals exhibited infarctions ranging from 17-28% of the risk region. Myocardial scarring was present in 35 % of the risk region for Ad5.IGF-1 treated pig 1289, however minimal (2%) or no scarring was evident in the remaining two Ad5.IGF-1 treated animals (#1292 and #1303). Thus, two Ad5.IGF-1 treated animals had minimal (2%) or no infarction of the LCx region while infarctions in the three control treated animals ranged from 17-28% of the LCx region at risk.

[0135] Hemodynamic measurements during the occlusion/reperfusion procedure revealed higher heart rates (100 $\pm$ 17 vs 74 $\pm$ 5 bpm; $p = 0.03$) and higher rate pressure products (8985 $\pm$ 1547 vs 6688 $\pm$ 513; $p = 0.03$) for the Ad5.IGF-1 treated pigs compared to control animals during the 60 minute occlusion period. This analysis is limited by the small group sizes (n=4) and influenced by the outlier response of pig 1273 who had an average heart rate of 120 bpm and a rate pressure product of 10,786. During the first 30 minutes of reperfusion, the two groups did not differ in heart rate and rate pressure product. No group differences were observed in mean arterial pressure and core body temperature during the occlusion or reperfusion period.

[0136] Echocardiographic findings are shown in Figs. 3-5. Pigs 1273 and 1278 are not included in these figures due to the technical problems described above. Pig 1273 exhibited no impairment in myocardial function which was consistent with residual flow distal to the occluding balloon and no MI. For pig 1278, myocardial function as measured by fractional shortening declined from 30% at day 7 and to 19% at day 14 post-MI. This is consistent with an extension of the MI resulting from sustained ventricular tachycardia on day 9 post-MI. Ad5.IGF-1 treated pigs 1292 and 1303 exhibited better regional and global myocardial function than control treated pigs at 7 and 14 days post-MI (Fig. 3). Left ventricular chamber dimensions were also smaller in pigs 1292 and 1303. The responses of Ad5.IGF-1 treated pig 1289 were comparable to those of controls. Visual assessment of myocardial function in the LCx region revealed normal function in Ad5.IGF-1 treated pigs 1292 and 1303 while control treated and Ad5.IGF-1 treated pig 1289 exhibited hypokinetic to akinetic function (Fig. 4).

[0137] Systolic function of the left circumflex region at risk was examined during a continuous infusion of dobutamine (20 μg/kg/min) (Fig. 5A-B). Systolic excursion of the lateral wall was markedly reduced at 7 and 14 days post-MI in the three control treated animals. The response of Ad5.IGF-1 treated pig 1289 was comparable to controls. All values for systolic excursion for these animals fell to less than 60% of the pre-MI value. Ad5.IGF-1 treated pigs 1292 and 1303 exhibited a sustained functional response to dobutamine at both 7 and 14 days post-MI. The systolic function for pig 1292 was increased above the pre-MI value at both 7 and 14 days post-MI while function for pig 1303 was sustained at 90% of the pre-MI value.

[0138] Thus, myocardial function was preserved in two of the Ad5.IGF-1 treated animals as confirmed by echocardiographic measurements as well as visual scoring of the LCx region.

Example 3: Exemplary *In Vitro* Biologic Activity of IGF-1 encoding Adenovirus

[0139] Biologic activity of the IGF-1 adenoviruses constructed as described in Example 4 was assessed in three relevant *in vitro* cell-based assays: apoptosis, hypertrophy, and proliferation.

[0140] Ventricular myocytes from neonatal rats were isolated and cultured using standard methods known in the art (for example, Zhu *et al.* (2000) *Cell Death Differ.* 7:773-784). To induce apoptosis, the neonatal rat cardiomyocytes were switched from standard medium with serum to serum-free medium and placed in a hypoxic chamber with 95% N_2 + 5% CO_2 for 24 hours. Neonatal cardiomyocytes displayed cell shrinkage and detachment from the culture dish after 24 h treatment with serum deprivation and hypoxia (Fig. 6B), suggesting that these cells have undergone apoptosis. Transduction of the neonatal cardiomyocytes with AdX.IGF-1 at MOI of 100 blocked the apoptotic morphologic changes under the same hypoxic, serum-deprived conditions (Fig. 6C), consistent with the notion that IGF-1 inhibits apoptosis in cardiomyocytes. Cardiomyocytes cultured with serum under normoxic conditions were used as controls (Fig. 6A).

[0141] An apoptosis hallmark is genomic DNA fragmentation in which genomic DNA is degraded into the lengths of multiple mononucleosomes, observed as ladders after electrophoresis on agarose gels. For a DNA fragmentation assay, genomic DNA was isolated from these cardiomyocyte cultures and analyzed by gel electrophoresis using standard methods. As shown in Fig. 6D, the hypoxia and serum deprivation-induced genomic DNA fragmentation was prevented by transduction with AdX.IGF-1, confirming the anti-apoptosis activity associated with AdX.IGF-1 transfection.

[0142] To determine whether IGF-1 expressed from the adenoviral vector functions in both autocrine and paracrine fashion, the DNA fragmentation apoptosis assay was performed using conditioned media that were produced from AdX.IGF-1-transduced neonatal cardiomyocytes. An α-adrenergic agonist, phenylephrine, was included as a positive control

(Zhu *et al.* (2000) *Cell Death Differ.* 7:773-784) and conditioned medium produced from Ad.Null-transduced cardiomyocytes was included as a negative control. To prepare the conditioned media, neonatal cardiomyocytes were cultured in plating medium at 2.5 x 10^6 cells/10-cm dish and transduced with 200 μl of AdX.IGF-1 (6.19 x 10^9 pfu/ml) for 18 hours. Free viral particles were removed by washing cells twice with phosphate-buffered saline (PBS) and the cardiomyocytes were cultured in minimal medium for either 24 or 48 hours before the medium was collected as conditioned medium. The conditioned medium (or control) was added to a new batch of neonatal cardiomyocytes cultured at 2.5 x 10^6 cells/10-cm dish for 24 hours and then followed by 24 hours of hypoxia and serum deprivation as described above. Genomic DNA was isolated from the cells and a DNA fragmentation assay was performed. As shown in Fig. 7, cardiomyocytes were protected against apoptosis by the AdX.IGF-1-produced conditioned medium but not the Ad.Null-produced conditioned medium. Thus, transduction with the AdX.IGF-1 vector is capable of both an autocrine and paracrine inhibition of apoptosis in neonatal cardiomyocytes.

[0143] A hypertrophy assay was performed to assess the effect of Ad5.IGF-1 transduction on cardiac myocyte hypertrophy. Neonatal rat cardiomyocytes were seeded in plating medium at 1 x 10^4 cells/well in 96-well BD BIOCOAT™ Collagen I plates (BD Biosciences) for 16 hours. Following one wash with plating medium to remove unattached cells, the cardiomyocytes were transduced with Ad5.IGF-1 or Ad.Null at MOI of 100 in four replicates for 6 hours. Free viral particles were removed by aspirating the plating medium and switching the cardiomyocytes to minimal medium. As a positive control, 50 mM phenylephrine (Sigma Chemical) was added to non-transduced cardiomyocytes in quadruplicates. After 72 hours, cell culture media were harvested and atrial natriuretic factor (ANF), a surrogate marker of hypertrophic growth, was quantified by ELISA using rat ANF EIA (Bachem). As shown in Fig. 8, Ad5.IGF-1 induced ANF expression in cardiomyocytes, confirming the hypertrophic effects Ad5.IGF-1. AdX.IGF-1 also was shown to induce hypertrophic growth in cardiomyocytes.

[0144] The proliferative effect of Ad5.IGF-1 on human coronary arterial endothelial cells (HCAEC) was quantified by the MTT tetrazolium method 72 hours after viral transduction. HCAEC from a single donor (Cell Applications, Inc., San Diego, CA) were maintained in 100 μl/well of EGM™-2 MV (Lonza) containing the following supplements: EGF, bFGF,

IGF-1, VEGF, ascorbic acid, hydrocortisone, gentamycin/amphotericin B and 5% fetal bovine serum (FBS) ("supplemented EGM™-2 MV"). HCAEC cells seeded at 2 x 10^3 cells/well in 96-well BD BIOCOAT™ Collagen I plates and triplicate wells were transduced for 16 h at MOI 100 with Ad5.IGF-1 adenoviruses. Cells were then washed once and placed in 100 µl/well EBM-2 (endothelial cell basal medium, Lonza) supplemented with 1% FBS. After 48 hours, cell proliferation was assessed by adding 20 µl/well of CellTiter 96® Assay reagent (Promega Corp.) and reading formazan absorbance at 490nm. The mean $\pm$ SD are represented on the graph in Fig. 9. In Fig. 9, "Control" represents cells cultured in EBM-2 supplemented with 1% FBS for the last 48 h without virus and "Growth Medium" represents cells cultured in supplemented EGM™-2 MV for the last 48 h. The statistical significance of the difference between the control and Ad5.IGI-I treated cells was detected Two-tailed by T-test (p=0.03).

[0145] Proliferative activity for Ad5.IGF-1 was significantly greater than control. The data confirm the angiogenic effects of the Ad5.IGF-1 vector by activation of the critical step of endothelial cell migration. These cell based assays demonstrated that the constructed adenovirus expressing IGF-1 protected against apoptosis in cardiomyocytes, induced hypertrophy in cardiomyocytes and induced proliferation of HCAEC cells. Ad5.IGF-1 was also shown to induce proliferation of human umbilical vein-derived endothelial cells (HUVEC).

Example 4: Preparation of IGF-1 Adenovirus Constructs

[0146] A recombinant adenovirus useful for expression of human IGF-1 protein, AdX.IGF-1, was constructed with an E1/E3-deleted adenoviral vector using the Adeno-X™ kit (Clontech Laboratories, Inc.) (Mizuguchi *et al.* (1999) *Hum. Gene Ther.* 10:2013-2017). To construct AdX.IGF-1, the complete coding sequence of human IGF-1 protein was amplified by high fidelity PCR using Pfu DNA polymerase (Stratagene) according to the vendor's protocol. A plasmid available from ATCC that contains the entire human IGF-1 coding sequence, pT7T3D-PAC-hIGF-1, was used as the template. The amplified IGF-1 coding sequence was cloned into the PCR-Script II vector (Stratagene) by T4 DNA ligase and recovered by Apa I and Kpn I double digestion. The IGF-1 cDNA was then cloned into the Apa I and Kpn I sites of the pShuttle vector (Clontech) between the CMV promoter and

bovine growth hormone polyadenylation signal (BGH polyA) by T4 DNA ligase. The CMV.IGF-1.BGH polyA expression cassette was isolated from the recombinant pShuttle vector by I-Ceu I and PI-Sce I double digestion and cloned into the I-Ceu I and PI-Sce I sites of the Adeno-X plasmid which contains the entire ΔE1/ΔE3 Ad5 genome. Following Pac I digestion to linearize the recombinant Adeno-X plasmid, transfection in HEK293 cells was conducted by the GeneSHUTTLE™ transfection kit (Qbiogene, Inc.) according to vendor's protocol to generate IGF-1 expressing adenovirus AdX.IGF-1. The CMV enhancer/promoter region of AdX.IGF-1 is 517 nucleotides in length.

[0147] Another recombinant adenovirus for expression of human IGF-1 protein, Ad5.IGF-1, was constructed with an E1-deleted adenoviral vector using the pΔE1SP1A/pJM17 system (Microbix Biosystems, Inc.) (McGrory *et al.* (1988) *Virology* 163:614-617). To construct Ad5.IGF-1, the complete IGF-1 coding sequence was amplified by Pfu DNA polymerase-mediated high fidelity PCR using the pT7T3D-PAC-hIGF-1 plasmid as the template. An EcoR I and Xba I sites were incorporated to the 5'- and 3'-end of the PCR product, respectively. Following restriction digestion with EcoR I and Xba I, the IGF-1 PCR product was cloned into the EcoR I and Xba I sites of the pCI vector (Promega Corp.) by T4 DNA ligase. The CMV.IGF-1.SV40 polyA expression cassette was isolated from the recombinant pCI vector by BamH I and Bgl II double digestion and cloned into the BamH I and Bgl II sites of the pΔE1SP1A vector (Microbix Biosystems, Inc.) by T4 DNA ligase. A mixture of the recombinant pΔE1SP1A and pJM17 (Microbix Biosystems, Inc.), which contains the entire Ad5ΔE1 genome, at a molar ratio of 1:1 was co-transfected in HEK293 cells by the FuGENE® 6 transfection kit (Roche Diagnostics Corp.) according to the vendor's protocol to generate Ad5.IGF-1.

[0148] Another recombinant adenovirus for expression of human IGF-1 protein, Ad5.eCMV.IGF-1, was constructed containing the eCMV promoter of about 1700 nucleotides in length (as described herein). Protein production from the IGF-1 adenoviral vectors with different CMV promoters was compared. In one exemplary experiment, HEK293 cells were transduced with Ad5.eCMV.IGF-1 virus or AdX.IGF-1 virus (with the tCMV promoter of about 517 nt) and, 48 hours later, the culture media was tested in an IGF-1 ELISA (R&D Systems). The amount of IGF-1 produced from the adenoviral vector with the eCMV promoter was about 15 fold greater than the amount of IGF-1 produced from the

vector with the tCMV promoter of about 517 nucleotides. In another experiment, Ad5.eCMV.IGF-1 virus and Ad5.IGF-1 virus (with a tCMV promoter) were tested for protein production on HUVEC cells at various MOI. At every MOI tested, the Ad5.eCMV.IGF-1 virus resulted in greater IGF-1 protein production in the cells than the Ad5.IGF-1 virus with the tCMV promoter. For example, a 15 fold increase (at an MOI of 30), a 6 fold increase (at an MOI of 100) and a three fold increase (at an MOI of 300) in IGF-1 protein production was obtained with the adenoviral vector with the eCMV promoter as compared to the adenoviral vector with the tCMV promoter.

Example 5: Construction of Exemplary Modified Polynucleotide Encoding hIGF-1

[0149] A modified polynucleotide in accordance with the present invention, encoding human IGF-1 and comprising one or more modified codons selected from Table 1, was constructed using a series of PCR reactions with a collection of oligonucleotides. The oligonucleotides used are provided below and were synthesized to incorporate codon changes to provide a construct comprising the sequence of modified hppIGF-1 polynucleotide 1 (SEQ ID NO:3) described herein. (Numbers in parentheses correspond to the nucleotide location within the corresponding component. AS = anti-sense; S = sense; and SP163 refers to a translation enhancer sequence used in the present construct.):

SP163 AS (193-174) (SEQ ID NO:7):
5' - GGG CAG GCT GCT GAT CTT G - 3'

SP 163 AS (193-82) (SEQ ID NO:8):
5' - GGG CAG GCT GCT GAT CTT GCC CAT GGC GGC GGT TTC GGA GGC CGT CCG GGG CCG GCG CGG CTC GCG CTC CCT CTC CGG CTC GGA CTG CGA GGC AGC CCG CTC TCT TGC GCG - 3'

SP163 S (1-18) (SEQ ID NO:9):
5' - AGC GCA GAG GCT TGG GGC - 3'

SP163 S (1-112) (SEQ ID NO:10):
5' - AGC GCA GAG GCT TGG GGC AGC CGA GCG GCA GCC AGG CCC CGG CCC GGG CCT CGG TTC CAG AAG GGA GAG GAG CCC GCC AAG GCG CGC AAG AGA GCG GGC TGC CTC GCA GTC C - 3'

AS (462-435) (SEQ ID NO:11):
5' - CTA CAT CCT GTA GTT CTT GTT GCC GGC - 3'

AS (462-271) (SEQ ID NO:12):
5' - GTG CGG GCC CAG GGC CAC ACC GAC ATG CCC AAG ACC CAG AAG GAG GTG CAC CTG AAG AAC GCC AGC AGA GGC AGC GCC GGC AAC AAG AAC TAC AGG ATG TAG - 3'

AS (390-372) (SEQ ID NO:13):
5' - GGG CAT GTC GGT GTG GCG - 3'

AS (390-271) (SEQ ID NO:14):
5' - GGG CAT GTC GGT GTG GCG CTG GGC CCG CAC GCT GCG GGC GCT CTT GGC GGG CTT CAG GGG GGC GCA GTA CAT CTC CAG CCT CCT CAG GTC GCA GCT CCG GAA GCA GCA CTC GTC CAC GAT - 3'

S (181-200) (SEQ ID NO:15):
5' - GCC CTG CAG TTC GTG TGC G - 3'

S (181-300) (SEQ ID NO:16):
5' - GCC CTG CAG TTC GTG TGC GGC GAC AGG GGC TTC TAC TTC AAC AAG CCC ACC GGC TAC GGC AGC AGC AGC CGG AGG GCC CCC CAG ACC GGC ATC GTG GAC GAG TGC TGC TTC CGG AGC TGC - 3'

AS (210-192) (SEQ ID NO:17):
5' - GCC CCT GTC GCC GCA CAC - 3'

AS (210-91) (SEQ ID NO:18):
5' - GCC CCT GTC GCC GCA CAC GAA CTG CAG GGC GTC CAC CAG CTC GGC GCC GCA CAG GGT CTC GGG GCC GGC GGT GGC GCT GCT GGT GAA GGT CAG CAG GCA CAG GGC CAG GTA GAA CAG GTG - 3'

S (-6-14) (SEQ ID NO:19):
5' - GCC GCC ATG GGC AAG ATC A - 3'

S (1-120) (SEQ ID NO:20):
5' - GCC GCC ATG GGC AAG ATC AGC AGC CTG CCC ACC CAG CTG TTC AAG TGC TGC TTC TGC GAC TTC CTG AAG GTG AAG ATG CAC ACC ATG AGC AGC AGC CAC CTG TTC TAC CTG GCC CTG TGC CTG CTG - 3'

[0150] Groups of four oligonucleotides: two larger oligonucleotides spanning the entire amplification region and having an internal complementary overlapping section, and two smaller oligonucleotides, were used in PCR reactions to amplify segments of the fulllength polynucleotide sequence. In order to permit construction of multiple hIGF-1encoding polynucleotide clones including different translation enhancers (eg. SP163), PCR reactions were first carried out to amplify individual components of the constructs followed by assembly of specific PCR products in subsequent PCR reactions. In the present example, the

construct was synthesized to encode the SP 163 translation enhancer followed by the modified version of the IGF-1 encoding polynucleotide. Four individual sequences of DNA were first synthesized, and then they were assembled into one complete sequence in a final PCR reaction.

[0151] Oligonucleotides were ordered from Trilink Biotechnologies (San Diego, CA) in lyophilized form. The oligonucleotides were hydrated with Tris-EDTA buffer (pH 8.0) to a final concentration of 100μM. The first PCR reaction was carried out with the following reaction mixture in a final volume of 50μl (oligonucleotides are referred to by their nucleotide location provided above): 5μl 10x Pfx buffer, 6μl 2.5mM dNTPs, 1μl 50mM $MgSO_4$, 1.5 μl 10μM (-6-14), 1.5 μl 10μM (210-192), 1 μl 100 μM S(1-120), 1 μl 100μM AS(91-210), 1μl Pfx enzyme (Invitrogen, Carlsbad, CA). The first cycle of the PCR program had a 2-minute denaturation step, followed by 1 minute of annealing at 50°C, followed by a 10-minute extension step at 68°C. Thirty-five additional cycles were conducted with 45 second denaturation, 30 second annealing, and 2-minute extension times. The resulting PCR product was resolved by agarose gel electrophoresis, gel purified using the Qiagen Qiaquick Gel Extraction Kit (Qiagen, Valencia, CA) and stored at -20°C. This fragment contained the sequences 1-120 of the final modified hppIGF-1 polynucleotide 1.

[0152] In order to synthesize the remaining pieces of the modified hppIGF-1 polynucleotide 1 sequence, two additional PCR reactions were carried out. One reaction had the following reaction mixture in a final volume of 500μl: 50μl 10x Pfx buffer, 60μl 2.5mM dNTPs, 10μl 50mM $MgSO_4$, 1.5μl 100μM S(181-200), 1.5μl 100μM AS(390-372), 10μl 100μM S(181-300), 10μl 100μM AS(390-271), 10μl Pfx enzyme. This PCR reaction was carried out according to the cycle described above. The other reaction mixture contained the same reaction components but different primers were used as follows: 1.5μl 1001μM SP163S(1-18), 1.5μl 100μM SP163AS(193-174), 10μl SP163S(1-112), 10μl 100μM SP163AS(193-82), and was carried out according to the cycle described above. These PCR products were resolved by agarose gel electrophoresis and purified as described above.

[0153] Individual DNA products were pieced together using additional PCR reactions. The first PCR reaction joined the SP163 sequence to the 1-210 nucleotide region of modified hppIGF-1 polynucleotide 1. The second PCR reaction joined the 181-390 region to the 361-

462 region of modified hppIGF-1 polynucleotide 1. The first reaction contained the following components in a final volume of 500μl: 50μl 10x Pfx buffer, 60μl 2.5mM dNTPs, 10μl 50mM $MgSO_4$, 5μl SP163, 5μl (1-210), 1.5μl 100μM SP163S(1-16), 1.5μl 100μM AS(210-92), 10μl Pfx enzyme. The other reaction mixture contained the same reaction components but different primers and template were used as follows: 6μl (181-390), 83μl 100μM (361-462), 1.5μl 100μM S(181-200), 1.5μl 100μM AS(462-435).

[0154] The final PCR reaction was done to synthesize the entire SP163 region coupled to the complete modified IGF-1 polynucleotide 1 sequence. The reaction conditions were as follows in a final volume of 500μl: 50μl 10x Pfx buffer, 60μl 2.5mM dNTPs, 10μl 50mM $MgSO_4$, 5μl (1-210), 18μl (181-462), 1.5μl 100μM SP163S(1-18), 1.5μl 100μM AS(462-435), 10μl Pfx, and subjected to the above described PCR cycle. PCR product was resolved by agarose gel electrophoresis and purified as described above. This product was labeled as SP163-IGF-1(1) and was subcloned into a TOPO cloning vector (Invitrogen) and used to transform One-Shot TOP10 competent cells (Invitrogen). Following amplification, the modified hppIGF-1 polynucleotide 1 was sequenced and any errors corrected via site-directed mutagenesis, using standard techniques, for example employing the QuickChange II Site-Directed Mutagenesis Kit from Stratagene, San Diego, CA.

Example 6: Additional Exemplary Modified Polynucleotides Encoding hIGF-1

[0155] Two additional modified polynucleotides encoding human IGF-1 and comprising one or more modified codons selected from Table 1, are constructed by the same method described in Example 5. These modified polynucleotides comprise the nucleotide sequences of modified hppIGF-1 polynucleotide 2 (SEQ ID NO:4, "hIGF-1(v2)") and modified hppIGF-1 polynucleotide 3 (SEQ ID NO:5, "IGF-1(v3)") provided herein. Exemplary oligonucleotides used to generate hIGF-1(v2) and hIGF-1(v3) polynucleotides are as follows (as above, numbers in parentheses correspond to the nucleotide location of the oligonucleotide within the modified hIGF-1 coding sequence; S = sense and AS = antisense. v2 and v3 refer to hIGF-l (v2) and hIGF-l (v3), respectively):

Oligonucleotides for construction of hIGF-1(v2):

v2AS (210-186) (SEQ ID NO:21):
5' - GCC GCG GTC TCC ACA CAC GAA TTG - 3'

v2 S (1-120) (SEQ ID NO:22):
5' - GCC GCC ATG GGC AAG ATC TCC AGC CTG CCC ACC CAG TTG TTC AAG TGC TGT TTT TGC GAC TTC CTG AAA GTG AAG ATG CAC ACC ATG AGC TCC TCT CAT CTC TTT TAC CTG GCC CTG TGC CTG CTC - 3'

v2 AS (462-428) (SEQ ID NO:23):
5' - CTA CAT CCT GTA GTT CTT ATT GCC GGC TGA TCC T - 3'

v2 S (181-300) (SEQ ID NO:24):
5' - GCC CTG CAA TTC GTG TGT GGA GAC CGC GGC TTC TAC TTC AAC AAG CCC ACT GGG TAT GGC AGC TCC TCT CGG AGG GCT CCA CAG ACA GGG ATC GTG GAC GAG TGC TGT TTT CGG TCA TGC - 3'

v2 AS (210-91) (SEQ ID NO:25):
5' - GCC GCG GTC TCC ACA CAC GAA TTG CAG GGC ATC GAC CAG TTC TGC ACC ACA AAG GGT CTC AGG CCC AGC TGT GGC GGA GCT GGT GAA AGT GAG CAG GCA CAG GGC CAG GTA AAA GAG ATG - 3'

Oligonucleotides for construction of hIGF-1(v3):

v3 S (-6-17) (SEQ ID NO:26):
5' -GCC GCC ATG GGC AAG ATC TCC AG - 3'

v3 AS (390-271) (SEQ ID NO:27):
5' - GGG CAT GTC GGT GTG GCG CTG GGC CCG CAC GCT GCG GGC GCT CTT GGC GGG CTT CAG GGG GGC GCA GTA CAT CTC CAG TCT CCT CAG GTC GCA GCT CCG GAA ACA GCA CTC GTC CAC GAT - 3'

v3 AS (210-191) (SEQ ID NO:28):
5' GCC CCT GTC GCC GCA CAC G- 3'

v3 AS (210-91) (SEQ ID NO:29):
5' - GCC CCT GTC GCC GCA CAC GAA CTG CAG GGC GTC CAC CAG CTC GGC GCC GCA CAG GGT CTC GGG GCC GGC GGT GGC GGA GCT GGT GAA GGT GAG CAG GCA CAG GGC CAG GTA GAA CAG GTG - 3'

v3 S (181-206) (SEQ ID NO:30):
5' -GCC CTG CAA TTC GTG TGT GGA GAC C - 3'

v3 S (181-300) (SEQ ID NO:31):
5' - GCC CTG CAG TTC GTG TGC GGC GAC AGG GGC TTC TAC TTC AAC AAG CCC ACC GGG TAC GGC AGC TCC TCT CGG AGG GCC CCC CAG ACC GGC ATC GTG GAC GAG TGC TGT TTC CGG AGC TGC - 3'

v3 S (390-411) (SEQ ID NO:32):
5' - GGG CAT GTC GGT GTG GCG CTG - 3'

v3 S (181-202) (SEQ ID NO:33):
5' - GCC CTG CAG TTC GTG TGC GGC - 3'

v3 S (1-120) (SEQ ID NO:34):
5' - GCC GCC ATG GGC AAG ATC TCC AGC CTG CCC ACC CAG CTG TTC AAG TGC TGC TTC TGC GAC TTC CTG AAG GTG AAG ATG CAC ACC ATG AGC TCC TCT CAC CTG TTC TAC CTG GCC CTG TGC CTG CTC - 3'

v3 AS (461-440) (SEQ ID NO:35):
5' - GGG CAT GTC GGT GTG GCG CTG - 3'

v3 AS (461-361) (SEQ ID NO:36):
5' - GGG CAT GTC GGT GTG GCG CTG GGC CCG CAC GCT GCG GGC GCT CTT GGC GGG CTT CAG GGG GGC GCA GTA CAT CTC CAG TCT CCT CAG GTC GCA GCT CCG GAA ACA GCA CTC GTC CAC GAT - 3'

The following oligonucleotides are for construction of both hIGF-1(v2) and hIGF1(v3):

v2v3 AS (390-271) (SEQ ID NO:37):
5' - GGG CAT GTC GGT GTG GCG CTG GGC CCG CAC GCT GCG GGC AGA CTT GGC AGG TTT GAG GGG TGC GCA ATA CAT CTC AAG TCT CCT CAG ATC GCA TGA CCG AAA ACA GCA CTC GTC CAC GAT - 3'

v2v3 AS (462-361) (SEQ ID NO:38):
5' - CTA CAT CCT GTA GTT CTT ATT GCC GGC TGA TCC TCT ACT AGC GTT CTT CAA GTG GAC TTC TTT CTG GGT CTT GGG CAT GTC GGT GTG GCG CTG GGC CCG CAC - 3'

CLAIMS

What is claimed is:

1. A method for improving cardiac function in a patient, comprising delivering to the heart of the patient a nucleic acid for inducing the synthesis of an insulin-like growth factor (IGF) in the heart of the patient.

2. The method of claim 1, wherein cardiac decompensation of the patient is ameliorated

3. The method of claim 1, wherein the nucleic acid is delivered following an acute coronary syndrome.

4. The method of claim 3, wherein the nucleic acid is delivered within twenty-four hours following a heart attack.

5. A method of claim 1, wherein the nucleic acid encodes insulin-like growth factor-1 (IGF-1).

6. The method of claim 5, wherein the nucleic acid encoding IGF-1 is comprised within a vector which vector is delivered to the myocardium.

7. The method of claim 6, wherein the vector is introduced by anterograde perfusion into at least one coronary artery supplying blood to the myocardium.

8. The method of claim 6, wherein the vector is introduced from a catheter conducted at least about 1 cm into the lumen of one or more coronary arteries supplying blood to the myocardium.

9. The method of claim 8, wherein the vector is infused from the tip of the catheter.

10. The method of claim 7, wherein the vector is introduced into at least one right coronary artery and at least one left coronary artery.

11. The method of claim 7, wherein the vector is also introduced into a saphenous vein graft and/or an internal mammary artery graft supplying blood to the myocardium.

12. The method of claim 6, wherein the vector is introduced into the myocardium by retrograde perfusion from a catheter placed into a conduit receiving blood from the myocardium.

13. The method of claim 6, wherein the vector is introduced into the myocardium by direct injection.

14. The method of claim 1, wherein the nucleic acid induces expression of an endogenous gene encoding IGF-1.

15. The method of claim 14, wherein the nucleic acid encodes a transcription factor.

16. The method of claim 14, wherein the nucleic acid encodes a zinc-finger DNA binding protein.

17. The method of claim 1, wherein the nucleic acid is first introduced onto a stent and the stent is placed in a coronary artery supplying blood to the myocardium.

18. The method of claim 1, wherein the nucleic acid is first introduced into a cell, ex vivo, and the cell is subsequently delivered to the myocardium.

19. The method of claim 6, wherein the vector is a viral vector.

20. The method of claim 19, wherein the vector is a replication-deficient viral vector.

21. The method of claim 20, wherein the vector is a replication-deficient adenovirus vector.

22. The method of claim 21, wherein about 10^7 to about 10^{13} adenovirus vector particles are delivered in vivo.

23. The method of claim 22, wherein about 10^9 to about 10^{12} adenovirus vector particles are delivered in vivo.

24. The method of claim 5, wherein the nucleic acid is operably linked to a cytomegalovirus (CMV) promoter.

25. The method of claim 24, wherein the CMV promoter is an enhanced CMV promoter (eCMV).

26. The method of claim 5, wherein the nucleic acid is operably linked to a tissue-specific promoter.

27. The method of claim 26, wherein the tissue-specific promoter is a cardiomyocyte-specific promoter.

28. The method of claim 27, wherein the cardiomyocyte-specific promoter is selected from the group consisting of a cardiomyocyte-specific myosin light chain promoter and a cardiomyocyte-specific myosin heavy chain promoter.

29. The method of claim 1, wherein the nucleic acid is predominantly localized to the heart.

30. The method of claim 6, wherein the vector predominantly transduces cardiac cells.

31. The method of claim 30, wherein expression of the nucleic acid occurs predominantly within the myocardium.

32. The method of claim 31, wherein expression of the nucleic acid occurs predominantly within cardiac myocytes.

33. A method according to one of claims 1-32, wherein the nucleic acid comprises an additional sequence inducing synthesis of a second protein factor in the heart of the patient.

34. The method of claim 33, wherein the second protein factor is selected from the group consisting of a factor which induces angiogenesis, a factor which promotes cardiac cell survival or function, and a factor which recruits cells within or to the heart.

35. The method of claim 34, wherein the second protein factor is selected from the group consisting of a fibroblast growth factor, a vascular endothelial growth factor, and a platelet-derived growth factor.

36. The method of claim 34, wherein the second protein factor is an anti-apoptotic factor.

37. The method of claim 33, wherein the second protein factor is granulocyte colony-stimulating factor.

38. The method of claim 33, wherein the protein factor is hepatocyte growth factor.

39. The method of claim 33, wherein the protein factor is endothelial PAS domain 1 (EPAS-1).

40. The method of claim 33, wherein the protein factor is a beta-adrenergic signaling protein (beta-ASP).

41. A method according to one of the preceding claims, further comprising the step of infusing a pharmacologic agent into one or more of the coronary arteries supplying blood to the myocardium.

42. The method of claim 41, wherein the pharmacologic agent is a protein factor selected from the group consisting of a factor which induces angiogenesis, a factor which promotes cardiac cell survival or function, and a factor which recruits cells within or to the heart.

43. The method of claim 42, wherein the protein factor is IGF-1 protein.

44. The method of claim 42, wherein the protein factor is selected from the group consisting of a fibroblast growth factor, a vascular endothelial growth factor, a platelet-

derived growth factor, a hypoxia-inducible factor, an angiogenic polypeptide regulator, and an insulin-like growth factor.

45. The method of claim 42, wherein the protein factor is an anti-apoptotic factor.

46. The method of claim 7, further comprising the step of infusing a vasoactive agent into one or more of the coronary arteries supplying blood to the myocardium.

47. The method claim 46, wherein the vasoactive agent is selected from the group consisting of histamine, a histamine agonist, sodium nitroprusside (SNP), an SNP agonist or a vascular endothelial growth factor.

48. The method of claim 3, wherein the acute coronary syndrome is myocardial infarction.

49. The method of claim 1, wherein the patient has atherosclerosis.

50. The method of claim 1, wherein the patient has myocardial ischemia.

51. The method of claim 18, wherein the cell is a stem cell.

52. A nucleic acid comprising a sequence encoding IGF-1 operatively linked to an eCMV promoter.

53. The nucleic acid of claim 52, wherein the nucleic acid is comprised within a viral vector.

54. A nucleic acid comprising a modified sequence encoding IGF-1, wherein the modified sequence comprises at least one alternate codon in place of the IGF-1 native codon.

55. The nucleic acid of claim 54, wherein the modified sequence encoding IGF-1 comprises at least 10 alternate codons.

56. The nucleic acid of claim 54, wherein the modified sequence encoding IGF-1 is operatively linked to an eCMV promoter.

57. The nucleic acid of claim 56, wherein the nucleic acid is comprised within a viral vector.

58. An isolated cell comprising the nucleic acid of claim 52.

59. An isolated cell comprising the nucleic acid of claim 54.

60. A kit for improving cardiac function following acute coronary syndrome in a patient, comprising a device for delivering an agent to the heart of the patient and a nucleic acid for inducing the synthesis of IGF in the heart of the patient.

61. The kit according to claim 60, wherein said device comprises a catheter.

62. The kit according to claim 60, wherein the nucleic acid encodes IGF-1.

63. The kit according to claim 62, wherein the nucleic acid encoding IGF-1 is comprised within a viral vector.

64. The kit according to claim 63, wherein said vector is a replication-deficient adenovirus vector.

65. The kit according to claim 64, wherein the kit comprises about 10^7 to about 10^{13} adenovirus vector particles.

66. The kit according to claim 64, wherein the nucleic acid is operably linked to a CMV promoter.

67. The kit according to claim 64, wherein the CMV promoter is an eCMV promoter.

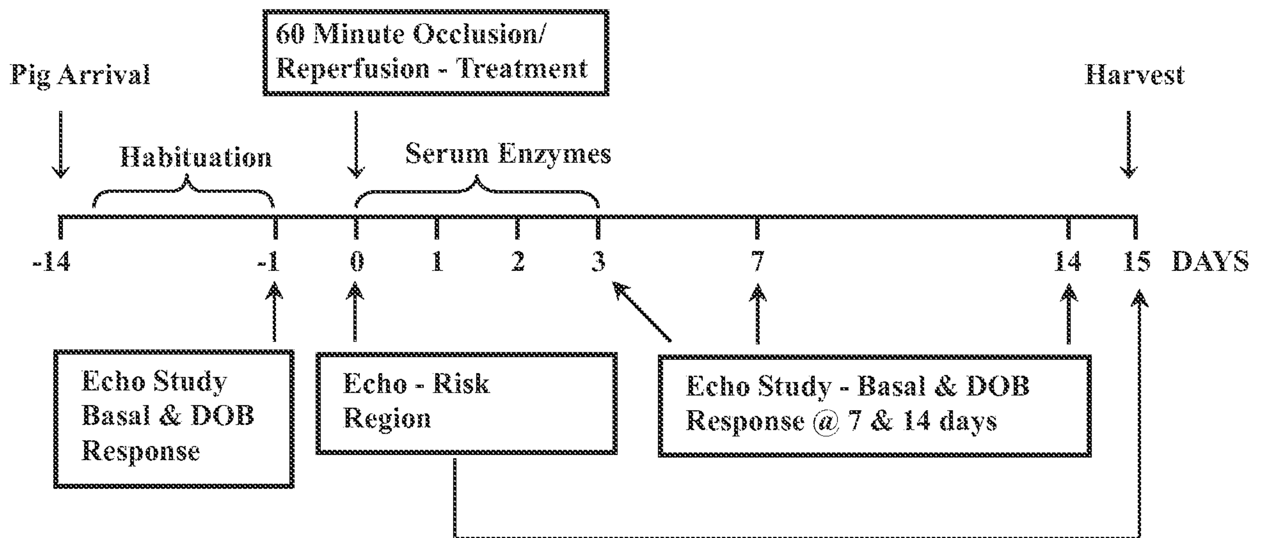

FIG. 1

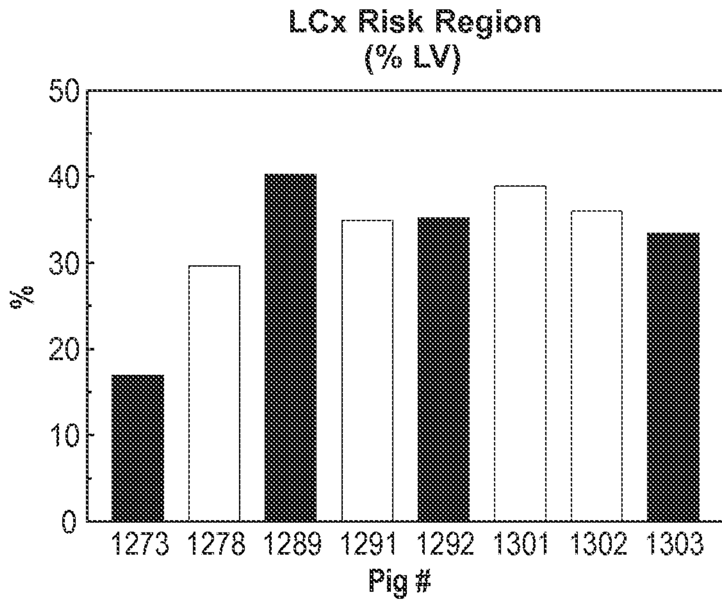

FIG. 2A

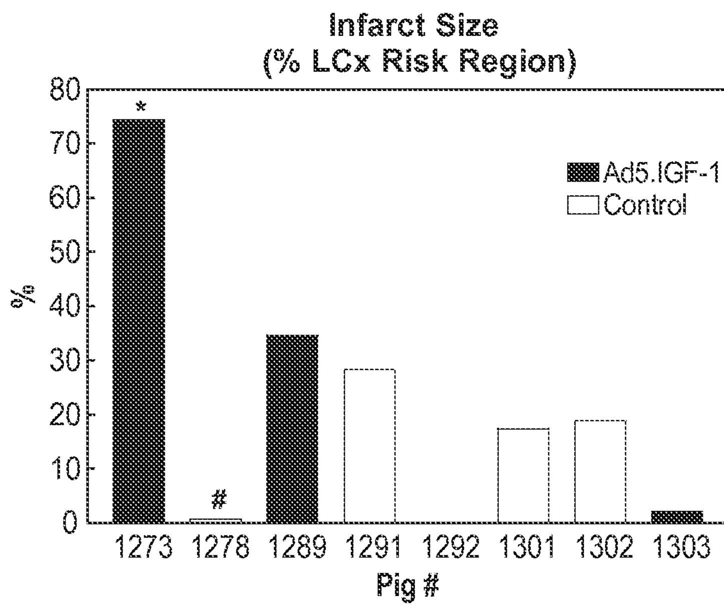

FIG. 2B

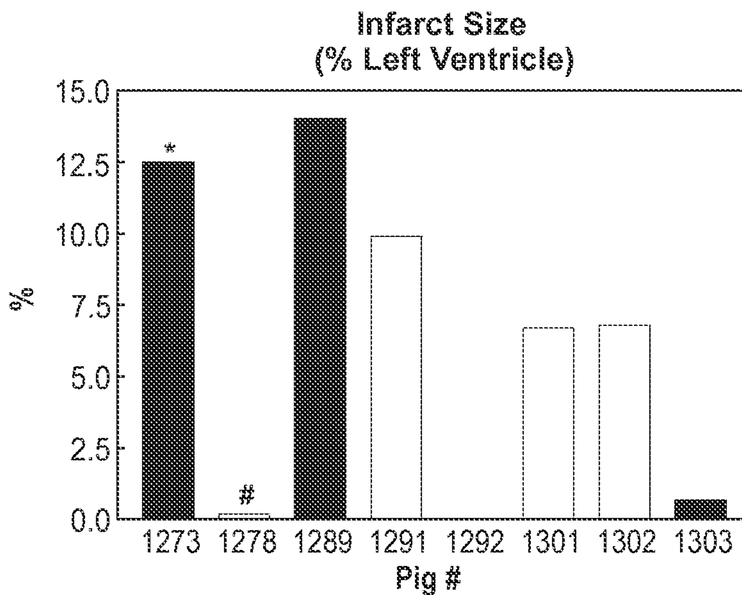

FIG. 2C

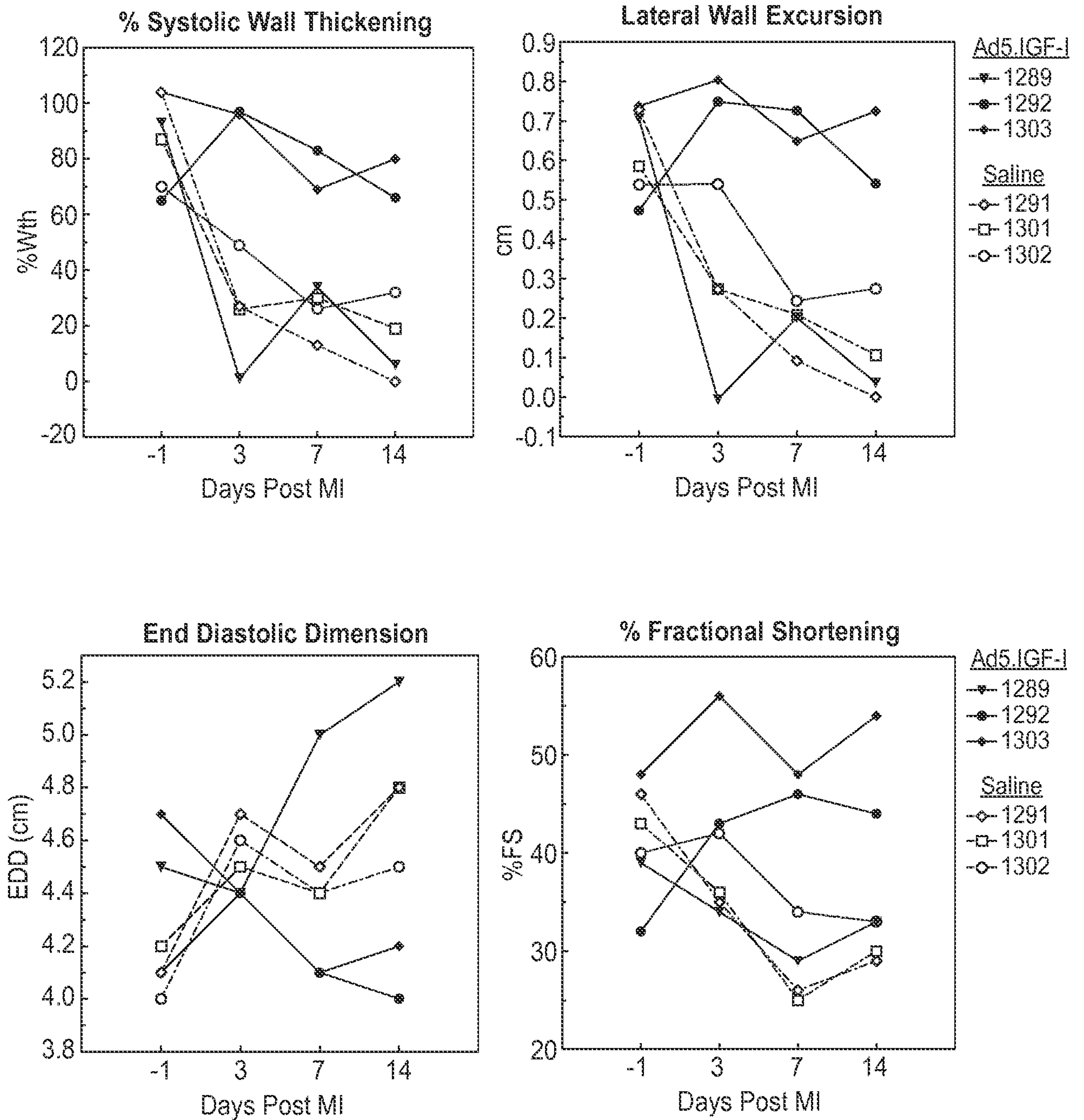

FIG. 3

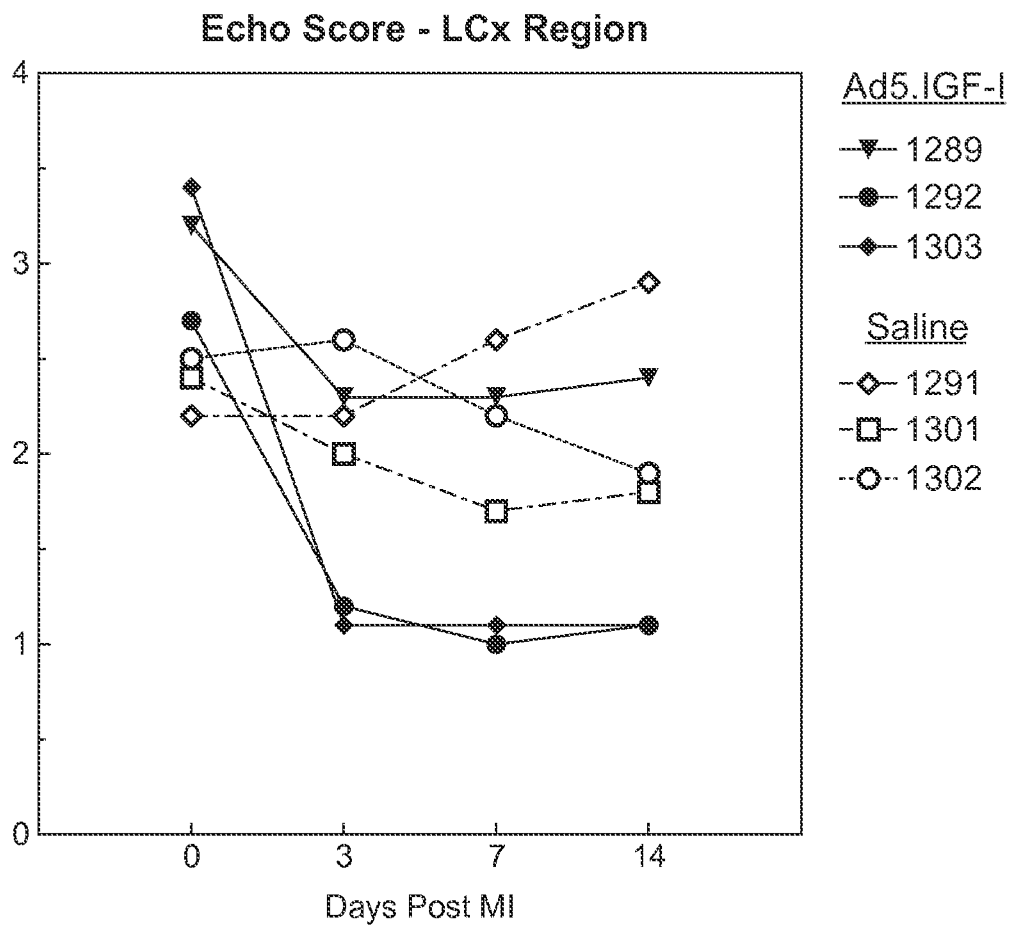

FIG. 4

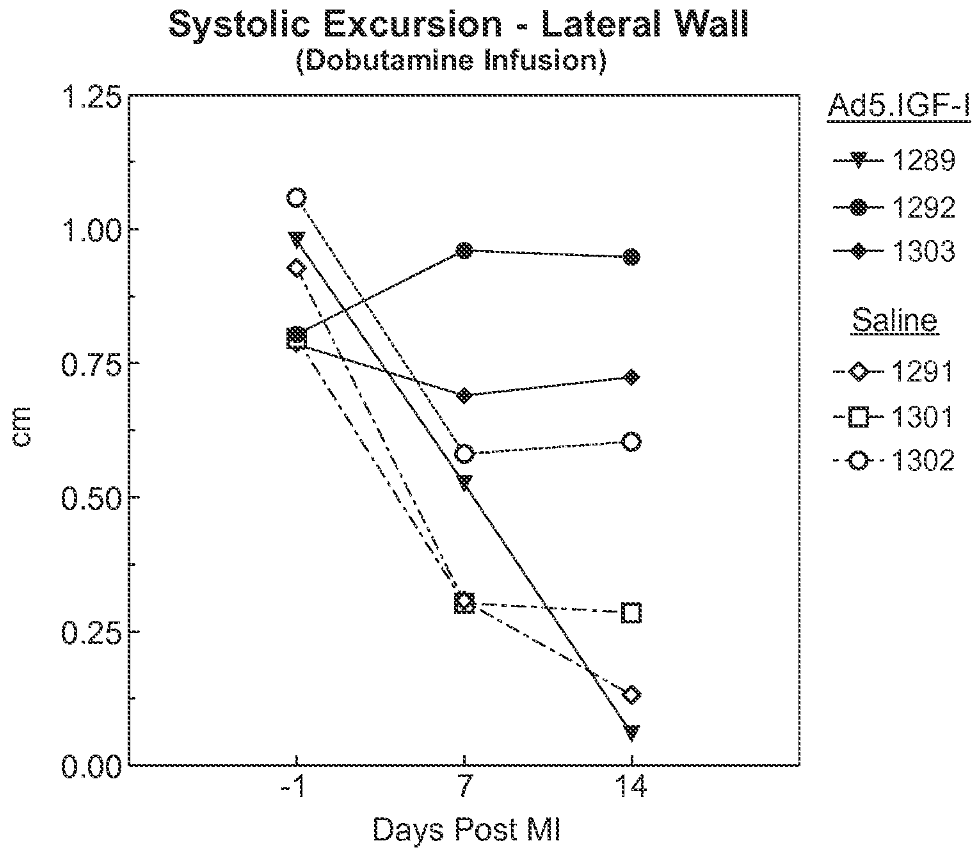

FIG. 5A

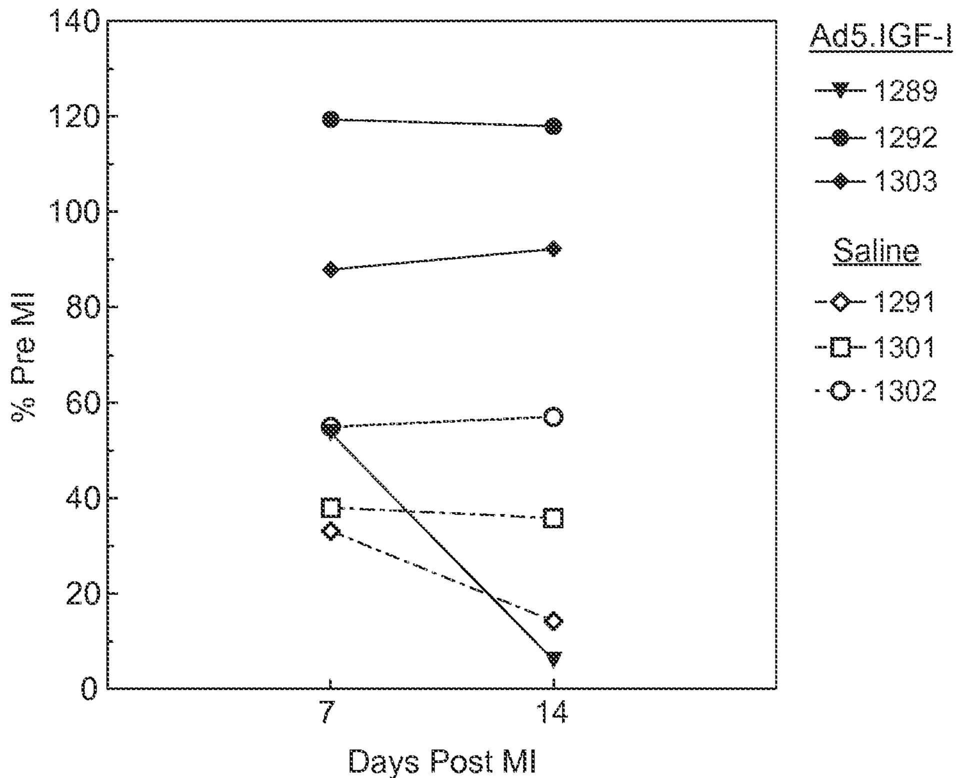

FIG. 5B

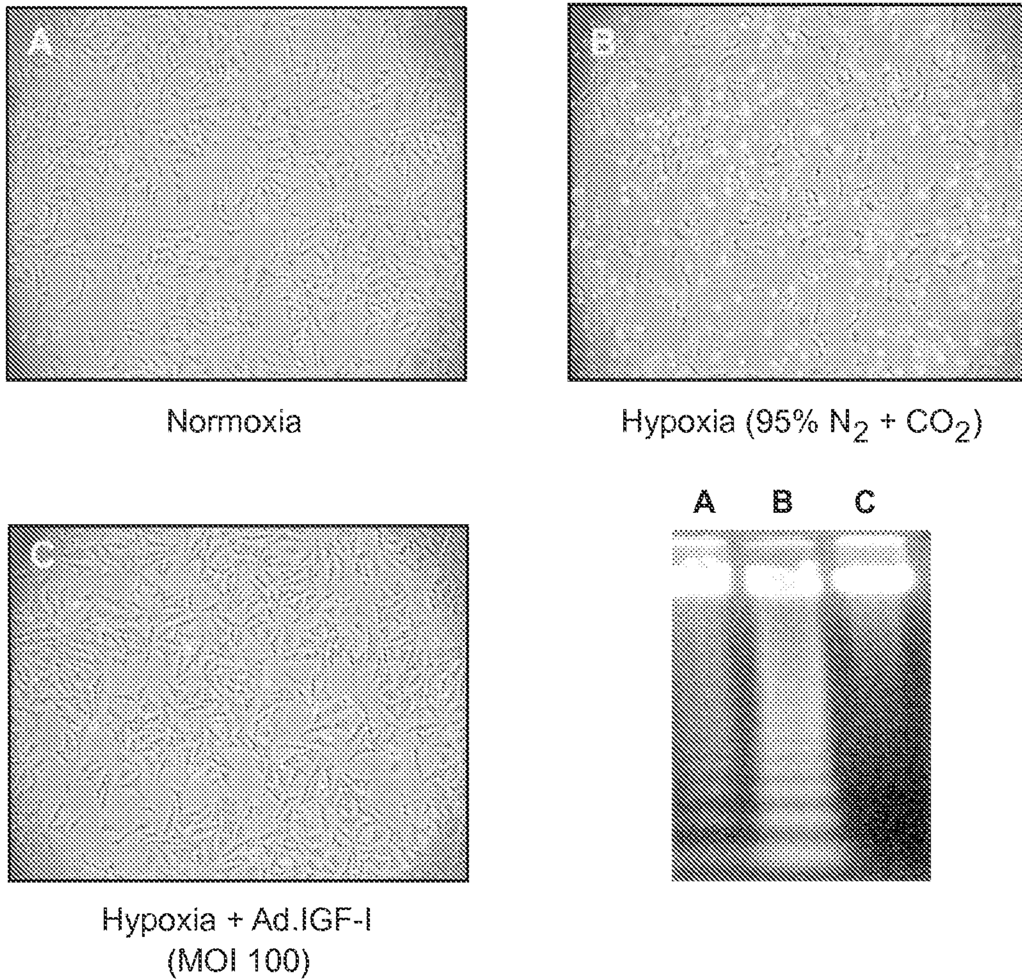

FIG. 6

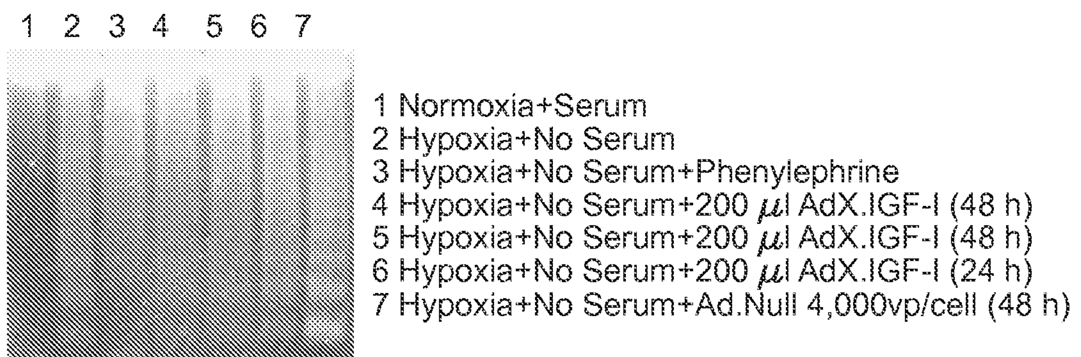

FIG. 7

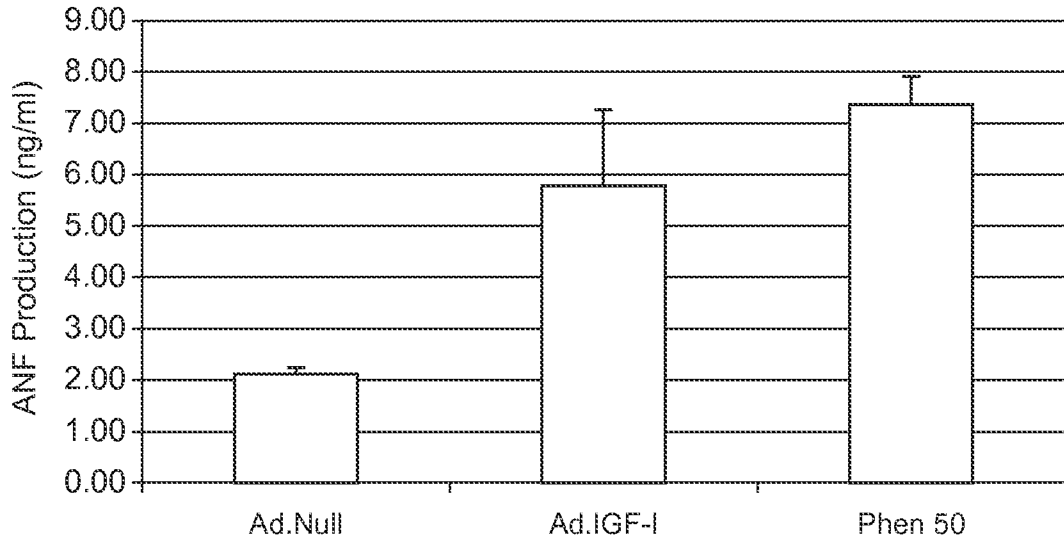

FIG. 8

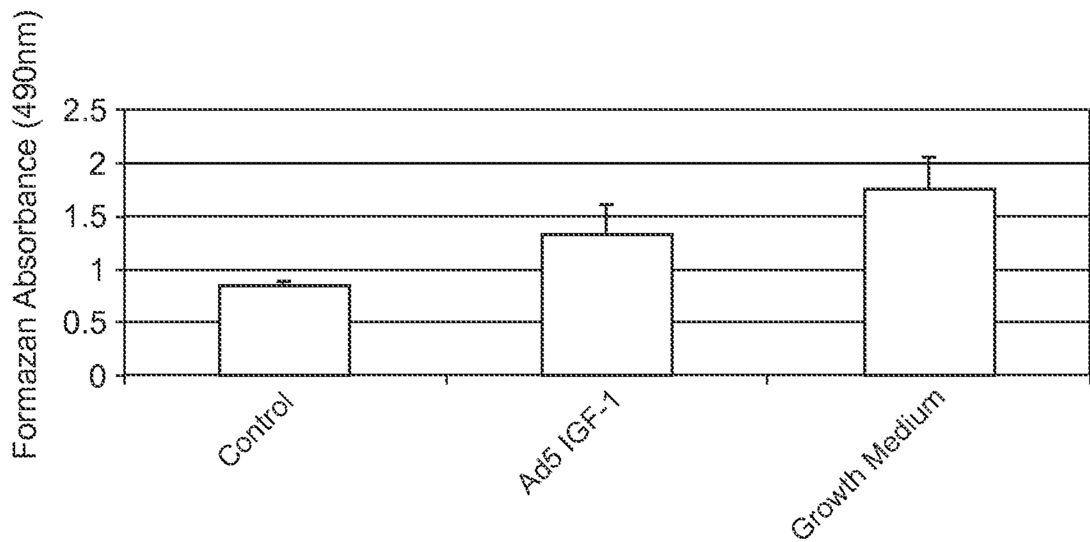

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/076733

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/19 A61K48/00 C07K14/65

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/081040 A (ADVISYS INC [US]; BAYLOR COLLEGE MEDICINE [US]; RABINOVSKY ERIC D [US]) 23 September 2004 (2004-09-23) the whole document	1-67
X	EP 1 810 696 A (ANGES MG INC [JP]; SAWA YOSHIKI [JP]) 25 July 2007 (2007-07-25) the whole document	1-67
X	WO 2004/080405 A (BIOEXPERTISE LLC [US]; MASCARENHAS DESMOND [US]) 23 September 2004 (2004-09-23) the whole document	1-67

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 January 2009	30/01/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sirim, Pinar

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SEROSE A ET AL: "Administration of insulin-like growth factor-1 (IGF-1) improves both structure and function of delta-sarcoglycan deficient cardiac muscle in the hamster" BASIC RESEARCH IN CARDIOLOGY, STEINKOPFF-VERLAG, DA, vol. 100, no. 2, 1 March 2005 (2005-03-01), pages 161-170, XP019383128 ISSN: 1435-1803 the whole document	1-67
X	CHAO WEI ET AL: "Strategic advantages of insulin-like growth factor-I expression for cardioprotection." THE JOURNAL OF GENE MEDICINE APR 2003, vol. 5, no. 4, April 2003 (2003-04), pages 277-286, XP002510458 ISSN: 1099-498X the whole document	1-67
X	SU E J ET AL: "Gene therapy vector-mediated expression of insulin-like growth factors protects cardiomyocytes from apoptosis and enhances neovascularization." AMERICAN JOURNAL OF PHYSIOLOGY, vol. 284, no. 4 Part 2, April 2003 (2003-04), pages H1429-H1440, XP002510460 ISSN: 0002-9513 the whole document	1-67
A	BOCCHI ET AL: "Beneficial effects of high doses of growth hormone in the introduction and optimization of medical treatment in decompensated congestive heart failure" INTERNATIONAL JOURNAL OF CARDIOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 110, no. 3, 28 June 2006 (2006-06-28), pages 313-317, XP005488416 ISSN: 0167-5273 the whole document	1-67

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCHULZE ET AL: "Insulin-like growth factor-1 and muscle wasting in chronic heart failure" INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, EXETER, GB, vol. 37, no. 10, 1 October 2005 (2005-10-01), pages 2023-2035, XP005042277 ISSN: 1357-2725 the whole document -----	1-67

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/076733

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